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Early School Age Outcomes after Exposure to
Repeat Antenatal Glucocorticoids

Christopher Joel Dorman McKinlay

A thesis submitted in fulfillment of the requirements
for the degree of Doctor of Philosophy in Paediatrics,

The University of Auckland, 2011.
Abstract

Background: Administration of repeat doses of antenatal betamethasone to women at risk of preterm birth 7 or more days after an initial course of glucocorticoids reduces the incidence of respiratory distress syndrome and combined serious morbidity, and has not been associated with any major adverse effects in early childhood. However, exposure to antenatal glucocorticoids could programme physiological changes that increase the risk of adult cardiovascular and metabolic disease.

Objective: To assess physiological risk factors for cardiovascular and metabolic disease in New Zealand children whose mothers participated in the Australasian Collaborative Trial of Repeat Doses of Corticosteroids (ACTORDS).

Methods: This study was performed as part of a wider follow-up of neurological and health outcomes in the whole ACTORDS cohort. Follow-up assessments at 6 to 8 years' corrected age included anthropometry, total body DEXA, frequently sampled intravenous glucose tolerance testing with minimal model analysis, 24-hour ambulatory blood pressure monitoring, renal function, and basal and stimulated cortisol concentrations.

Results: A total of 301 New Zealand children (92% of survivors) underwent anthropometric assessment, and 264 (88% of those eligible) completed at least one physiological investigation. There were no significant differences between children exposed to repeat doses of antenatal betamethasone and those exposed to a single course of antenatal glucocorticoids (placebo group) in growth, body size, body composition, minimal model indicators of glucose and insulin metabolism, ambulatory blood pressure, renal function, and basal diurnal salivary cortisol concentrations. Children exposed to repeat antenatal betamethasone had slightly reduced plasma cortisol responses to insulin-induced mild hypoglycaemia. In non-randomised analyses, reduced birthweight for gestational age was associated with decreased lean mass in childhood, but gestation length was not significantly associated with physiological outcomes.

Conclusions: Exposure to repeat doses of antenatal betamethasone does not alter physiological risk factors for cardiovascular and metabolic disease. Clinicians should consider using repeat doses of betamethasone in women at risk of very preterm birth, 7 or more days after an initial course of glucocorticoids because of the short-term neonatal benefits, which are likely to outweigh any potential long-term harm.
Für Michelle, von ganzem Herzen,

für den Rest der gemeinsamen Reise.
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<th>Description</th>
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<tbody>
<tr>
<td>11β-HSD2</td>
<td>11-beta-hydroxysteroid dehydrogenase type 2</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ACTORDS</td>
<td>Australasian Collaborative Trial of Repeat Doses of Corticosteroids</td>
</tr>
<tr>
<td>AIR</td>
<td>Acute insulin response to glucose</td>
</tr>
<tr>
<td>APCI</td>
<td>Atmospheric pressure chemical ionisation</td>
</tr>
<tr>
<td>AVP</td>
<td>Arginine vasopressin</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin releasing hormone</td>
</tr>
<tr>
<td>DHEA</td>
<td>Dehydroepiandrosterone</td>
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<tr>
<td>DI</td>
<td>Disposition index</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DXA</td>
<td>Dual energy X-ray absorptiometry</td>
</tr>
<tr>
<td>EDD</td>
<td>Estimated date of delivery</td>
</tr>
<tr>
<td>G6P</td>
<td>Glucose-6-phosphatase</td>
</tr>
<tr>
<td>GA</td>
<td>Gestational age</td>
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<tr>
<td>GLUT4</td>
<td>Glucose transporter type 4</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HMP</td>
<td>2-hydrazino-1-methylpyridine</td>
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<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal axis</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
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<tr>
<td>IDDM</td>
<td>Insulin-dependent diabetes mellitus</td>
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<tr>
<td>IGF1</td>
<td>Insulin-like growth factor 1</td>
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<tr>
<td>IGF2</td>
<td>Insulin-like growth factor 2</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Insulin-like growth factor binding protein 3</td>
</tr>
<tr>
<td>IMI</td>
<td>Intramuscular injection</td>
</tr>
<tr>
<td>Kg</td>
<td>Glucose disappearance constant</td>
</tr>
<tr>
<td>MACS</td>
<td>Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study</td>
</tr>
<tr>
<td>MD</td>
<td>Mean difference</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>PEPCK</td>
<td>Phospho-phenolpyruvate carboxykinase</td>
</tr>
<tr>
<td>PPROM</td>
<td>Preterm premature rupture of membranes</td>
</tr>
<tr>
<td>ROP</td>
<td>Retinopathy of prematurity</td>
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<tr>
<td>RR</td>
<td>Risk ratio</td>
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<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SE</td>
<td>Standard error</td>
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<tr>
<td>Sg</td>
<td>Glucose effectiveness</td>
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<td>Si</td>
<td>Insulin sensitivity index</td>
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<tr>
<td>SP-A</td>
<td>Surfactant protein A</td>
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<td>Surfactant protein B</td>
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<td>SP-C</td>
<td>Surfactant protein C</td>
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<td>SP-D</td>
<td>Surfactant protein D</td>
</tr>
<tr>
<td>SRM</td>
<td>Selective reaction monitoring</td>
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<tr>
<td>TB</td>
<td>Tuberculosis</td>
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1 Introduction

1.1 Overview

Preterm birth is a major and growing health problem affecting both high- and low-income countries. One of the most significant advances in the management of this condition was the introduction of antenatal glucocorticoids to accelerate fetal maturation. This has been responsible for a substantial reductions in neonatal mortality and morbidity (Roberts 2006). Liggins and Howie who conducted the first and largest randomised controlled trial of single course antenatal glucocorticoids, the Auckland Steroid Trial, pioneered this treatment in New Zealand in the 1970s (Howie 1977; Liggins 1972). The National Institutes of Health later concluded that antenatal glucocorticoids are a “rare example of a technology that yields substantial cost savings in addition to improving health” (1994).

Despite more than 40 years of research on antenatal glucocorticoids many questions remain about how this treatment should be employed. One important issue is whether glucocorticoid treatment should be repeated in women who do not give birth shortly after an initial course. Randomised trials conducted over the past decade have shown that there is potential to maximise neonatal benefit by giving repeat doses (McKinlay 2012). One such trial was the Australasian Collaborative Trial of Repeat Doses of Corticosteroids (ACTORDS), which showed that administration of a weekly dose of betamethasone to women at risk of preterm birth at <32 weeks’ gestation 7 or more days after an initial course of glucocorticoids significantly reduced neonatal respiratory and other major morbidity (Crowther, Haslam, 2006). However, animal experimental data (Aghajafari, Murphy, Matthews, 2002) and other evidence from the field of developmental origins of disease (Seckl 2001) have raised concern that increased fetal exposure to glucocorticoids could adversely affect neurological development, lung growth, and cardiovascular and metabolic function.

This thesis is based on data from a follow-up study of children in the ACTORDS trial at 6 to 8 years’ corrected age. The aim of this study was to determine whether there are long-term health gains without adverse effects following exposure to repeat doses of antenatal betamethasone. This thesis concentrates on whether repeat antenatal glucocorticoid treatment has any long-term effects on physiological risk factors for cardiovascular and metabolic disease.
This chapter provides an overview of the health issues related to preterm birth, the scientific basis of antenatal glucocorticoid treatment, and evidence regarding the short and long-term effects of glucocorticoids on clinical outcomes and physiological function. It also briefly discusses the potential role of glucocorticoids in the developmental programming of adult disease. The aims, methods and previous results of the ACTORDS trial are outlined in chapter two.

1.2 Preterm birth

1.2.1 Definitions
Preterm birth is defined as delivery before 37 completed weeks’ gestation, timed from the first day of the mother’s last menstrual period. Because this date may be uncertain or unknown, especially if conception occurs during oral contraceptive use, fetal ultrasound is commonly used to confirm the gestational age and expected date of delivery. Measurement of the crown-rump-length or biparietal diameter in late first trimester provides the most accurate estimate of gestational age, with a 95% CI of ±5 days in women with regular menstrual cycles and certain menstrual dates (Hohler 1984; Verburg 2008). In later pregnancy, prediction of gestational age from fetal measurements (usually biparietal diameter or femur length) is less accurate, although this may be improved by the use of various ancillary measures (Gottlieb 2008). Postnatal estimates of gestational age are unreliable, especially in very preterm infants (Wariyar 1997).

Preterm infants are further classified as being very preterm if born at <32 weeks’ gestation or extremely preterm if born at <28 weeks’ gestation (Engle 2004). More recently, the term late preterm has been used to refer to infants born from 34 to 36 weeks’ gestation (Engle 2006), with those born from 32 to 33 weeks’ gestation described as moderately preterm (Goldenberg 2008; Kirkby 2007).

Newborn infants are also frequently classified by birthweight. Low birthweight is defined as <2500 g, and is further divided into very low birthweight (<1500 g) and extremely low birthweight (<1000 g) (Tucker 2004). Although preterm infants frequently have low birthweight, only two thirds of low birthweight infants are born preterm. However, very low birthweight infants are invariably preterm. Infants with a birthweight <10th percentile for gestational age are generally regarded as being small for gestational age, although some authorities restrict this term to babies whose birthweight is more than two standard deviations below the gestation-specific mean (Lee 2003). Some small for gestational age infants are constitutionally small, but many are small because of in utero growth.
restriction, which is associated with an increased risk of an adverse perinatal outcome (de Jong 1999). Customisation of centile curves for maternal characteristics, such as body size, parity and ethnicity, can help to distinguish between these two types of small for gestational age infants (Gardosi 2006).

1.2.2 Epidemiology

Preterm birth is a major global health problem with an estimated 13 million preterm babies born annually and in developed countries preterm birth is responsible for approximately 40% of all neonatal deaths (Lawn 2010). Since the early 1980s, the incidence of preterm birth in many high income countries has gradually increased (Barros 2005; Callaghan 2006; Craig 2002; Joseph 1998), such that between 6% and 12% of all live births now occur preterm, with the highest rates recorded in the United States (Hamilton 2007; Wen 2004). This is largely due to increased rates of moderate and late preterm births, while rates of very preterm birth have remained relatively static (Craig 2002; Joseph 1998; Joseph 2009). The reason for this rise is not fully understood, but contributory factors include increased multiple births, the greater use of assisted reproductive technologies, and increased obstetric intervention to prevent perinatal death (Ananth 2005; Goldenberg 2008; Joseph 2009; Magowan 1998). Approximately 5% of all preterm infants are extremely preterm, 15% are born from 28 to 32 weeks’ gestation, 20% are moderately preterm, and 60% to 70% are late preterm (Goldenberg 2008). In New Zealand in 2005, 4055 infants were born preterm (7.1% of live births), of which 752 were very preterm (1.3% of live births) (New Zealand Health Information Service 2008). There is little reliable evidence for the incidence of preterm birth in developing countries, although it is likely to be higher than that of developed countries (Haas 2006; Lawn 2010).

1.2.3 Pathogenesis

Up to a third of all preterm births and nearly 50% of births between 28 to 31 weeks’ gestation are iatrogenic (Goldenberg 2008; Steer 2005). The most common indications for elective delivery are preeclampsia, placental bleeding, and acute or chronic fetal compromise (fetal distress or severe growth restriction) (Iams 2008; Tucker 2004). Of spontaneous preterm births, up to 50% follow preterm premature rupture of membranes (PPROM), which is defined as spontaneous rupture of membranes at <37 weeks’ gestation, at least 1 hour before the onset of contractions (Goldenberg 2008; Simhan 2005). PPROM is usually followed by spontaneous preterm labour within several days, and 70% to 80% of women with PPROM deliver by one week (Mercer 1997). The remainder of spontaneous preterm births are due to spontaneous preterm labour with intact membranes.
Normal term parturition involves a common uterine pathway consisting of activation of the decidua and fetal membranes, cervical dilatation and effacement, and increased myometrial contractility (Romero 2006). Inflammatory mediators have an important physiological role in regulating parturition, and disruption or premature initiation of inflammatory cascades is thought to be central to the pathogenesis of PPROM and spontaneous preterm labour (Park 2005; Romero 2007). This is most commonly triggered by intrauterine bacterial infection, (Goldenberg 2000), which usually occurs secondary to ascending infection from the lower genital tract. Bacteria first enter the choriodecidual space before crossing the membranes to enter the amniotic cavity. It is estimated that intrauterine infection accounts for 25% to 40% of all preterm births, but it is more common in spontaneous births at <30 weeks’ gestation than with late preterm birth (Goldenberg 2000). However, precise determination of infection rates in preterm birth is difficult as infection is frequently asymptomatic, bacteria of the genital tract are often difficult to culture, and infection may be confined to the decidua or fetal membranes (Andrews 1995; Yoon 2003). Furthermore, the decidua and membranes can be colonised without inducing an inflammatory response. For example, it is not uncommon to detect bacteria in the fetal membranes in asymptomatic women at term (Steel 2005). Colonisation may occur early in gestation or even before pregnancy, and the factors that determine whether inflammation or invasive infection develop are not well known (Cassell 1983; Goldenberg 2000).

Maternal systemic infections, such as pyelonephritis, pneumonia and appendicitis, are also associated with an increased risk of preterm birth (Goldenberg 2000; Romero 2006). This could be due to haematogenous spread of bacteria to the uterus, but it is more likely that systemic endotoxin or cytokines have a proinflammatory effect on the uterus (Gibbs 2001). Similarly, maternal periodontal disease has been associated with an increased risk of preterm birth (Shub 2006; Vergnes 2007; Xiong 2006), but it is unclear whether the relationship is causal or reflects shared variations in the inflammatory response to microorganisms in the oral and genital tracts (Ferguson 2007).

In addition to infection, other pathological processes that can contribute to preterm birth include placental bleeding, uteroplacental ischaemia, uterine overdistension, cervical insufficiency or disease, uterine anomalies, and progesterone deficiency (Goldenberg 2000; Romero 2006). Numerous epidemiological risk factors have also been identified but correlation with underlying mechanisms has not been well described. These include young or old maternal age, low body mass index, smoking, low socio-economic status, African-American, Maori, or Indian ethnicity, assisted reproduction, multiple births, social stress
and suboptimal working conditions (Ancel 1999; Craig, Mitchell, 2004; Filicori 2005; Goldenberg 2008; Mantell 2004; Tucker 2004). Up to 50% of twin and almost all triplet pregnancies deliver preterm (Bornstein 2009; Lee, Cleary-Goldman, 2006). The two most important predictors of preterm birth are a history of previous preterm birth and low socio-economic status (Ancel 1999; Bloom 2001; Esplin 2008). Genetic differences in immune function and regulation of inflammation may account for some of the variation in risk of preterm birth (Varner 2005).

1.2.4 Diagnosis
Preterm labour is defined as regular contractions with cervical dilatation or effacement at < 37 weeks’ gestation. However, the clinical diagnosis of true preterm labour is not straightforward, as many women with labour symptoms, even with cervical change, do not progress to preterm delivery. Furthermore, some women who deliver preterm present with acute cervical ripening and minimal contractions. In the tocolysis trials for preterm labour, approximately 40% of control women gave birth within 48 hours and 50% by 7 days, but 30% continued to term (Gyetvai 1999).

Currently no diagnostic tests can accurately predict when a woman with symptoms of preterm labour will deliver. The risk of preterm birth is increased if fetal fibronectin is detected in cervicovaginal secretions or if there is cervical shortening on transvaginal ultrasound. However, while these tests have high negative predictive value, especially if used in combination, their positive predictive value for impending preterm birth is only about 50% (Leitich 2005). Furthermore, routine use of such tests has not been clearly shown to be clinically beneficial (Berghella 2008; Berghella 2009). A number of other biomarkers for preterm labour have been identified, such as phosphophorylated insulin-like growth factor binding protein-1 and various inflammatory cytokines, but these tests are not widely available and may not have better predictive value (Kurkinen-Raty 2001; Leitich 2005).

1.2.5 Prevention
Interventions to prevent preterm birth can be divided into those that target low-risk women (primary prevention) or women with known risk factors (secondary prevention). Primary prevention is important as more than 50% of preterm births occur in women with no obvious risk factors (Haas 2005; Herron 1982; Mercer 2005). However, most interventions in low-risk women have proven to be ineffective with the exception of smoking cessation programmes in pregnancy (Lumley 2004), and possibly screening and treatment of
asymptomatic bacteriuria (Smaill 2007) and improvement of working conditions for pregnant women (Launer 1990; Pompeii 2005; Saurel-Cubizolles 2004). Screening and treatment of genital tract infection or colonisation has yielded conflicting results. Although most studies have shown that treatment of asymptomatic bacterial vaginosis, *Ureaplasma urealyticum* and group B streptococcal infection does not reduce the incidence of preterm birth in low-risk women (Eschenbach 1991; Klebanoff 1995; McDonald 2007), one large trial found that routine screening and treatment of any asymptomatic vaginal infection, especially before 20 weeks’ gestation, was beneficial (Kiss 2004; Sangkomkamhang 2008). Similarly, treatment of periodontal disease in pregnancy reduced the incidence of preterm birth in one trial (Polyzos 2009) but not in others (Macones 2010; Newnham 2009; Offenbacher 2009). Nutritional supplementation (Czeizel 1994; Kramer 2003; Peña-Rosas 2009), fetal fibronectin testing and antibiotic treatment (Andrews 2003), and cerclage for short cervix (Berghella 2005) have no effect on the incidence of preterm birth in low-risk women. Programmes that involve intensive antenatal care and general maternal health promotion have not been adequately assessed for their effect on the incidence of preterm birth (Papiernik 1985; Whitworth 2009).

Secondary prevention strategies are generally targeted at women with multiple pregnancy or a history of preterm birth. Progesterone has a role in maintaining pregnancy and progesterone supplementation has been shown to reduce the risk of preterm birth in women with a short cervix identified on ultrasound (Fonseca 2007) or history of preterm birth (Dodd 2008; Meis 2003), but not in those with multiple pregnancy (Caritis 2009; Norman 2009; Rouse 2007) or other risk factors for preterm birth (Dodd 2008). However, data on important maternal and neonatal outcomes (Dodd, Flenady, 2006; Dodd 2008) and long-term effects (Doyle 2009; Northen 2007) are limited, and work is ongoing to clarify the role of antenatal progesterone in the prevention of preterm birth (Dodd 2009). Women with a short cervix and a history of preterm birth may also benefit from cerclage, although the evidence is inconclusive (Berghella 2005; Drakeley 2003). Omega-3 supplementation may have a modest effect on gestation length (Makrides 2006) but has not been shown to have any additional effect on the incidence of preterm birth when combined with progesterone (Harper 2010). Low-dose aspirin treatment reduces the risk of preeclampsia and hence can decrease indicated preterm birth in women with risk factors for preeclampsia (Duley 2007). Calcium supplementation also helps to prevent preeclampsia but has not been associated with a reduced incidence of preterm birth (Hofmeyr 2006). Interconceptional home visits, counselling and antibiotics have no effect on recurrence of preterm birth in
women with a history of preterm birth (Andrews 2006; Lumley 2006). Screening and treatment of bacterial vaginosis also does not appear to be effective in preventing recurrent preterm birth (McDonald 2007).

Given the strong causative association between infection and preterm birth, the failure of antibiotics in primary and secondary prevention is surprising. One possible explanation is that while antibiotics are effective at treating mucosal infection, they may not be effective at preventing or treating chorioamnionitis (Goldenberg 2006; Ugwumadu 2006). Another possibility is that maternal factors, such as smoking or genetic variations in inflammatory responses, influence the risk of preterm birth of infectious origin, irrespective of antibiotic treatment (Iams 2008).

### 1.2.6 Management

Women with acute risk of preterm delivery most commonly present with PPROM, symptoms of labour, or vaginal bleeding (Iams 2008). Medical interventions have generally proven to be ineffective in preventing preterm birth in this setting. The main aim of management is thus to reduce neonatal mortality and morbidity associated with preterm birth. The most commonly used agents in women with acute risk of preterm birth are tocolytics, antibiotics and glucocorticoids, and more recently magnesium sulphate. The role of antenatal glucocorticoids is discussed later in this chapter.

Tocolytic drugs suppress myometrial contractility and include calcium channel blockers, beta-2 adrenoceptor agonists, oxytocin antagonists (Atosiban), cyclo-oxygenase inhibitors (Indomethacin), and nitroglycerin. However, with the exception of indomethacin, none of these agents has been shown to reduce the risk of preterm birth (King 2005; Zuckerman 1984). The aim of tocolysis is thus to delay delivery long enough to allow administration of antenatal glucocorticoids and antenatal transfer to a hospital with a specialist neonatal unit. Beta-2 adrenoceptor agonists and nitroglycerin reduce delivery within 48 hours and nitroglycerin reduces delivery before 28 weeks’ gestation (Anotayanonth 2004; Smith 2007). Calcium channel blockers are a little more effective in delaying delivery and are better tolerated than beta-2 adrenoceptor agonists, but they have not been compared with placebo treatment (King 2003). The data on the effectiveness of Atosiban are inconclusive (Papatsonis 2005). The routine use of tocolysis is controversial, as maternal side effects are relatively common and neonatal benefit has not been clearly demonstrated, although infant outcomes have been poorly studied (Fisk 2003; Gyetvai 1999; Smith 2003). If women do
not deliver immediately, no further benefit is achieved by giving prolonged tocolytic treatment (Crowther 1998; Dodd, Crowther, 2006).

In PPROM, antibiotic treatment is associated with delayed delivery and a reduced incidence of chorioamnionitis, neonatal infection, respiratory distress syndrome, intraventricular haemorrhage and major cerebral abnormalities on ultrasound (Cousens 2010; Kenyon 2003). However, in a large multi-centre trial, antibiotic treatment did not alter developmental and educational outcomes at 7 years of age (Kenyon 2008). Erythromycin is preferred over amoxycillin / clavulanic acid as the latter has been associated with an increased risk of necrotising enterocolitis (Kenyon 2003). The use of tocolysis in women with PPROM but without uterine activity is controversial and has not been adequately studied (Mercer 2007). The role of antibiotics for prolonging pregnancy in late preterm PPROM is also unclear (Kominiarek 2009), as is the optimum time to achieve delivery (Buchanan 2010). Clinical trials are currently underway to evaluate these questions (Morris 2006).

Routine use of antibiotics in women with symptoms of preterm labour, intact fetal membranes, and no clinical signs of infection does not prolong pregnancy and may actually be harmful to the fetus (Hutzal 2008; King 2002). In this setting, antibiotic use has been associated with a trend towards increased risk of neonatal death (King 2002) and cerebral palsy (Kenyon 2008). However, intrapartum penicillin prophylaxis is widely recommended for women with threatened or established labour who are colonised with group B streptococcus (Schrag 2002), although the evidence that this prevents neonatal group B streptococcal sepsis is limited (Ohlsson 2009).

Magnesium sulphate is ineffective as a tocolytic agent and is associated with increased neonatal mortality when used for this purpose (Crowther 2002). However, meta-analysis of randomised trials suggests that it reduces the incidence of cerebral palsy and motor dysfunction in offspring when administered to women at imminent risk of very preterm birth (Doyle, Crowther, 2009). The use of magnesium sulphate for fetal neuroprotection at <30 weeks’ gestation has now been recommended in several practice guidelines, including an Australian national guideline (www.adelaide.edu.au/arch/MagnesiumSulphate2010). Apart from glucocorticoids, there are no other known antenatal neuroprotective agents. Phenobarbitone was once thought to be indicated for this purpose but has since been found to be ineffective (Crowther 2010).
1.2.7 Consequences of preterm birth

1.2.7.1 Preterm lung disease

1.2.7.1.1 Human lung development

By 16 to 17 weeks’ gestation, the conducting airways and terminal bronchioles are formed, cartilage, smooth muscle and mucus glands appear, and the primitive respiratory epithelium differentiates into columnar ciliated cells proximally and immature type II alveolar (cuboidal) cells distally (Jeffrey 1998; Joshi 2007; Merkus 1996). Further branching of the respiratory bronchioles continues until 24 weeks’ gestation and is accompanied by increased vasculogenesis in the mesenchyme adjacent to the airways. By 20 to 22 weeks’ gestation, both type I and type II alveolar cells can be identified in distal airways and from 24 weeks’ gestation lamellar bodies containing surfactant are present in type II cells. From 24 to 26 weeks’ gestation, thinning of the walls of terminal ducts results in the formation of terminal saccules (primitive alveoli), which become surrounded by capillary networks. It is only at this stage that pulmonary gas exchange becomes theoretically possible, although antenatal stress may accelerate lung development and be a key factor in the survival of extremely preterm infants (Jobe 2010).

Alveolar development occurs by secondary septation of the terminal saccules, beginning at 26 to 28 weeks’ gestation (Harding and Hooper 2001). This involves the protrusion of crests from the saccule wall into the lumen, which progressively increase in height and subdivide the saccule into individual alveoli. The crests contain capillaries, fibroblasts, and other connective tissue components, which contribute to the process of septation. Mature alveoli do not appear until 32 to 36 weeks’ gestation, following remodelling of the secondary septa (Hislop 1986; Langston 1984). This requires loss of interstitial cells, the laying down of supporting elastin fibres, and maturation of a single alveolar capillary bed. There is also further differentiation of alveolar epithelial cells and a gradual increase in surfactant in type II cells. However, significant amounts of surfactant are not produced until 35 weeks’ gestation (Gluck 1973).

In the final weeks of pregnancy, alveolar multiplication progresses rapidly so that by term the total number of alveoli is 20% to 50% of that present in adults. Most postnatal alveolarisation occurs within the first 6 months after term birth, but alveolar multiplication continues until 2 to 3 years of age (Burri 1984; Thurlbeck 1982). Subsequent lung growth occurs by enlargement of existing alveoli.
Fetal lung fluid, which is secreted from the lung epithelium, plays an important role in normal lung growth by providing mechanical support to developing lung tissue (Harding and Hooper 2001). The volume of fetal lung fluid at term is considerably greater than the functional residual capacity after birth. Lung fluid is reabsorbed by the respiratory epithelium during labour, stimulated by stress hormones such as adrenaline and arginine vasopressin. Lung growth is also dependent on fetal breathing movements, which affect the pressure gradient across the lung and help to regulate the efflux of lung fluid via the trachea (Harding 2006).

1.2.7.1.2 Surfactant

After birth, the alveolar surface is lined with a liquid layer that protects epithelial cells from dehydration but generates high surface tension, causing alveoli to collapse on expiration (Harding and Hooper 2001). This is prevented by secretion of surfactant from type II alveolar cells, consisting of a complex mixture of lipids (~90%) and proteins (~10%). The major lipid component is phospholipid, especially phosphatidylcholine, which forms a monolayer at the air-liquid interface due to association of the hydrophilic polar heads with the liquid layer or hypophase and extension of the hydrophobic fatty acid tails into the alveolar air space. This reduces alveolar surface tension, which prevents atelectasis during expiration and promotes lung inflation during inspiration (Parmigiani 2005). This in turn increases lung compliance and gas volume, and decreases the work of breathing. The alveolar surface tension is inversely correlated with the concentration of phospholipids in surfactant (Brumley 1967). Surfactant also prevents alveolar oedema and contributes to the innate immunity of the lung. All infants require at least some surfactant for successful respiration.

Although proteins make up only a small part of total surfactant mass, they play a critical role in the normal function of the phospholipid monolayer. Four surfactant-associated proteins have been identified, of which surfactant proteins B (SP-B) and C (SP-C) are the most important. Both are small hydrophobic polypeptides that interact with surfactant lipid to enhance the absorption of phospholipids into the surface film and maintain its integrity when compressed during expiration (Serrano 2006). SP-B also has wider effects on surfactant production, promoting the intracellular storage of surfactant in lamellar bodies and the processing of SP-C precursor protein. Thus, genetic deficiency of SP-B leads to fatal respiratory failure at birth (Nogee 1994).
Surfactant proteins A (SP-A) and C (SP-C) are hydrophilic and are part of the collectin family of proteins (Kingma 2006; Takahashi 2006). Collectins have a similar quaternary structure to the first component of complement (C1q) and therefore function as opsonins, enhancing the phagocytosis of a wide range of microbes. For example, in mice, deficiency of SP-A and SP-D is associated with increased pulmonary infection and inflammation but not respiratory distress. However, SP-A also enhances the function of SP-B and SP-C, resists inactivation of surfactant by plasma proteins, and is essential for the formation of tubular myelin, a bilayer reservoir of surfactant components in the hypophase (Parmigiani 2005; Yukitake 1995). SP-D may have a role in surfactant recycling pathways (Ikegami 2005).

1.2.7.1.3 Respiratory Distress Syndrome

Lung immaturity is the most serious consequence of preterm birth and poses the greatest threat to survival, especially in extremely preterm or extremely low birthweight infants. Respiratory distress syndrome is the term used to describe the increased work of breathing and impaired gas exchange that preterm infants suffer due to structural lung immaturity and qualitative and quantitative deficiencies of surfactant. Compared to term infants, preterm infants have lower surfactant pools, less surfactant-associated proteins and saturated phospholipids, decreased synthesis and recycling of surfactant components and surfactant is more readily inactivated (Zimmermann 2005). In very preterm infants, gas exchange must occur via terminal saccules and immature alveoli that are thick-walled, prone to atelectasis, and have poorly developed microvasculature. The lung epithelium also leaks fluid and proteins, which inactivate surfactant, and has limited ability to resorb fluid due to reduced expression of sodium channels (O'Brodovich 1996). Preterm infants also have a compliant chest wall and weaker respiratory muscles, which contribute to respiratory inefficiency.

Respiratory distress syndrome typically presents shortly after birth with tachypnoea, expiratory grunt, chest retraction, and cyanosis without added oxygen. Dyspnoea and oxygen requirement worsen over the first 24 to 36 hours of life but start to abate after 36 to 48 hours as the production of surfactant increases (Kanto 1976). In uncomplicated cases, the respiratory distress resolves after seven days. Histologically, there is atelectasis and over-distension of terminal and respiratory bronchioles with epithelial necrosis and formation of hyaline membranes composed of coagulated plasma proteins. The chest radiograph shows a diffuse, fine granular opacification with air bronchograms due to widespread atelectasis (Hjalmarsone 1981). However, there is considerable variation in the
severity of preterm lung disease, and infants with mild initial distress and short exposure to low oxygen concentrations may also be included in the diagnosis of respiratory distress syndrome (Jobe 2010). Furthermore, infants who receive exogenous surfactant do not have typical clinical or radiographic findings, which can make the categorisation of lung disease difficult when surfactant is given prophylactically. Therefore, the diagnosis of respiratory distress syndrome is imprecise.

Gestational age is the most important determinant of the incidence and severity of respiratory distress syndrome. In the absence of antenatal glucocorticoids, the incidence is approximately 80% to 100% at <28 weeks’ gestation, 40% to 60% at 28 to 31 weeks’, 20% to 30% at 32 to 33 weeks’, and 15% at 34 weeks’ (Lewis 1996; Roberts 2006; Robertson 1992; Usher 1971). With the surge in surfactant production at 35 weeks’ gestation, the incidence of respiratory distress syndrome drops to about 5% and it is rare after 36 weeks’ gestation (Gluck 1973; Lewis 1996; Robertson 1992). However, the routine use of antenatal glucocorticoids has altered the presentation and course of preterm lung disease such that even very preterm infants may have no or only mild respiratory distress (Jobe 2010).

Preterm lung disease is also influenced by a number of other perinatal factors. Male infants have a higher incidence and mortality from respiratory distress syndrome (Hjalmarson 1981; Rubaltelli 1998), which may be due to the fact that surfactant maturation is delayed by about one week compared to females (Fleisher 1985). Infants of poorly controlled diabetic mothers also have a higher incidence of respiratory distress syndrome, as fetal hyperinsulinaemia impairs type II epithelial cell differentiation and surfactant production (Piper 1993). Other risk factors for respiratory distress syndrome in preterm infants include intrauterine growth restriction, asphyxia, hypothermia, caesarean delivery before labour, and being the second twin (Gerten 2005; Pallotto 2006). Finally, the risk of respiratory distress syndrome varies among ethnic groups. For example, African-American infants have a lower incidence of respiratory distress syndrome at a given gestation than Caucasian infants (Kavvadia 1998), which may be due to allelic variation in surfactant-associated proteins (Krizkova 1994).

Respiratory distress syndrome is treated with oxygen, assisted ventilation and exogenous surfactant, but there is uncertainty about the optimal use of each of these therapies. While oxygen is often needed to correct cyanosis, it is also toxic to preterm babies. Maintaining high arterial oxygen tension increases the risk of retinopathy of prematurity (ROP) and
pulmonary complications, and prolongs the need for supplemental oxygen (STOP-ROP Multicenter Study Group 2000; Tin 2007). Oxygen therapy is usually titrated according to pulse oximetry but there is considerable variability in arterial partial pressure at a given oxygen saturation (Brockway 1998), and the ideal saturation range for preterm infants is yet to be established. Recent randomised trials in extremely preterm infants suggest that targeting low (85% to 89%) compared with high (91% to 95%) oxygen saturations reduces the incidence of retinopathy of prematurity but reduces survival (Stenson 2011; SUPPORT Study Group 2010).

Mechanical ventilation is required in infants with apnoea, poor respiratory effort, and severe respiratory failure. Although there have been many advances in neonatal ventilation such as patient triggering, volume targeting, and high frequency oscillation, ventilator-induced lung injury remains a concern (Greenough 2002; Jobe and Ikegami 1998). In spontaneously breathing infants, continuous positive airway pressure, usually via a nasal interface, is widely promoted as an alternative means of respiratory support as it helps to avoid mechanical ventilation, prevents apnoea, reduces oxygen use, decreases lung injury and promotes alveolarisation (Jobe 2002; Thomson 2004; Thomson 2005). There is increasing evidence from observational studies (Aly 2005; Ammari 2005; Gittermann 1997; Lindner 1999; Sahni 1998), and randomised trials (Morley 2008; SUPPORT Study Group 2010) that nasal continuous positive airway pressure can be used to manage respiratory distress syndrome in a large proportion of even the most immature infants. However, antenatal glucocorticoid treatment appears to be important for the success of non-invasive management strategies.

Surfactant therapy was introduced for the treatment of respiratory distress syndrome in the 1980s but did not become widely available until the mid-1990s (Halliday 2005). It has been shown to improve oxygenation, reduce requirements for ventilatory support, decrease air leak and increase neonatal survival (Seger 2009). Surfactant is most effective if given early (Morley 1997; OSIRIS Collaborative Group 1992; Soll 2001; Stevens 2007), but its prophylactic use is controversial, as this has traditionally required intubation, which may destabilise an infant. Furthermore, many extremely preterm infants with respiratory distress syndrome do not need surfactant if treated with antenatal glucocorticoids and early nasal continuous positive airway pressure (Morley 2008; SUPPORT Study Group 2010). There is ongoing research into the composition and pharmacology of surfactant replacement (Pfister 2007) and less invasive methods of administration (Dargaville 2011; Finer 2010; Kribs 2008).
1.2.7.1.4 Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia is a form of chronic lung disease occurring in preterm infants with prolonged postnatal dyspnoea and oxygen dependency, and is the most common severe complication of preterm birth (Jobe 2011). When first described in the 1960s, it was a well-characterised disease entity with clinical, pathological and radiological correlates (Nash 1967; Northway 1967). Antenatal glucocorticoids were not in use at the time, and infants who developed bronchopulmonary dysplasia usually had severe hyaline membrane disease with respiratory failure. This was treated with high-pressure mechanical ventilation and high concentrations of oxygen that resulted in lung injury, culminating in patchy lung fibrosis and cystic hyperinflation. Most very preterm infants died in the first postnatal week. Surviving infants remained ventilator and oxygen dependent for prolonged periods and frequently suffered chronic obstructive airway disease (Berman 1986; Edwards 1979; Northway 1990; Saigal 1987).

However, with the widespread use of antenatal glucocorticoids and improved management of respiratory distress syndrome, this type of severe hyaline membrane disease and mechanical lung injury is now uncommon (Philip 2009). Instead, bronchopulmonary dysplasia occurs primarily in extremely preterm infants and is due to the effect of air breathing on an immature lung, resulting in disruption of normal lung development (Bancalari 2006; Jobe 1999). Currently, it is defined as oxygen dependency for 28 days or more, with further classification of severity according to the need for oxygen or ventilatory support at 36 weeks’ postmenstrual age (Ehrenkranz 2005; Jobe 2001; Shennan 1988; Walsh 2004). With the introduction of surfactant, the presentation of bronchopulmonary dysplasia has become milder and radiographic changes are less specific. Furthermore, up to 40% of very preterm infants who develop bronchopulmonary dysplasia have minimal or no initial respiratory distress (Charafeddine 1999; Panickar 2004; Streubel 2008). In New Zealand, the incidence of moderate or severe bronchopulmonary dysplasia is approximately 45% in babies born at 24 to 25 weeks’ gestation, 30% at 26 to 27 weeks’, 8% at 28 to 31 weeks’, and is uncommon after 32 weeks’ gestation (Cust 2003). In the past decade, the incidence of bronchopulmonary dysplasia in extremely preterm infants has remained relatively static (Stoll 2010).

Bronchopulmonary dysplasia, as it now presents, is characterised histologically by impaired alveolarisation, abnormal pulmonary microvascular development and minimal but diffuse interstitial fibroproliferation (Coalson 1995; Coalson 1999; Coalson 2003; Husain 1998; Thibeault 2004). Airspace size is increased, but alveoli number and lung
surface area are decreased. The bronchiolar lesions that were common in classical bronchopulmonary dysplasia are generally minimal or absent. Infants with bronchopulmonary dysplasia have reduced functional residual capacity and gas mixing efficiency, consistent with impaired alveolarisation (Greenough, Dimitriou, 2005; Hjalmarsen 2005; Kavvadia 2000; Shao 1998). The main factors that disrupt alveolar development in very preterm infants are oxygen toxicity, mechanical ventilation, and inflammation and infection (Davies 2006; Jobe 2011; Speer 2006). The focus of much current research is directed towards understanding the regulation of alveologenesis and angiogenesis, and to identify novel therapeutic targets for promoting normal lung growth (Been 2010; Bourbon 2009; Wright 2011).

The prognostic value of a diagnosis of bronchopulmonary dysplasia, as it is currently defined, has been questioned (Lefkowitz 2008), as many extremely preterm infants without this diagnosis have recurrent respiratory symptoms and illness in early childhood (Davis 2002; Ehrenkranz 2005; Greenough, Limb, 2005; Vrijlandt 2007). However, there is evidence that even the milder contemporary form of bronchopulmonary dysplasia is associated with persistent and significant deficits of airway and peripheral lung function, whereas extremely preterm infants who do not have chronic oxygen dependency tend to show some improvement in lung function in early childhood (Balinotti 2010; Fakhoury 2010; Fawke 2010; Filippone 2009; Lum 2011). Infants with bronchopulmonary dysplasia are also at greater risk of other major complications of preterm birth (Ehrenkranz 2005; Sahni 2005) and long-term neurodevelopmental disability (Anderson 2006; Hintz 2005; Vohr 2005) compared with infants without bronchopulmonary dysplasia.

1.2.7.2 Non-respiratory morbidity

In addition to incomplete lung development, preterm infants commonly have immaturity in multiple other organ systems. Physiological insufficiency is common in those born very preterm, and this results in a number of neonatal problems and pathologies (Table 1.1). Apart from bronchopulmonary dysplasia, the most serious morbidities include intraventricular haemorrhage (grade 3 and 4), periventricular leukomalacia, sepsis, retinopathy (grade ≥3), and necrotising enterocolitis. The burden of disease is greatest in extremely preterm infants, and is directly related to gestational age at birth (Table 1.2). Overall, nearly two-thirds of surviving extremely preterm infants experience at least one major neonatal complication (Stoll 2010). This decreases to about 7% by 30 to 31 weeks’ gestation (Cust 2003), and serious neonatal morbidity is uncommon after 32 weeks’ gestation. Nevertheless, compared with term infants, those born moderately and late
preterm have higher rates of suspected or proven sepsis, grade 1 or 2 intraventricular haemorrhage (Khashu 2009; McIntire 2008), and possibly periventricular leukomalacia (Kinney 2006). As in very preterm infants, these complications are strongly associated with the development of respiratory distress syndrome (As-Sanie 2003).

Intraventricular haemorrhage and periventricular leukomalacia are important risk factors for long-term neurological impairment in preterm infants. Intraventricular haemorrhage is bleeding that originates in the fragile vascular network of the periventricular subependymal germinal matrix of the brain (grade 1), which can extend into the ventricular system (grade 2, or grade 3 if the ventricle is distended) or brain parenchyma (grade 4) (Papile 1978). Grade 3 and 4 intraventricular haemorrhage can lead to hydrocephalus and grade 4 lesions cause parenchymal infarction and porencephalic cysts. The risk of bleeding is greatest in the first three days following preterm birth, after which the germinal matrix vessels become more robust (Ment 1991). Respiratory distress syndrome and its associated complications, such as hypercapnia, pneumothorax, patent ductus arteriosus and hypotension, increase the risk of germinal matrix bleeding due to fluctuations in blood flow and pressure in the cerebral circulation, which has limited autoregulatory capacity in preterm infants (Boylan 2000; Cooke 1981; Funato 1992; Perlman 1983).

Impaired cerebral perfusion also contributes to cystic periventricular leukomalacia, which represents focal ischaemic necrosis of the deep cerebral white matter, typically involving the corticospinal tracts, and optic and acoustic radiations. This region is particularly susceptible to hypoxic-ischaemic injury because blood is supplied by deep penetrating end arteries and because the immature oligodendroglial precursor cells are vulnerable to free radical damage (Volpe 2001). Inflammatory cytokines and bacterial products also contribute to cytotoxic injury in these cells, and hence systemic infection is an important risk factor for leukomalacia (Bass 2011). Magnetic resonance imaging has shown that cystic periventricular leukomalacia is at the severe end of a spectrum of white matter injuries in very preterm infants, and that diffuse white matter volume loss, not usually evident on head ultrasound, is relatively common (Volpe 2001; Woodward 2006).

The retina is one of the last organs to be vascularised in the fetus. By 27 weeks’ gestation the capillary plexus of the inner retina has only reached about 70% of the distance from the optic disc to the ora serrata, and vascularisation is not complete until 36 weeks’ on the nasal side and 40 weeks’ on the temporal side (Madan 2005). Preterm birth interrupts the vascular development of the inner retina as relative hyperoxia and low postnatal levels of
insulin-like growth factor type 1 inhibit angiogenesis (Fleck 2009). As insulin-like growth factor levels increase with postnatal growth, vascular endothelial growth factor mediated angiogenesis resumes. However, this can be excessive, leading to extra-retinal vessel growth into the vitreous (grade 3 retinopathy) and dilation and tortuosity of posterior retinal vessels (plus disease). If left untreated, this can cause retinal detachment and blindness (grade 4 or 5 retinopathy). The risk of retinopathy of prematurity in very preterm infants is increased not only by excessive oxygen exposure but also by fluctuations in tissue oxygen tension (Fleck 2009).

Necrotising enterocolitis is characterised by inflammation, oedema, haemorrhage, and ulceration of the intestinal mucosa, culminating in coagulation necrosis. It is one of the most devastating illnesses to affect very preterm infants and is associated with high mortality and morbidity, and increased risk of neurological impairment (Rees 2006). The pathophysiology is not completely understood, but involves an aberrant response of the immature gut and immune system to enteral feeding and bacteria (Jesse 2006).

### 1.2.7.3 Survival and neurological sequelae

With the introduction of antenatal glucocorticoids, assisted ventilation, and surfactant, the survival of preterm infants improved dramatically from the 1970s to 1990s (Moster 2008). Further improvements in neonatal care led to increased survival of extremely preterm infants throughout the 1990s, but this was associated with higher neonatal morbidity and an increased incidence of neurodevelopmental impairment (Hack 2008; Wilson-Costello 2005). Follow-up studies of extremely preterm survivors born in the 1990s found that around a quarter had substantial neurosensory deficits including cerebral palsy, hearing and visual impairment, and intellectual disability (Saigal 2008). Furthermore, even in the absence of major disability these children had a high prevalence of developmental problems that persisted into adolescence, including impaired coordination and visuo-spatial skills, inattention, hyperactivity, reduced executive function, emotional difficulties, anxiety, and lower educational achievement (Saigal 2008).
Table 1.1 Common neonatal complications of very preterm infants.

<table>
<thead>
<tr>
<th>Organ or system</th>
<th>Physiological insufficiency</th>
<th>Problem or disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>No mature alveoli, poor microvascular development, limited surfactant, immature respiratory control</td>
<td>Respiratory distress syndrome, bronchopulmonary dysplasia, apnoea of prematurity, air leak</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>Limited auto-regulation of cerebral circulation, fragile germinal matrix vessels, incomplete myelination</td>
<td>Intraventricular haemorrhage, periventricular leukomalacia (white matter injury), hydrocephalus</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Low renal blood flow and glomerular filtration rate, impaired tubular function</td>
<td>Electrolyte and acid-base disturbance</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Limited ability to increase cardiac output, immature autonomic control of circulation</td>
<td>Hypotension, patent ductus arteriosus, pulmonary hypertension</td>
</tr>
<tr>
<td>Intestine</td>
<td>Low digestive enzymes, poorly developed peristalsis, abnormal bacterial flora</td>
<td>Necrotising enterocolitis, growth failure, feeding intolerance</td>
</tr>
<tr>
<td>Immune</td>
<td>Antibody deficiency, immune system poorly responsive to infection</td>
<td>Perinatal infection, late onset sepsis</td>
</tr>
<tr>
<td>Eyes</td>
<td>Incompletely vascularised retina</td>
<td>Retinopathy of prematurity</td>
</tr>
<tr>
<td>Haematologic</td>
<td>Reduced haematopoiesis</td>
<td>Anaemia of prematurity, transfusions</td>
</tr>
<tr>
<td>Endocrine and metabolic</td>
<td>Limited fat and glycogen stores, decreased ketogenesis and gluconeogenesis, impaired bilirubin conjugation</td>
<td>Hypoglycaemia, jaundice</td>
</tr>
</tbody>
</table>

(Eichenwald 2008; Jobe 2010; Ward 2003)

Table 1.2 Mortality and major neonatal morbidity in extremely preterm infants.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Gestational age at birth (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Severe intraventricular haemorrhage (grade 3 or 4)</td>
<td>26</td>
</tr>
<tr>
<td>Cystic periventricular leukomalacia</td>
<td>3</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia (moderate or severe)</td>
<td>69</td>
</tr>
<tr>
<td>Necrotising enterocolitis</td>
<td>15</td>
</tr>
<tr>
<td>Sepsis</td>
<td>55</td>
</tr>
<tr>
<td>Retinopathy of prematurity grade ≥3</td>
<td>42</td>
</tr>
<tr>
<td>Survival to discharge</td>
<td>55</td>
</tr>
<tr>
<td>Survival without major neonatal morbidity</td>
<td>5</td>
</tr>
</tbody>
</table>

Data are percent and are from the National Institute of Child Health and Human Development Neonatal Research Network for years 2003 to 2007 (Stoll 2010).
These complications show an inverse and graded association with gestation length but preterm infants who experience major neonatal morbidity, especially with brain lesions on ultrasound, are at increased risk of long-term sequelae. Cystic periventricular leucomalacia is strongly associated with cerebral palsy, especially spastic diplegia (Bass 2011; Woodward 2006). Infants who develop grade 3 or 4 intraventricular haemorrhage also have higher rates of cerebral palsy and intellectual impairment, and in some studies grade 2 haemorrhage has been associated with a modest increased risk of neuromotor impairment (Futagi 2006; Ment 2005; Patra 2006; Sherlock 2005; Woodward 2006).

Since 2000, the survival of extremely preterm infants has remained static. Currently, about half of infants born at 24 weeks’ gestation and admitted to neonatal intensive care survive the neonatal period. With each subsequent week of gestation neonatal mortality decreases further, such that by 28 weeks’ survival is increased to 92% (Table 1.2) and by 30-31 weeks’ to 97% (Cust 2003). There is some evidence that the incidence of major neonatal morbidities in extremely preterm infants has declined over the past decade, and that this has been associated with a decrease in the incidence of cerebral palsy (Hack 2008; Robertson 2007; Wilson-Costello 2007). Nevertheless, in a regional cohort of very preterm New Zealand children born from 1998 to 2000, there was a high incidence of neuromotor, cognitive and behaviour problems compared with term controls, and it seems unlikely that this profile has changed substantially in recent years. At four years of age, very preterm children were 16 times more likely to have cerebral palsy and three times more likely to have impairments in multiple developmental domains than term control children (Woodward 2009). Although neurodevelopmental impairment was most frequent in children born at <28 weeks’ gestation, those born at 28 to 33 weeks’ also had high rates of developmental problems. By school age, very preterm children were five times more likely than control children to have pervasive emotional and behavioural problems and two to three times more likely to have educational delay and learning disability, even in those without neurological disability (Bora 2011; Pritchard 2009).

Although preterm infants born after 31 weeks’ gestation have low mortality and morbidity compared with those born very preterm, outcomes at later gestations are important from a public health perspective because these individuals represent an increasingly large proportion of the general population (Kramer 2000; Shapiro-Mendoza 2009). Moderate and late preterm infants are two to three times more likely to have cerebral palsy and have a modest increased risk of developmental delay compared to term infants (McGowan 2011). By school age, they require more special education services and have slightly slower
academic progress in the first few years of school but appear to have similar educational attainment in later years compared with term-born peers (Chyi 2008). Whether or not these differences are due to preterm birth *per se* or associated antenatal factors is unclear. However, the third trimester is a critical period for brain development in humans, with 35% of brain mass and 50% of cortical volume accrued in the last four to six weeks of gestation and a five-fold increase in myelination over this time (Guihard-Costa 1990; Huppi 1998). Thus, the brain of a late preterm infant is still immature, and central nervous system development may be affected by the altered extra-uterine environment.

### 1.3 Antenatal glucocorticoids

#### 1.3.1 Historical background

It was first recognised during the 1950s that glucocorticoids induced maturation of the fetal intestine (Halliday 1958; Moog 1955). In 1968, Buckingham (1968) hypothesised that similar effects may occur in the fetal lung because these organs had a common endodermal origin. At about the same time Liggins (1969) was studying parturition in sheep in Auckland and made the chance observation that the lungs of preterm lambs exposed to antenatal glucocorticoids (cortisol or dexamethasone) were partially aerated, whereas the lungs of control animals were collapsed and airless. He speculated that glucocorticoids induced enzymes responsible for surfactant synthesis. Within several years, further animal studies by Avery and others confirmed that glucocorticoids directly accelerated fetal lung maturation in late gestation, including epithelial cell differentiation and surfactant production, and reduced respiratory distress in prematurely delivered animals (Avery 1975).

Remarkably, within just months of completing his animal work, Liggins and his colleague Howie embarked on the first human randomised trial of antenatal glucocorticoids for the prevention of neonatal respiratory distress syndrome at National Women’s Hospital, Auckland (Auckland Steroid Trial). Women at risk of preterm birth from 24 to 36 weeks’ gestation were randomised to an intramuscular injection of placebo (cortisone acetate) or 12mg betamethasone (mixed preparation of short-acting phosphate and long-acting acetate), which was repeated after 24 hours. Betamethasone was used because it was freely available, and the dosage regimen was based on Liggins’ animal work and knowledge of human fetal adrenal function, with the aim of achieving a 48-hour fetal effect (Reynolds 2005). It was subsequently shown that this dose resulted in fetal plasma glucocorticoid concentrations that closely approximated physiologic stress responses in preterm infants.
An interim report of the first 287 women recruited to the trial was published in 1972 (Liggins), but results for the complete trial involving 1142 women are only available in conference proceedings (Howie 1977; Liggins 1976). The trial demonstrated that antenatal betamethasone treatment resulted in marked reductions in the incidence of neonatal death, respiratory distress syndrome, and intraventricular haemorrhage.

Antenatal glucocorticoid treatment was widely adopted in New Zealand and Australia in the 1970s, but in other parts of the world uptake was slow due to skepticism about the findings of the trial and concerns about long-term safety (Reynolds 2005). Consequently, a large number of similar randomised trials were performed, including 11 in the 1970s and 1980s, and 10 after 1990 (Roberts 2006). However, with the exception of the Collaborative Group Trial (1981), most were small and under-powered. This, combined with inappropriate use of subgroup analysis and over-reliance on observational data, led to many erroneous conclusions (Robertson 1982). In 1989, Crowley performed the first meta-analysis of 12 randomised trials, which showed conclusively that antenatal glucocorticoid treatment decreased the incidence of neonatal death, respiratory distress syndrome, and other major neonatal morbidities; that it was effective in major clinical subgroups; and that it did not appear to be harmful to the mother or baby (Crowley 1990). This led to a marked increase in the use of antenatal glucocorticoids in the United Kingdom (Wilson 2002), but in the United States, it was not until after the 1994 Consensus Development Conference that attitudes changed (Avery 1995; National Institutes of Health 1994). Antenatal glucocorticoid therapy is now used routinely for threatened or planned preterm labour in Western countries, but in some developing countries usage continues to be low (Pattanittum 2008).

1.3.2 Role of glucocorticoids in parturition

When Liggins discovered that fetal glucocorticoid exposure induced fetal lung maturation, it was already known that activation of the fetal hypothalamic-pituitary-adrenal axis was involved in the onset of parturition in sheep (Bassett 1969; Liggins 1967; Liggins 1968). From these observations and studies that followed, it was established that fetal glucocorticoids have a key role in achieving synchrony between the timing of birth and the maturation of fetal organs, thereby ensuring that the fetus is prepared for life outside the womb. In all species studied, including humans, there is a surge in fetal circulating glucocorticoid concentration for periods of up to 10 to 15 days before birth (Fowden 1998). This is primarily due to increased cortisol secretion from the adrenal gland,
although in some species, such as the rat and horse, free cortisol concentrations are increased by a fall in plasma cortisol-binding globulin concentrations (Liggins 1994). Glucocorticoids induce a wide range of structural and functional maturational changes in numerous fetal tissues, which are essential for successful physiological adaptation and survival at birth (Table 1.3). In particular, the gut, liver and lungs must replace the placenta as the source of energy supply and gaseous exchange. Thus, maturation of these tissues is highly cortisol-dependent (Liggins 1994). In precocial species, tissue maturation closely mirrors the rise in endogenous fetal glucocorticoid concentrations before birth, and ablation of the fetal pituitary or adrenal gland prolongs gestation and prevents tissue maturation (Fowden 1998). In general, cortisol promotes cellular differentiation at the expense of proliferation and thus in some species, such as sheep, the late gestation rise in plasma cortisol concentration is associated with a transient slowing of fetal growth (Fowden 1996).

The range of fetal proteins and enzymes induced by glucocorticoids is reasonably consistent across species with the notable exception of placental 17-alpha-hydroxylase, which is fundamental to species differences in the mechanism of parturition (Liggins 1994). In sheep, induction of this enzyme alters placental steroidogenesis, resulting in an increase in placental oestrogen and decrease in maternal plasma progesterone concentration. This triggers myometrial prostaglandin synthesis and contractions (Challis 2000; Thorburn 1991). In contrast, glucocorticoids do not induce placental 17-alpha-hydroxylase in primates, although fetal adrenal activation still stimulates placental oestrogen production indirectly due to increased synthesis of dehydroepiandrosterone sulfate, a precursor for placental aromatisation (Challis 2000). This difference is important clinically, as it enables glucocorticoids to be administered to promote fetal maturation without precipitating preterm labour. Although antenatal glucocorticoids may initially cause myometrial contractions (Elliott 1995; Yeshaya 1996), fetal adrenocortical activity is subsequently suppressed and placental oestrogen production and myometrial activity are decreased (Challis 2000).
<table>
<thead>
<tr>
<th>Organ</th>
<th>Morphological effects</th>
<th>Functional effects</th>
<th>Protein or enzyme induced</th>
</tr>
</thead>
</table>
| Lung  | Epithelial cytodifferentiation  
Thinning of alveolar septae  
Increased alveolar airspace  
Increased elastin and collagen content  
Maturation of alveolar capillaries | Increased tissue and alveolar surfactant  
Increased antioxidant activity  
Enhanced clearance of fetal lung fluid  
Increased glycogenolysis (provides substrate for phospholipid synthesis)  
Increased catecholamine induced surfactant synthesis and clearance of lung fluid  
Reduced vascular permeability | Surfactant proteins A, B, C, D  
Lipogenic enzymes (fatty acid synthetase, phosphatidyl acid phosphatase, lyso PC acyl CoA acyltransferase)  
Fibroblast pneumocyte factor (mediates some glucocorticoid effects)  
Superoxide dismutase, catalase, glutathione peroxidase  
Sodium-potassium ATPase subunits, epithelial sodium channel subunits  
Beta adrenergic receptors |
| Liver | Increased bile canaliculi | Increased glycogen deposition  
Increased gluconeogenesis  
Enhanced protein and lipid metabolism  
Increased synthesis of plasma proteins  
Induction of hepatic receptors  
Increased conversion of thyroxine (T4) to triidothyronine (T3) | Glycogen synthetase  
Phospho-phenolpyruvate carboxykinase, glucose-6-phosphatase  
Fatty acid synthetase, aminotransferases  
Cortisol binding globulin  
Growth hormone, beta adrenoreceptors  
5'-monodeiodinase |
<table>
<thead>
<tr>
<th>Organ</th>
<th>Morphological effects</th>
<th>Functional effects</th>
<th>Protein or enzyme induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Decreased expression of some hormones</td>
<td>Increased renal blood flow</td>
<td>Insulin-like growth factor 2, angiotensinogen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased glomerular filtration rate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased tubular sodium reabsorption</td>
<td>Sodium-potassium ATPase, sodium-hydrogen exchanger</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhanced sodium regulation</td>
<td>Renin (increased stimulated secretion)</td>
</tr>
<tr>
<td>Gut</td>
<td>Increased villus height and density</td>
<td>Increased stomach acid secretion</td>
<td>Gastrin</td>
</tr>
<tr>
<td></td>
<td>Maturation of glands in stomach and small intestine</td>
<td>Enhanced digestive activity of intestine</td>
<td>Pancreatic amylase and trypsin, brush border hydrolases</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td>Reduced permeability to large proteins</td>
<td></td>
</tr>
<tr>
<td>Adrenal</td>
<td></td>
<td>Increased adrenaline content of medulla</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased cortical response to adrenocorticotrophic hormone</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>Keratinisation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>Regression of lymphoid tissue in thymus and spleen</td>
<td>Switch from liver to bone marrow as primary site of haematopoiesis</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>Enhanced blood-brain barrier</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maturation of microvascular circulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>Increased cardiac output</td>
<td>Myocardial adenylyl cyclase, sodium-potassium ATPase alpha-isoforms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhanced ductus arteriosus closure with prostaglandins</td>
<td></td>
</tr>
</tbody>
</table>

(Ballard 1995; Fowden 1998; Grier 2004; Liggins 1994)
Importantly, the administration of physiological doses of glucocorticoids to preterm fetuses accelerates the normal sequence of organ development occurring near term (Fowden 1998). However, some of these effects are dependent on the developmental stage of tissues. For example, stimulation of phosphatidylcholine synthesis is greatest at the point in gestation just prior to normal differentiation, but is limited in the early third trimester and at term (Ballard 1986). Similarly, in sheep and pigs intestinal maturation is most marked in later gestation near the time when cortisol concentrations normally rise (Trahair 1997).

1.3.3 Mechanism of action

At the molecular level, glucocorticoids primarily act by binding to the glucocorticoid receptor in the cytosol of target cells. This receptor contains a glucocorticoid binding domain at the C-terminal end, two zinc fingers in the middle region that interact with DNA and an N-terminal domain that binds transcription factors (Venkatesh 1991). When glucocorticoids attach to the receptor, it dissociates from a heat shock protein, which enables it to enter the nucleus. Glucocorticoids can affect the expression of proteins and enzymes in several ways. Some glucocorticoid sensitive genes contain glucocorticoid response elements in their promoter region and binding of the activated glucocorticoid receptor at this site directly increases or decreases transcription rate (Venkatesh 1991). This includes genes for SP-B, insulin-like growth factor-2, angiotensinogen, tropoelastin, beta-1 subunit of the sodium-potassium ATPase and alpha subunit of the epithelial sodium channel (Barquin 1997; Champigny 1994; Li 1998; Olson 1991; Pierce 1995; Venkatesh 1993). However, in other genes the activated glucocorticoid receptor regulates transcription indirectly by interacting with or inducing other nuclear transcription factors (Venkatesh 1991). Alternatively, glucocorticoids can affect gene expression at the post-transcriptional level by altering the stability of mRNA (Venkatesh 1993), and can enhance post-translational processing of the protein product (Ballard 2000).

Glucocorticoid action in utero is influenced by the expression of glucocorticoid receptors in fetal tissues, which is developmentally regulated. For example, from mid to late gestation, glucocorticoid receptor expression is high in airways, lungs, intestine, pituitary and thymus; intermediate in the heart, liver and periventricular regions of the brain; low in other brain regions, the gonads, adrenal gland and vertebral column; and transient in the pancreas (Speirs 2004). In addition, tissue and cell responsiveness to glucocorticoids may be determined by chromatin conformation, which influences the accessibility of promoter regions and glucocorticoid response elements of genes (Venkatesh 1991).
The effect of glucocorticoids on fetal tissues has been studied extensively in the lungs, where glucocorticoids induce both biochemical and structural maturation. Functional effects include increased synthesis of surfactant proteins and phospholipids, antioxidant enzymes that prevent free radical injury, and subunits of the epithelial sodium channel and basal sodium-potassium pump that are critical for clearance of alveolar fluid (Grier 2004). These effects are due to direct or indirect transcriptional regulation of the corresponding proteins or enzymes (Table 1.3). Changes in lung structure include differentiation of alveolar type I and II epithelial cells, decreased interstitial tissue and thinning of the alveolar wall, deposition of supportive connective tissue matrix (elastin and collagen), enlargement of alveolar airspaces, and maturation of alveolar capillary vessels (Pinkerton 1997; Polglase 2007; Pua 2005; Willet 1999). Consequently, there is a decrease in secondary septal division and formation of alveolae. It is not entirely clear how glucocorticoids change lung architecture, but they reduce cell division by inhibiting DNA synthesis, induce a variety of growth factors, and counteract the effects of lung retinoids that promote alveolarisation (Bolt 2001; Grier 2004). The net effect of these biochemical and structural changes is increased lung volume and stability, reduced work of breathing and enhanced gaseous exchange.

Remarkably, studies in sheep found that improvements in lung function were detectable as early as 15 hours after glucocorticoid exposure, before significant increases in surfactant had occurred (Ikegami, Polk and Jobe 1996; Ikegami, Polk, Jobe, 1996). This suggests that the initial improvement in lung compliance is due to absorption of lung fluid or changes in lung architecture.

The kinetics of glucocorticoid-induced protein synthesis have been studied in most detail for components of surfactant. Although responses vary for different genes, there are some common patterns of action. First, the effect of glucocorticoids on gene transcription is rapid. For example, in second trimester human lung explants exposed to dexamethasone, maximal transcription rates for SP-B and SP-C occurred after 2 hours and 8 hours, respectively, and were maintained with ongoing glucocorticoid exposure (Ballard 1996). Transcription rates were maximal at very low concentrations of dexamethasone, approximately 20 to 30 nM. Messenger ribonucleic acid (mRNA) content peaked after about 24 to 48 hours of dexamethasone exposure (Liley 1989; Venkatesh 1993). Similarly, in preterm lambs (~0.8 gestation) lung tissue SP-B and SP-C mRNA content was maximal after two maternal betamethasone injections given 24 and 48 hours before delivery (Tan 1999), and tissue SP-B concentration was increased 48 hours after treatment (Ballard
Lung phosphatidylcholine concentration increased more slowly, because glucocorticoids must first induce the relevant lipogenic enzymes (Ballard 1997; Moss 2009).

Second, glucocorticoid induction of gene transcription is quickly and fully reversible. For example, in lung explants exposed to cortisol for 24 hours, transcription rates for SP-B and SP-C genes were decreased by 40% to 50% four hours after removal of cortisol, and mRNA content returned to control values within two to three days (Ballard 1996; Liley 1989). In preterm lambs, lung tissue SP-A, SP-B and SP-C mRNA content had returned to control levels when delivery occurred ≥7 days after maternal betamethasone injection (Tan 1999). However, the duration of physiological effect depends on the half-life of the induced proteins and enzymes. In preterm lambs, SP-A and SP-B remained elevated in lung tissue and alveolar fluid for up to two weeks but returned to control levels by about three weeks (Ballard 1997; Ikegami, Polk, Jobe, 1996). Phosphatidylcholine appears to persist for slightly longer in the fetal lung (Jobe, Newnham, 1998; Willet 2001).

While protein synthesis is reversible, it is not clear whether the structural changes induced by glucocorticoids persist or slowly revert to the baseline fetal state. However, a study in preterm lambs (0.8 and 0.9 gestation) found that lung morphometry was similar between control animals and those exposed to maternal betamethasone 3 weeks previously (Willet 2001). In rat pups treated with dexamethasone, withdrawal of glucocorticoid resulted in redevelopment of double capillary networks and septal crests with resumption of alveolarisation, although recovery was incomplete (Tschanz 1995; Tschanz 2002). These data suggest that if the fetus remains in utero after glucocorticoid exposure that there is a slow return to the normal developmental state rather than a permanent shift in the trajectory of maturation (Jobe 2006).

Third, in individual animals exposed to glucocorticoids there are strong correlations between concentrations of different surfactant proteins and phospholipids in lavage samples, indicating that glucocorticoid treatment results in a coordinated induction of multiple genes (Ballard 2000). This is similar to the way in which glucocorticoid-induced structural maturation represents an acceleration of the normal sequence of lung development.

In addition to the glucocorticoid receptor, there is increasing evidence that glucocorticoids can affect cell function and metabolism through a number of other mechanisms. For example, glucocorticoids can impair cation transport due to physicochemical effects on the
cell membrane (Buttgereit 1999) and directly influence mitochondrial oxidation (Du 2009). Glucocorticoids can also influence intracellular signal transduction pathways, such as mitogen activated protein kinases, which may result in non-specific effects on gene transcription by altering the phosphorylation of transcription factors (Chen 2001). These so-called non-genomic effects probably occur at higher doses, whereas receptor-mediated effects predominate at lower doses (Buttgereit 1999). Importantly, the propensity of a particular glucocorticoid for non-genomic effects is not necessarily related to its affinity for the glucocorticoid receptor (Buttgereit 1999).

1.3.4 Pharmacology

Two synthetic glucocorticoids are used clinically for acceleration of fetal maturation, betamethasone and dexamethasone (Jobe 2004). These fluorinated steroids are stereoisomers, differing only by the position of a methyl group on carbon 16. The placental enzyme 11-beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2) normally provides a functional barrier to the transfer of maternal glucocorticoids to the fetus by converting cortisol to its inactive 11-keto metabolite cortisone (Benediktsson 1997). Data regarding the extent to which betamethasone and dexamethasone are metabolised by the placenta are conflicting (Blanford 1977; Levitz 1978; Murphy 2007), but clinical studies have shown that these drugs readily cross the placenta. For example, betamethasone cord blood concentrations are, on average, one-third of maternal serum concentrations (Anderson 1977; Ballard 1975). Betamethasone and dexamethasone are potent agonists of the glucocorticoid receptor but, unlike cortisol, have weak affinity for the mineralocorticoid receptor (Matthews 2001).

The standard maternal treatment regimens for acceleration of fetal maturation consist of either two 12 mg doses of betamethasone 24 hours apart or four 6 mg doses of dexamethasone 12 hours apart, given by intramuscular injection (National Institutes of Health 1994). Although oral dexamethasone has good bioavailability and reduces the incidence of respiratory distress syndrome, oral treatment is not recommended as it is less effective at preventing neonatal sepsis and intraventricular haemorrhage compared with intramuscular injection (Egerman 1997; Egerman 1998). Betamethasone is usually given as a mixture of betamethasone sodium phosphate and betamethasone acetate, in equal parts (Jobe 2009). Betamethasone sodium phosphate is a soluble ester that is rapidly absorbed and pharmacologically active, while betamethasone acetate is only slightly soluble and becomes a repository for slow absorption. Using this preparation, serum betamethasone concentrations peak within 1 hour in both the maternal and fetal circulation and have a
half-life of approximately 6 and 12 hours, respectively (Ballard 1975). Betamethasone is completely cleared from the maternal and fetal circulations two days after the second injection (Ballard 1975). Dexamethasone is only available as a phosphate ester and its half-life in maternal circulation after intramuscular injection is 3 to 4 hours (Ballard 1986). With the two-dose mixed betamethasone regimen, it is estimated that total free glucocorticoid activity in fetal plasma increases about five-fold at peak betamethasone concentration and remains above baseline for about 60 hours (Ballard 1980). Compared with betamethasone, the four-dose dexamethasone regimen results in slightly lower peak free glucocorticoid activity, but the duration of elevated glucocorticoid activity is about 12 hours longer (Ballard 1986). Dexamethasone also has higher affinity for the glucocorticoid receptor than betamethasone (seven and five-fold compared to cortisol, respectively) (Ballard 1986).

Given that glucocorticoid receptor binding is saturated at low nanomolar concentrations of glucocorticoids, it is likely that these treatment regimens provide near-maximal induction of transcription in glucocorticoid-regulated genes (Ballard 1986). Accordingly, in the Auckland Steroid Trial it was shown that doubling the dose of betamethasone (two doses of 24 mg) did not improve infant outcomes (Howie 1977). However, the lowest effective dose in humans is not known. It appears that a sustained increased in fetal glucocorticoid activity, even if only modest, is more important for clinical efficacy than peak glucocorticoid concentrations. For example, hydrocortisone and prednisone cross the placenta when given in sufficient amounts, but are rapidly cleared from fetal circulation (half-life 1 to 2 hours), which may explain why they are not effective at inducing lung maturation (Ballard 1986). In sheep, a single maternal injection of betamethasone acetate was as effective at inducing fetal lung maturation as two doses of mixed betamethasone or four doses of betamethasone phosphate even though maternal and fetal plasma betamethasone concentrations were substantially lower (Jobe 2009). In a human trial that directly compared a mixed preparation of betamethasone with betamethasone phosphate, point estimates for treatment effect favoured the mixed preparation, although confidence intervals were wide due to the small sample size (Subtil 2003). It is possible that a lower dose of betamethasone acetate may be effective at inducing fetal maturation in humans and reduce total glucocorticoid exposure to mother and baby, but this could only be determined in clinical trials.
1.3.5 Clinical effects of a single course of antenatal glucocorticoids

1.3.5.1 Short-term effects
In 2006, Roberts and Dalziel updated Crowley’s systematic review (1995) on antenatal glucocorticoid therapy to include six new randomised trials and the complete data from the Auckland Steroid Trial using an intention to treat analysis. They also excluded three trials from the earlier review; two because they had more than 20% post-randomisation exclusions and one because it was quasi-randomised. The updated review included 21 randomised trials, published from 1972 to 2002, and 4269 infants, over a quarter of who were from the Auckland Steroid Trial. Eight trials that were included in the main meta-analysis permitted the use of repeat doses, but sensitivity analysis showed that this did not alter the results. Six trials used dexamethasone, 14 used betamethasone and one trial did not specify the type of steroid given. Antenatal glucocorticoid treatment was compared to a placebo treatment in 18 trials and to expectant management in the remainder.

The updated review confirmed that a single course of antenatal glucocorticoids, given for threatened or planned preterm birth, was associated with substantial neonatal benefit including a decrease in the incidence of respiratory distress syndrome, perinatal and neonatal death, intraventricular haemorrhage, early neonatal sepsis and necrotising enterocolitis (Table 1.4). These benefits were achieved without an increase in the incidence of intrauterine or neonatal infection. Antenatal glucocorticoids did not reduce the incidence of bronchopulmonary dysplasia, but there was significant heterogeneity for this outcome. Respiratory benefits appeared to require at least 24 to 48 hours of antenatal glucocorticoid exposure, but infants born within 24 hours of treatment had a clear survival advantage over those not so exposed. The beneficial effect of glucocorticoids on intraventricular haemorrhage may be due to improved cardiovascular stability and maturation of the cerebral microvasculature (Liu 2008; Stonestreet 1999).

About a quarter of all data in the review came from trials that completed recruitment after 1990, and the relative and absolute benefits of treatment in this subgroup were at least as good as, if not better, than the benefits of treatment for infants born in earlier decades (Roberts 2006). Thus, despite many improvements in neonatal care since the first trials were performed in the 1970s, antenatal glucocorticoid treatment continues to have an important influence on infant outcome. Even though antenatal glucocorticoid treatment did not significantly decrease the use of surfactant, observational evidence indicates that antenatal glucocorticoids improve the effectiveness of this therapy (Jobe, Mitchell, 1993; Sen 2002).
Subgroup analysis by gestational age at first dose showed that antenatal glucocorticoid treatment reduced respiratory morbidity from 26 to 34 weeks’ gestation, and decreased the incidence of neonatal death or intraventricular haemorrhage from 26 to 32 weeks’ gestation (Table 1.5). The benefits and risks of treatment before 26 weeks’ gestation could not be adequately determined in this systematic review as there were <30 infants in this subgroup (Roberts 2006). However, reports from large series of non-randomised extremely preterm infants have consistently demonstrated that antenatal glucocorticoid treatment is beneficial at very early gestations, primarily due to reductions in the incidence of neonatal death and intraventricular haemorrhage (Abbasi 2010; Costeloe 2006; Foix-L’Helias 2008; Manktelow 2010). Although antenatal glucocorticoids have minimal impact on the incidence of respiratory distress syndrome in extremely preterm infants, due to the severe deficit of mature alveoli, there is clear evidence from animals and human fetal lung explants that glucocorticoids induce surfactant synthesis and structural changes during the saccular phase of lung development (Ballard 1996; Liley 1989; Willet 2001). There is currently no evidence that antenatal glucocorticoids decrease neonatal respiratory morbidity when administered to women after 34 weeks’ gestation (Porto 2011), except perhaps before elective caesarean section (Stutchfield 2005), though results from several trials are awaited (NCT01222247, NCT01206946, NCT00446953).

Subgroup analysis in the systematic review by Roberts and Dalziel (2006) showed that antenatal glucocorticoids were effective in women with ruptured membranes at trial entry (reduced incidence of neonatal death, respiratory distress syndrome, intraventricular haemorrhage) without increasing the incidence of intrauterine or neonatal infection. Antenatal glucocorticoids were also effective in the context of pre-eclampsia (Roberts 2006). No benefit was seen in infants born from multiple pregnancy, but this is likely to represent a type 2 error, as few data were available for this subgroup analysis and point estimates of treatment effect generally favoured glucocorticoid treatment. Although one study found that maternal clearance of glucocorticoid was increased in multiple pregnancy (Ballabh 2002), this was not confirmed in others (Della Torre 2010; Gyamfi 2010), and there is currently no evidence that fetal glucocorticoid exposure is substantially altered in multiple pregnancy (Gyamfi 2010). There are insufficient data from randomised trials to evaluate effectiveness in other clinical subgroups, such as in women with growth-restricted fetuses (Torrance 2009).

Observational studies and indirect evidence have suggested that betamethasone is more effective than dexamethasone, and that dexamethasone may increase the incidence of
periventricular leukomalacia (Baud 1999; Feldman 2007; Jobe 2004; Lee, Stoll, 2006). However, several small trials that directly compared these two agents showed that they had equivalent effects on perinatal death and respiratory distress syndrome, while dexamethasone tended to prevent more intraventricular haemorrhage (Brownfoot 2008). Animal studies have also suggested that they may differ in their potential to influence long-term physiological function (Dunn 2010). There are a number of theoretical reasons why the effect of these drugs may be dissimilar, including differences in pharmacokinetics, affinity for the glucocorticoid receptor, potency of non-genomic actions, effects on placental 11β-HSD2 function, and the preservatives used in drug preparations (Jobe 2004). A large trial is currently underway to compare the clinical effects of antenatal dexamethasone and betamethasone treatment for preterm birth (ACTRN12608000631303).

Table 1.4: Perinatal effects of antenatal glucocorticoids compared with placebo or no treatment in women at risk of preterm birth.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Total infants or women</th>
<th>Risk ratio (95% CI)</th>
<th>Number needed to treat to benefit (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perinatal death</td>
<td>3627</td>
<td>0.77 (0.67, 0.89)</td>
<td>23 (16, 48)</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>3956</td>
<td>0.69 (0.58, 0.81)</td>
<td>22 (16, 36)</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>4038</td>
<td>0.66 (0.59, 0.73)</td>
<td>12 (10, 15)</td>
</tr>
<tr>
<td>Moderate or severe</td>
<td>1686</td>
<td>0.55 (0.43, 0.71)</td>
<td>14 (11, 21)</td>
</tr>
<tr>
<td>Ventilatory support</td>
<td>569</td>
<td>0.69 (0.53, 0.90)</td>
<td>10 (7, 31)</td>
</tr>
<tr>
<td>Surfactant use</td>
<td>456</td>
<td>0.72 (0.51, 1.03)</td>
<td>NS</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia</td>
<td>818</td>
<td>0.86 (0.61, 1.22)</td>
<td>NS</td>
</tr>
<tr>
<td>Intraventricular haemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>2872</td>
<td>0.54 (0.43, 0.69)</td>
<td>21 (17, 30)</td>
</tr>
<tr>
<td>Severe (grade 3 or 4)</td>
<td>572</td>
<td>0.28 (0.16, 0.50)</td>
<td>7  (8, 12)</td>
</tr>
<tr>
<td>Necrotising enterocolitis</td>
<td>1675</td>
<td>0.46 (0.29, 0.74)</td>
<td>30 (23, 29)</td>
</tr>
<tr>
<td>Early neonatal sepsis</td>
<td>1319</td>
<td>0.56 (0.38, 0.85)</td>
<td>27 (19, 78)</td>
</tr>
<tr>
<td>Any proven neonatal sepsis</td>
<td>2607</td>
<td>0.83 (0.66, 1.04)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean birthweight–g</td>
<td>2588</td>
<td>-17 (-62, 27)</td>
<td>NS</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>378</td>
<td>0.96 (0.63, 1.44)</td>
<td>NS</td>
</tr>
<tr>
<td>Maternal chorioamnionitis</td>
<td>2485</td>
<td>0.91 (0.70, 1.18)</td>
<td>NS</td>
</tr>
<tr>
<td>Maternal puerperal sepsis</td>
<td>1003</td>
<td>1.35 (0.93, 1.95)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data from Roberts and Dalziel (2006). NS, non-significant effect; CI, confidence interval.
Table 1.5: Influence of gestational age on effects of antenatal glucocorticoids compared with placebo or no treatment in women at risk of preterm birth.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>&lt;26 (wk)</th>
<th>26 to 29 (wk)</th>
<th>30 to 32 (wk)</th>
<th>33 to 34 (wk)</th>
<th>35 to 36 (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory distress syndrome</td>
<td>2.86 (0.37, 21.87)</td>
<td>0.49 (0.34, 0.72)</td>
<td>0.56 (0.36, 0.87)</td>
<td>0.53 (0.3, 0.91)</td>
<td>0.61 (0.11, 3.26)</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>1.87 (0.61, 5.72)</td>
<td>0.67 (0.45, 0.99)</td>
<td>0.51 (0.23, 1.11)</td>
<td>1.11 (0.49, 2.48)</td>
<td>0.62 (0.06, 6.76)</td>
</tr>
<tr>
<td>Intraventricular haemorrhage</td>
<td>1.2 (0.24, 6.06)</td>
<td>0.45 (0.2, 0.95)</td>
<td>0.23 (0.03, 2.00)</td>
<td>1.11 (0.23, 5.40)</td>
<td>No events</td>
</tr>
</tbody>
</table>

Data are relative risk (95% confidence interval); from Roberts and Dalziel (2006).

1.3.5.2 Long-term effects

In the systematic review by Roberts and Dalziel (2006), the use of antenatal glucocorticoids in women with threatened or planned preterm birth was associated with a trend towards a decrease in the incidence of developmental delay (RR 0.49, 95% CI 0.24 to 1.00) and cerebral palsy (RR 0.6, 95% CI 0.34 to 1.03). However, only about one-quarter of neonatal survivors had neurological follow-up (5 trials), so these effects may have been significant with a larger sample. Despite this apparent reduction in disability, the Auckland and Collaborative Group trials found no differences between treatment groups in cognitive function at 3 to 4 years of age (Collaborative Group on Antenatal Steroid Therapy 1984; MacArthur 1981). At 6 years of age, Auckland children exposed to betamethasone performed less well on one subtest of the Raven Progressive Matrices test (abstract thinking), but were otherwise similar to control children in cognitive function and academic achievement (MacArthur 1982). This finding was interpreted as a type 1 error, and was not replicated in a small Dutch trial that assessed cognitive function at 10 to 12 years of age (Schmand 1990). These trials included few very preterm children, but observational studies have variously shown that at very early gestations antenatal glucocorticoids have beneficial (Doyle, Ford, Rickards, 2000) or no long-term effect on neurodevelopmental (Foix-L’Helias 2008).

Follow-up of subjects in early adulthood from the Auckland Steroid Trial (192 [19%] neonatal survivors) and Dutch trial (81 [74%] neonatal survivors) found no differences between those exposed to antenatal betamethasone or placebo in cognitive function, education, psychiatric morbidity, socioeconomic status, employment, sexual function, and health related quality of life (Dalziel, Lim, 2005; Dessens 2000). In the Auckland trial
there were also no long-term effects of antenatal betamethasone exposure on lung function or the prevalence of wheeze and asthma (Dalziel, Rea, 2006). Long-term outcomes relating to growth, metabolism and cardiovascular function are described later in this chapter.

1.3.6 Clinical effects of repeat doses of antenatal glucocorticoids

1.3.6.1 Rationale for repeat doses

The idea that antenatal glucocorticoid treatment should be repeated was first proposed by Liggins and Howie, based on secondary analysis of Auckland Steroid Trial data. This showed that in very preterm infants there was a trend towards an increase in the incidence of respiratory distress syndrome in those born between 7 and 20 days compared with those born at <7 days after betamethasone treatment (Howie 1977). On this basis, they concluded that:

This suggests that if very premature delivery has not occurred and remains apparently imminent, therapy should be repeated at intervals of not less than seven days, at least up to 32 weeks. There is probably no point in repeating therapy after 32 weeks unless liquor analyses show that the fetus is still at risk.

Following this advice, repeat doses were routinely given in Auckland. As other centres around the world adopted antenatal glucocorticoids, it became common practice to use repeat dose therapy (Brocklehurst 1999; Quinlivan, Evans, 1998). However, by the early 2000s there was mounting evidence from animal experiments and human observational studies that excess exposure to glucocorticoids may have adverse effects on fetal growth, brain development and other physiologic outcomes (Aghajafari 2001; Aghajafari, Murphy, Matthews, 2002; French 1999; Kay 2000; National Institutes of Health 2000). Furthermore, rat pups exposed to glucocorticoids had permanently impaired alveolarisation leading to decreased total alveolar number (Blanco 1989; Massaro 1986; Tschanz 1995), although this was not found in sheep (Willet 2001). Despite these concerns, the question of whether antenatal glucocorticoid treatment should be repeated remains important as approximately 40% of women who receive an initial course of treatment remain undelivered after 7 days but may have ongoing risk of preterm birth (Roberts 2006). This led to evaluation of repeat dose therapy in randomised trials, as discussed below.

It is unclear why there was an apparent increase in the incidence of respiratory distress syndrome in very preterm infants in the Auckland trial who had remote glucocorticoid exposure, but it is widely accepted that the clinical benefits of treatment diminish with time. This assumption is supported by the tissue and animal work previously described, which showed that the effects of glucocorticoids on protein synthesis, and possibly tissue
differentiation, were reversible. However, the precise duration of clinical effect in humans is not known, and it cannot be determined from subgroup analysis of randomised trials, as the relevant data needed to perform appropriate analyses are not available (Gates 2007). In an observational study of very preterm infants, it appeared that improvements in respiratory compliance were maintained for about 14 days after glucocorticoid treatment (McEvoy 2008). Similarly, in preterm lambs improvements in lung function were maintained when delivery occurred 14 days after treatment, and modest benefits were still evident after 21 days (Ikegami 1997; Jobe, Newnham, 1998).

Because protein synthesis can be repetitively induced (Tan 1999), repeating glucocorticoid administration at regular intervals ensures that biochemical induction of the lung is maintained around the time of preterm birth. Furthermore, studies in preterm lambs have shown that administration of two to four doses of glucocorticoids at weekly intervals from the beginning of the third trimester onwards results in a stepwise increase in surfactant pools (Ballard 1997; Jobe, Newnham, 1998), antioxidant enzymes (Walther 1998), lung function (Ikegami 1997; Jobe, Newnham, 1998) and possibly structural maturation (Pua 2005). Similarly, in monkeys, rabbits and mice, longer courses of antenatal glucocorticoids had a greater effect on pulmonary maturation than a single dose (Engle 1996; Pratt 1999; Stewart 1998). However, it is possible that there is a biphasic response, and that surfactant pools may be decreased after too many doses (Ballard 1997). As pregnancy progresses to term, the differences between control animals and those treated with repeat doses become less evident due to normal developmental maturation of the fetal lung (Jobe, Newnham, 1998).

1.3.6.2 Randomised trials
As background to this thesis, a Cochrane systematic review was performed of randomised trials that compared the effectiveness and safety of one or more doses of antenatal glucocorticoids with placebo or no treatment in women at risk or preterm birth 7 or more days after an initial course of glucocorticoids (Crowther 2011; McKinlay 2012). This review identified 10 such trials, including ACTORDS, involving over 4730 women and 5700 infants (Table 1.6). The trials were of high methodological quality and had a low risk of bias, except for one study in which participants and clinicians were not blinded to treatment allocation (Mazumder 2008). However, this trial was small and contributed few data to the review.
Women were included in these trials if they were considered to be at high risk of preterm birth at least 7 days after an initial course of glucocorticoids. Three trials specified additional inclusion criteria (Aghajafari, Murphy, Ohlsson, 2002; Guinn 2001; Peltoniemi 2007). One of these trials targeted women at imminent risk of preterm birth and 79% of the participants in this study gave birth within 24 hours of trial entry (Peltoniemi 2007). In the remaining trials, women were included if their responsible clinician judged them to be at high risk for preterm birth. The most common reasons for inclusion were symptoms of preterm labour, preterm premature rupture of membranes, antepartum haemorrhage, maternal medical complications, and cervical incompetence. However, rates of these and other risk factors for preterm birth, where reported, varied considerably between trials.

There were some important differences in study design among the ten trials. The protocols of seven trials permitted multiple repeat treatments, either weekly (Aghajafari, Murphy, Ohlsson, 2002; Crowther, Haslam, 2006; Guinn 2001; Mazumder 2008; McEvoy 2002; Wapner 2006) or fortnightly (Murphy 2008), whereas three trials planned only a single repeat treatment (Garite 2009; McEvoy 2010; Peltoniemi 2007). All trials used betamethasone as the study drug, although the preparation used varied. Two trials gave a single dose of 12 mg betamethasone per repeat treatment (Crowther, Haslam, 2006; Peltoniemi 2007), while the remainder gave two doses per repeat treatment. Six trials excluded women with chorioamnionitis (Aghajafari, Murphy, Ohlsson, 2002; Mazumder 2008; McEvoy 2010; Murphy 2008; Peltoniemi 2007; Wapner 2006) and two trials excluded women with preterm premature rupture of membranes (Garite 2009; Wapner 2006). One trial was a small pilot study (Aghajafari, Murphy, Ohlsson, 2002) and two were performed primarily to assess effects on early postnatal lung function (McEvoy 2002; McEvoy 2010), although they provided relevant clinical outcomes.

Our systematic review showed that infants exposed to repeat dose(s) of betamethasone compared to a single course of antenatal glucocorticoids had a reduced incidence of respiratory distress syndrome (risk ratio [RR] 0.83, 95% confidence interval [CI] 0.75 to 0.91; eight trials, 3,206 infants) and combined serious outcome (RR 0.84, 95% CI 0.75 to 0.94; seven trials, 5,094 infants) (Table 1.7). The composite outcome was variously defined in the trials but included severe lung disease, chronic lung disease, severe intraventricular haemorrhage, periventricular leukomalacia, necrotising enterocolitis, retinopathy of prematurity, proven sepsis, patent ductus arteriosus requiring treatment, and perinatal death. For respiratory distress syndrome, the direction of treatment effect favoured the repeat betamethasone group in all but one of the eight trials that contributed
data for this outcome (Peltoniemi 2007). This study differed from the others in that most of the women gave birth less than 24 hours after trial entry. However, subgroup analysis of infants born 24 hours or more after entry into this trial found that those exposed to a repeat dose of betamethasone tended to have a reduced risk of respiratory distress syndrome compared with infants exposed to a single course 7 or more days previously. In subgroup analyses there was no apparent dose response for respiratory distress syndrome. There was a similar magnitude of effect observed in trials administering single versus multiple repeat treatment and in trials administering 12 mg versus 24 mg of betamethasone per repeat treatment.

In keeping with the decrease in respiratory distress syndrome, infants exposed to repeat betamethasone had a lower incidence of oxygen use (RR 0.92, 95% CI 0.85 to 0.99; two trials, 3,448 infants), surfactant use (RR 0.78, 95% CI 0.65 to 0.95, random effects; nine trials, 5,525 infants), mechanical ventilation (RR 0.84, 95% CI 0.71 to 0.99, random effects; six trials, 4,918 infants), patent ductus arteriosus (RR 0.80, 95% CI 0.64 to 0.98; six trials, 4,356 infants), and inotrope use (RR 0.80, 95% CI 0.66 to 0.97; two trials, 1,470 infants).

Six trials reported results for severe respiratory distress syndrome or lung disease, including one large trial (Murphy 2008) that did not contribute data to the analysis of respiratory distress syndrome (Table 1.7). In several of these trials, greater benefit was seen for severe lung disease than for all respiratory distress syndrome (Crowther, Haslam, 2006; Guinn 2001; Wapner 2006). However, in two trials, no significant difference was seen between groups in the incidence of severe lung disease, and the direction of treatment effect favoured infants exposed to a single course of glucocorticoids (Murphy 2008; Peltoniemi 2007). Consequently, there was significant statistical heterogeneity for this outcome, even after excluding the one trial with a higher risk of bias (Mazumder 2008). Heterogeneity was not explained by differences in trial design including the planned interval between treatments, the planned number of repeat treatments, or the planned drug exposure per repeat treatment or per week. For most trials, outcome data were not available for other subgroups such as maternal risk factors for preterm birth, gestational age at trial entry, and the number of treatments received. An individual patient data meta-analysis is currently in progress that will examine possible reasons for this heterogeneity in detail (http://adelaide.edu.au/arch/research/res_synthesis/#IPD).
There were no significant differences between treatment groups in the incidence of the chronic lung disease, perinatal death, intraventricular haemorrhage or maternal infection (Table 1.7). The effect of repeat betamethasone treatment on growth and other physiological outcomes is discussed below.

Four trials reported follow-up at two to three years of age involving 4,370 infants (Asztalos 2010; Crowther 2007; Peltoniemi 2009; Wapner 2007). Assessment rates ranged from 80% to 92% of children presumed to be alive at the time of follow-up. There were no significant differences between children exposed to repeat betamethasone and those exposed to a single course of glucocorticoids in the incidence of disability, cerebral palsy or cognitive impairment (Table 1.8). One trial that assessed behaviour using the Child Behavior Checklist found no differences between groups in either mean scores or in the proportion of children with scores in the clinical range (Crowther 2007). Another trial found that scores for the Behaviour Rating Scale of the Bayley Scales of Infant Development were similar between groups (Asztalos 2010). Repeat betamethasone treatment also did not affect the incidence of hospital admission in childhood, or asthma and recurrent wheeze (McKinlay 2012).
Table 1.6: Randomised trials comparing repeat doses of antenatal glucocorticoids with placebo or no treatment in women at risk of preterm birth 7 or more days after an initial course of glucocorticoids.

<table>
<thead>
<tr>
<th>Study &amp; location</th>
<th>GA at entry (wk)</th>
<th>Minimal interval from first steroids to entry (d)</th>
<th>Exclusion criteria</th>
<th>Intervention</th>
<th>Number of women randomised (live fetuses)</th>
<th>Mean GA at birth (wk)</th>
<th>Number of study treatments received</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinn (2001), USA</td>
<td>25 to &lt;34</td>
<td>7</td>
<td>Major fetal anomaly, documented fetal lung maturity, need for immediate delivery, active maternal TB or HIV infection</td>
<td>Betamethasone 24 mg (divided) or placebo IMI every 7 d until 34 wk GA if undelivered</td>
<td>502 (589)*</td>
<td>33</td>
<td>1 = 2% 2 = 35% 3 = 22% ≥4 = 41%</td>
</tr>
<tr>
<td>Aghajafari (2002), Canada</td>
<td>24 to 30</td>
<td>7</td>
<td>Major fetal anomaly, chorioamnionitis, chronic steroid use</td>
<td>Betamethasone 24 mg (divided) or placebo IMI every 7 d until 33 wk GA if undelivered</td>
<td>12 (16)</td>
<td>31 repeat, 35 placebo</td>
<td>1 = 33% 2 = 25% ≥3 = 42%</td>
</tr>
<tr>
<td>McEvoy (2002), USA</td>
<td>25 to 33</td>
<td>7</td>
<td>Major fetal anomaly, IDDM, drug addiction, multiple pregnancy</td>
<td>Betamethasone 24 mg (divided) or placebo IMI every 7 d until 34 wk GA if undelivered†</td>
<td>37 (37)</td>
<td>32</td>
<td>1 = 44% 2 = 28% 3 = 22% ≥4 = 6%</td>
</tr>
<tr>
<td>Wapner (2006), USA</td>
<td>23 to &lt;32</td>
<td>7 (up to 10)</td>
<td>Major fetal anomaly, chorioamnionitis, chronic steroid use, IDDM, PPROM, documented fetal lung maturity, non-reassuring fetal status</td>
<td>Betamethasone 24 mg (divided) or placebo IMI every 7 d if undelivered &lt;34 wk GA; after the first 67 women total study courses limited to 4</td>
<td>495 (594)‡</td>
<td>34</td>
<td>1 = 12% 2 = 9% 3 = 17% ≥4 = 62%</td>
</tr>
<tr>
<td>Study &amp; location</td>
<td>GA at entry (wk)</td>
<td>Minimal interval from first steroids to entry (d)</td>
<td>Exclusion criteria</td>
<td>Intervention</td>
<td>Number of women randomised (live fetuses)</td>
<td>Mean GA at birth (wk)</td>
<td>Number of study treatments received</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------</td>
<td>--------------</td>
<td>------------------------------------------</td>
<td>----------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Crowther (2006) (ACTORDS), Australia &amp; New Zealand</td>
<td>&lt;32</td>
<td>7</td>
<td>Documented fetal lung maturity, chorioamnionitis requiring urgent delivery, second stage of labour</td>
<td>Betamethasone 11.4 mg or placebo IMI every 7 d if undelivered &lt;32 wk GA†</td>
<td>982 (1146)</td>
<td>32</td>
<td>1 = 42% 2 = 23% 3 = 12% 4 = 22%</td>
</tr>
<tr>
<td>Peltoniemi (2007), Finland</td>
<td>&lt;34</td>
<td>7</td>
<td>Major fetal anomaly, chorioamnionitis, chronic steroid use</td>
<td>Betamethasone 12 mg or placebo IMI, single dose</td>
<td>249 (326)</td>
<td>30</td>
<td>1 = 100%</td>
</tr>
<tr>
<td>Mazumder (2008), India</td>
<td>26 to 33</td>
<td>7</td>
<td>Major fetal anomaly, chorioamnionitis, chronic steroid use, unreliable EDD, not available for follow-up</td>
<td>Betamethasone 24 mg (divided) IMI every 7 d if undelivered &lt;34 wk GA or no treatment</td>
<td>76 (84)§</td>
<td>30</td>
<td>1 = 8% 2 = 39% 3 = 18% 4 = 34%</td>
</tr>
<tr>
<td>Murphy (Murphy 2008)(MACS), 20 countries</td>
<td>25 to 32</td>
<td>14 (up to 21)</td>
<td>Major fetal anomaly, chorioamnionitis, chronic steroid use, multiple pregnancy with fetal death &gt;13 wk GA</td>
<td>Betamethasone 24 mg (divided) or placebo IMI every 14 d until 33 wk GA if undelivered (or until 32 wk GA if PPROM)¶</td>
<td>1858 (2309)</td>
<td>34</td>
<td>1 = 40% 2 = 31% 3 = 17% 4 = 10%</td>
</tr>
<tr>
<td>Garite (2009), USA</td>
<td>25 to &lt;33</td>
<td>14</td>
<td>Major fetal anomaly, chronic steroid use, triplet pregnancy, cervical dilatation ≥5 cm, PPROM, documented fetal lung maturity, active maternal TB or HIV infection</td>
<td>Betamethasone 24 mg (divided) or placebo IMI, single course¶</td>
<td>437 (577)</td>
<td>33</td>
<td>1 = 98%</td>
</tr>
<tr>
<td>Study &amp; location</td>
<td>GA at entry (wk)</td>
<td>Minimal interval from first steroids to entry (d)</td>
<td>Exclusion criteria</td>
<td>Intervention</td>
<td>Number of women randomised (live fetuses)</td>
<td>Mean GA at birth (wk)</td>
<td>Number of study treatments received</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------------------------------------</td>
<td>------------------</td>
<td>-------------</td>
<td>------------------------------------------</td>
<td>----------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>McEvoy (2010), USA</td>
<td>26 to &lt;34</td>
<td>14</td>
<td>Major fetal anomaly, triplet pregnancy, IDDM, chorioamnionitis, chronic steroid use</td>
<td>Betamethasone 24 mg (divided) or placebo IMI, single course†</td>
<td>85 (113)</td>
<td>32</td>
<td>1 = 100%</td>
</tr>
</tbody>
</table>

ACTORDS, Australasian Collaborative Trial of Repeat Dose Antenatal Corticosteroids; MACS, Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study; IMI, intramuscular injection; TB, tuberculosis; HIV, Human Immunodeficiency Virus; PPROM, preterm premature rupture of membranes; NA, not available; EDD, estimated date of delivery; IDDM, insulin-dependent diabetes mellitus; GA, gestational age. * For multiple pregnancy one infant was randomly selected for analysis. † Mixed preparation of betamethasone acetate and betamethasone sodium phosphate. ‡ For multiple pregnancy the worst infant outcome was used for analysis of categorical data. § Results reported only for first-born infants. ¶ 31 women received an equivalent dose of dexamethasone.
Table 1.7 Perinatal effects of repeat doses of antenatal betamethasone compared with placebo or no treatment given to women at risk of preterm birth 7 or more days after an initial course of glucocorticoids.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of trials</th>
<th>Repeat group n / N (%)</th>
<th>Single group n / N (%)</th>
<th>Relative risk or mean difference, fixed effect (95% CI)</th>
<th>Heterogeneity</th>
<th>Number needed to treat to benefit (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory distress syndrome</td>
<td>8</td>
<td>463 / 1603 (29)</td>
<td>565 / 1603 (35)</td>
<td>0.83 (0.75, 0.91)</td>
<td>No</td>
<td>17 (11, 32)</td>
</tr>
<tr>
<td>Severe lung disease</td>
<td>6</td>
<td>267 / 2427 (11)</td>
<td>321 / 2399 (13)</td>
<td>0.83 (0.72, 0.96)</td>
<td>Yes*</td>
<td>NA</td>
</tr>
<tr>
<td>Perinatal death</td>
<td>9</td>
<td>96 / 2791 (3)</td>
<td>102 / 2763 (4)</td>
<td>0.94 (0.71, 1.23)</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>8</td>
<td>181 / 2709 (7)</td>
<td>170 / 2684 (6)</td>
<td>1.06 (0.87, 1.30)</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Intraventricular haemorrhage</td>
<td>6</td>
<td>129 / 1533 (8)</td>
<td>137 / 1532 (9)</td>
<td>0.94 (0.75, 1.18)</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Composite serious infant outcome†</td>
<td>7</td>
<td>438 / 2561 (17)</td>
<td>506 / 2533 (20)</td>
<td>0.84 (0.75, 0.94)</td>
<td>No</td>
<td>33 (20, 83)</td>
</tr>
<tr>
<td>Maternal chorioamnionitis</td>
<td>6</td>
<td>140 / 2152 (7)</td>
<td>118 / 2109 (6)</td>
<td>1.16 (0.92, 1.46)</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Maternal puerperal sepsis</td>
<td>5</td>
<td>72 / 1565 (5)</td>
<td>61 / 1526 (4)</td>
<td>1.15 (0.83, 1.60)</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Birth size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight–g</td>
<td>9</td>
<td>NA / 2820</td>
<td>NA / 2806</td>
<td>-76 (-118, -34)</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Length–cm</td>
<td>6</td>
<td>NA / 2279</td>
<td>NA / 2271</td>
<td>-0.6 (-0.9, -0.2)</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Head circumference–cm</td>
<td>9</td>
<td>NA / 2820</td>
<td>NA / 2806</td>
<td>-0.3 (-0.5, -0.2)</td>
<td>No</td>
<td>NA</td>
</tr>
</tbody>
</table>

CI, confidence interval; NA, not applicable. *$I^2 = 76\%$. Random effects risk ratio = 0.80, 95% CI 0.56 to 1.14; $\tau^2 = 0.12$, 95% prediction interval 0.4 to 1.56. †Variously defined but includes severe lung disease, chronic lung disease, severe intraventricular haemorrhage, periventricular leukomalacia, necrotising enterocolitis, retinopathy of prematurity, proven sepsis, patent ductus arteriosus requiring treatment, and perinatal death. Data from McKinlay et al (2012).
Table 1.8 Early childhood effects of repeat doses of antenatal betamethasone compared with placebo or no treatment given to women at risk of preterm birth 7 or more days after an initial course of glucocorticoids.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of trials</th>
<th>Repeat group n / N (%)</th>
<th>Single group n / N (%)</th>
<th>Relative risk or mean difference, fixed effect (95% CI)</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total deaths</td>
<td>4</td>
<td>96 / 2190 (4)</td>
<td>90 / 2180 (4)</td>
<td>1.06 (0.80, 1.41)</td>
<td>No</td>
</tr>
<tr>
<td>Survival free of any neurosensory disability*</td>
<td>2</td>
<td>1241 / 1584 (78)</td>
<td>1215 / 1571 (77)</td>
<td>1.00 (0.97, 1.04)</td>
<td>No</td>
</tr>
<tr>
<td>Survival free of major neurosensory disability†</td>
<td>2</td>
<td>557 / 642 (87)</td>
<td>572 / 675 (85)</td>
<td>1.03 (0.98, 1.07)</td>
<td>Yes‡</td>
</tr>
<tr>
<td>Composite serious outcome§</td>
<td>2</td>
<td>352 / 1593 (22)</td>
<td>356 / 1571 (23)</td>
<td>0.99 (0.87, 1.12)</td>
<td>No</td>
</tr>
<tr>
<td>Cerebral palsy</td>
<td>4</td>
<td>54 / 1909 (3)</td>
<td>52 / 1891 (3)</td>
<td>1.03 (0.71, 1.50)</td>
<td>No</td>
</tr>
<tr>
<td>Mental developmental index (Bayley Scales of Development II)</td>
<td>2</td>
<td>NA / 562</td>
<td>NA / 600</td>
<td>1 (-1, 3)</td>
<td>No</td>
</tr>
<tr>
<td>Score between 1 and 2 SD below mean</td>
<td>3</td>
<td>175 / 789 (22)</td>
<td>176 / 806 (22)</td>
<td>1.00 (0.83, 1.20)</td>
<td>No</td>
</tr>
<tr>
<td>Score &lt;2 SD below mean</td>
<td>4</td>
<td>174 / 1754 (10)</td>
<td>180 / 1742 (10)</td>
<td>0.94 (0.77, 1.15)</td>
<td>No</td>
</tr>
<tr>
<td>Weight–kg</td>
<td>3</td>
<td>NA / 883</td>
<td>NA / 893</td>
<td>-0.03 (-0.21, 0.15)</td>
<td>No</td>
</tr>
<tr>
<td>Height–cm</td>
<td>3</td>
<td>NA / 883</td>
<td>NA / 893</td>
<td>-0.1 (-0.6, 0.3)</td>
<td>No</td>
</tr>
<tr>
<td>Head circumference–cm</td>
<td>3</td>
<td>NA / 883</td>
<td>NA / 893</td>
<td>-0.1 (-0.2, 0.1)</td>
<td>No</td>
</tr>
<tr>
<td>Asthma or recurrent wheeze</td>
<td>3</td>
<td>176 / 853</td>
<td>204 / 867</td>
<td>0.89 (0.74, 1.06)</td>
<td>Yes‡‡</td>
</tr>
</tbody>
</table>

CI, confidence interval; SD, standard deviation. * Denominator includes total deaths. Neurosensory disability defined as cerebral palsy, blindness, deafness or a mental developmental index score more than one standard deviation (SD) below the mean in one trial, and cerebral palsy or a developmental quotient more than two SD below the mean in the other trial. † Denominator includes total deaths. Major neurosensory disability defined as severe cerebral palsy, blindness or a mental developmental index more than three SD below the mean in one trial, and cerebral palsy, blindness, deafness or a mental developmental index score more than two SD below the mean in the other trial. ‡ I² = 88%. Random effects risk ratio = 1.01, 95% CI 0.92 to 1.11; τ² = 0.00. § Variousy defined but includes total deaths and cerebral palsy, blindness, deafness, or cognitive delay. ‡‡ I² = 62%. Random effects risk ratio = 0.89, 95% CI 0.63 to 1.27; τ² = 0.06. Data from McKinlay et al (2012).
1.3.7 Physiological effects of antenatal glucocorticoids: growth, metabolism and cardiovascular function

1.3.7.1 Somatic growth

1.3.7.1.1 Short-term effects

In animals, exposure to high doses of antenatal glucocorticoids invariably inhibits fetal somatic growth (Ballard 1986). However, at physiological doses this effect is not universal and varies among species. For example, in guinea pigs neither single nor repeat doses of antenatal glucocorticoids affected offspring birthweight (Banjanin 2004; Dean 1999; Liu 2001; McCabe 2001), whereas in rabbits birthweight was reduced in a dose-related manner, even after a single dose (Pratt 1999; Roubliova 2008; Sun 1993). In some species, effects on growth depend on the duration or frequency of glucocorticoid exposure. Thus in rodents, one or two doses of antenatal glucocorticoids did not affect birthweight, but four or more doses did cause fetal growth restriction (Nyirenda 2001; Okajima 2001; Stewart 1997; Stewart 1998). Similarly, in monkeys exposure to a single dose or short course of antenatal glucocorticoids did not affect birthweight, even when placental weight was reduced, whereas a prolonged course reduced placental, total body, and organ weight (Bramlage 2009; Engle 1996; Epstein 1977; Hauser 2007; Johnson 1979; Johnson 1981; Uno 1990). In sheep, several studies demonstrated a 15% to 20% reduction in birthweight after a single dose of betamethasone at mid to late gestation (Ikegami 1997; Jobe, Wada, 1998; Moss 2009; Newnham 1999; Pua 2005), but in other studies this did not occur (Moss 2001; Moss 2002; Moss, Nitsos, 2003; Quinlivan, Archer, 1998). However, treatment of ewes with repeat injections of betamethasone consistently resulted in a 20% to 30% reduction in birthweight of preterm and term lambs (Ikegami 1997; Jobe, Newnham, 1998; Moss 2001; Moss 2002; Moss, Nitsos, 2003; Pua 2005; Quinlivan, Archer, 1998). Therefore, in some species a single course of antenatal glucocorticoids can impair fetal growth but in others a more sustained elevation of fetal glucocorticoid concentrations is required before somatic growth is affected.

When given in sufficient amounts, antenatal glucocorticoids cause a generalised stunting of fetal growth with effects on the skeleton and most organ tissues (Fowden 1996; Mosier 1981; Newnham 1999; Quinlivan, Archer, 1998). However, soft tissues appear to be more sensitive to the effects of glucocorticoids than bone. For example, in monkeys a prolonged course of antenatal betamethasone reduced preterm fetal weight but not crown-rump or crown-heel length (Johnson 1979; Johnson 1981). Likewise, solid organ weights were reduced in lambs after a single dose of antenatal betamethasone but femur length was
reduced only after repeat doses (Newnham 1999). In addition, when the normal late gestation decline in lamb growth due to rising endogenous cortisol was prevented by adrenalectomy, the increment in body weight was more pronounced than that of crown-rump length (Fowden 1996).

In humans, a single course of antenatal glucocorticoids appears to have little effect on birthweight or the incidence of being born small-for-gestational age (Table 1.4), though it should be noted that in the Auckland Steroid Trial infants born more than 48 hours after glucocorticoid treatment tended to weigh about 100 g less than control infants (Roberts 2006). Meta-analysis of repeat-dose trials found that infants exposed to repeat antenatal betamethasone had slightly reduced size at birth, including weight, length and head circumference (McKinlay 2012). However, in three of the trials that showed the largest differences, infants in the repeat dose group had slightly shorter gestation length, which may have explained their smaller size at birth (Guinn 2001; Murphy 2008; Peltoniemi 2007). In the few studies that corrected for gestational age, there were no differences between groups for birthweight Z-scores or multiples of the median birthweight (McKinlay 2012). Similarly, in baboons, three courses of antenatal betamethasone had no effect on fetal somatic growth (Schlabritz-Loutsevitch 2009).

Two human observational studies found reduced cord blood concentrations of biochemical markers of collagen synthesis, suggesting that antenatal glucocorticoids inhibit fetal bone formation (Koivisto 2007; Saarela 2001). However, in a randomised trial, infants exposed to repeat doses compared to a single course of antenatal glucocorticoids showed evidence of reduced bone degradation, but not reduced formation (Fonseca 2009). In subgroup analysis, it appeared that this occurred only in infants exposed to four or more courses of betamethasone. The significance of these changes in fetal bone metabolism is not clear, and they are probably transient (Korakaki 2007; Korakaki 2011).

Fetal growth is governed by a complex interaction between the mother, placenta and fetus, although the main determinant of growth in late gestation is fetal nutrient supply, principally of glucose, lactate, and amino acids (Bloomfield 1998). This is influenced to some extent by maternal diet and metabolism but more importantly by placental transfer of nutrients from the maternal to the fetal circulation. The placenta also synthesises several key fetal nutrients such as lactate and glycine, but if substrates are limited, the metabolic demands of the placenta may compete with those of the fetus (Bloomfield 1998; Hay 1994). Accretion of tissue mass is affected by the relative drive to catabolic and anabolic
metabolism and whether the cell cycle is targeted towards growth or differentiation. Reduced tissue weight is ultimately due to reduced cellular growth, particularly cell number. Various hormones act to promote and co-ordinate feto-placental growth, the most important of which are fetal insulin and the insulin-like growth factors (Fowden 2009; Hill 1998). Glucocorticoids can influence cell growth through a number of mechanisms, including direct effects on cellular physiology and by indirect effects on fetal metabolism and nutrient supply. They can also decrease fetal tissue water content, especially with repeat administration (Stonestreet 2004).

The most important cellular effect is to shift the cell cycle from proliferation to differentiation. As discussed above, glucocorticoids promote maturation and differentiation in a wide range of cells, upregulating numerous enzymes and proteins that are important for postnatal life (Table 1.3). However, this occurs at the expense of DNA synthesis and cell division, leading to reduced tissue accretion, particularly in the heart, lungs, skeletal muscle, kidneys, adrenal glands and liver (Ballard 1986). Furthermore, in lymphoid cells glucocorticoids inhibit glucose uptake and DNA and protein synthesis, leading to apoptosis and tissue involution (Ballard 1986). Thymus and spleen weight are thus particularly affected by glucocorticoid-induced fetal growth restriction (Newnham 1999; Quinlivan, Archer, 1998).

Glucocorticoids have both anabolic and catabolic effects in the fetus, but their predominant effect is catabolic (Fowden 2009). They induce fetal proteolysis and reduce the uptake of alpha-amino nitrogen (Barbera 1997; Marconi 2010; Milley 1995; Verhaeghe 2007). For some amino acids, such as serine and glutamate, betamethasone treatment has been associated with net delivery from the fetal circulation to the placenta (Marconi 2010). These catabolic effects on protein metabolism contribute to reduced fetal tissue accretion.

Glucocorticoids can also influence placental nutrient transfer. In many animal models, glucocorticoid-induced fetal growth restriction is accompanied by reduced placental weight, and the effect on the placenta is often greater than the effect on the fetus (Fowden 2009). In rodents, this has been attributed to apoptosis of placental trophoblast cells (Ain 2005; Baisden 2007; Sugden and Langdown 2001). While compensatory increases in placental efficiency may occur, such as increased expression of glucose transporters (Langdown 2001), smaller placental size is likely to contribute to glucocorticoid induced intra-uterine growth restriction (Fowden 2009). Although decreased placental weight has not been observed in all animal studies, the transfer and synthesis of specific nutrients,
such as lactate, may still be affected (Moss 2005). Relatively little is known of the effects of antenatal glucocorticoids on specific placental amino acid transport systems (Fowden 2009). In a human randomised trial of repeat courses of betamethasone, infants exposed to four or more betamethasone courses had reduced birthweight (Wapner 2006), and there was some evidence that this was associated with reduced placental growth (Sawady 2007).

Interestingly, fetal growth restriction in sheep has been associated with administration of maternal glucocorticoids, but not with direct fetal injection (Ikegami 1995; Jobe, Polk, 1993; Jobe, Newnham, 1998; Moss 2001; Moss, Nitsos, 2003; Newnham 1999; Polk 1995; Polk 1997). While this may be due to differences in duration of fetal glucocorticoid exposure (Moss, Doherty, 2003), it may also relate to differential effects on placental function. For example, in sheep, one study found that maternal but not fetal dosing reduced placental weight (Newnham 1999). In another study, although placental weight was unchanged, repeat maternal but not fetal betamethasone treatment reduced fetal plasma lactate and calcium concentrations, suggesting altered placental function (Moss, Nitsos, 2003).

The effects of glucocorticoids on feto-placental growth are likely to be partly mediated by changes in the expression or action of the insulin-like growth factors (IGF), which have a key role in regulating intra-uterine growth (Fowden 2003; Gluckman 2003). IGF1 and IGF2 are both potent mitogens (Jones 1995), though their expression and specific actions differ. IGF2 is widely expressed in fetal tissues and the placenta (Han 2000; Hill 1990), and although it is the main IGF in fetal circulation it appears to act in a predominantly paracrine manner (Fowden 2003; Hill 1991). It is an important determinant of placental growth (Ain 2005; Baker 1993; Constancia 2002; DeChiara 1990) and may provide the main constitutive drive for fetal growth through direct actions in tissues and indirectly by effects on the placenta (Fowden 2003). IGF1 is expressed at lower levels than IGF2 in fetal life but tissue and circulating concentrations change in response to fetal nutrient and oxygen supply (Bennet 2001; Muaku 1995; Oliver 1993; Owens 1994). IGF1 has anabolic effects, particularly on fetal amino acid metabolism, thereby promoting tissue accretion and fetal growth (Boyle 1998; Harding 1994; Javaid 2004; Jensen 2000). Therefore, an important role of IGF1 is to ensure that fetal growth rate is commensurate with nutrient supply, and in this regard, IGF1 acts synergistically with insulin (Fowden 1995; Oliver 1996). In contrast, IGF2 gene expression is less responsive to nutritional stimuli. IGF1 has relatively little effect on placental size (Baker 1993), but it influences placental nutrient utilisation and transfer (Harding 1994; Liu 1994). Because of differences in the expression
and action of the IGFs, size at birth is highly correlated with concentrations of circulating IGF1 but not IGF2 (Ashton 1985; Chiesa 2008; Giudice 1995; Gluckman, Johnson-Barrett, 1983; Kajantie 2001; Lassarre 1991; Ong, Kratzsch, 2000; Verhaeghe 2003), even though inactivation of either gene has a similar net affect on fetal weight (DeChiara 1990; Liu 1993).

In sheep, the prepartum surge in endogenous fetal plasma cortisol concentration results in changes in the fetal IGF axis. IGF gene expression was reduced in several tissues, such as skeletal muscle and adrenal glands, but in the liver IGF1 was up-regulated while IGF2 was down-regulated (Li 1993; Li 1996; Li 2002; Lu 1994). The prepartum cortisol rise was also accompanied by a fall in fetal plasma IGF2 concentration (Gluckman and Butler 1983). The effects on both growth and IGF expression were prevented by adrenalectomy but restored by fetal cortisol infusion (Gluckman and Butler 1983). These data suggest that one of the mechanisms by which glucocorticoids influence tissue growth and maturation is to initiate a switch in the regulation of the somatotrophic axis from predominantly paracrine IGF production \textit{in utero} to growth hormone-dependent hepatic endocrine IGF1 production characteristic of adult life (Fowden 2003). Suppression of tissue and circulating IGF reduces the drive for fetal growth, while falling tissue levels of IGF2 may act as a signal for cell differentiation or programmed apoptosis (Ain 2005; Fowden 2003; Hill 1998).

In keeping with this, fetal rabbits that were growth restricted because of maternal dexamethasone treatment had increased liver expression of IGF1, although there was also a transient increase in fetal plasma IGF1 concentration (Thakur 2000). However, in sheep, maternal cortisol infusion decreased fetal plasma IGF1 concentration (Jensen, Gallahere, 2002). In rats, a short course of high dose antenatal glucocorticoids also reduced fetal plasma IGF1 concentration (Mosier 1987), but a more prolonged course induced hepatic expression of IGF binding protein 1 (Price 1992), which in turn can reduce IGF bioactivity (Watson 2006). In fetal lambs, repeat but not single doses of maternal betamethasone caused growth restriction and changes to the IGF axis, including reduced plasma concentrations of IGF1 and IGF binding protein 3 (Gatford, Owens, 2008). IGF binding protein 3 is the main carrier protein for IGF1 in circulation and may potentiate its action (Rechler 1998). These observations support the hypothesis that glucocorticoid-induced growth restriction is mediated, at least in part, by altered fetal IGF activity.

In humans, a single course of antenatal glucocorticoids was associated with reduced cord blood concentrations of IGF1 in one observational study (Verhaeghe 2007) and reduced
IGF2 in another (Ahmad 2006), even though infant size was not altered. Nevertheless, reduced fetal plasma IGF1 concentration was associated with a catabolic response (Verhaeghe 2007). Conversely, in another study that included infants exposed to multiple courses of antenatal glucocorticoids, cord blood IGF1 concentrations were positively correlated with the number of doses received, although this relationship could have been influenced by gestation length (Kajantie 2001). There are no data from randomised trials on the effects of antenatal glucocorticoids on IGF function.

In addition to effects on the somatotrophic axis, glucocorticoids can alter the production of other feto-placental hormones that have a role in regulating fetal growth. In sheep, maternal antenatal betamethasone treatment reduced placental production of placental lactogen (Braun 2007), a growth-promoting and anabolic hormone that enhances nutrient transfer to and uptake by the fetus, and stimulates IGF secretion (Handwerger 2000). Human data on the effect of antenatal glucocorticoids on placental lactogen have been conflicting with one study showing reduced hormone concentrations (Lange 1980), but another showing no change (Maltau 1979). Endogenous and exogenous glucocorticoids also modify leptin expression, which is involved in fetal energy balance and regulation of adipose mass (Forhead 2009). However, differences in leptin physiology between species and with the stage of fetal development make assessment of the interaction between glucocorticoids and leptin difficult. For example, antenatal glucocorticoid exposure reduced fetal plasma leptin concentration in rats (Smith 2002; Smith and Waddell 2003; Sugden, Langdown, 2001), increased it in sheep (Forhead 2002; O'Connor 2007) and had no effect in baboons (Schlabritz-Loutsevitch 2009). Results from human observational studies have also been conflicting with reports of both increased (Shekhawat 2000) and decreased (Marinoni 2008) cord blood leptin concentrations after antenatal betamethasone exposure.

1.3.7.1.2 Long-term effects
In animals, glucocorticoid-induced fetal growth restriction was followed by catch-up growth within one to two weeks in rodents (O'Regan 2004; Stewart 1998) and one to six months in sheep (Gatford, Owens, 2008; Moss 2001). This occurred despite ongoing suppression of the somatotrophic axis in sheep (Gatford, Owens, 2008). There was no further effect of antenatal glucocorticoid exposure on adult body size in mice, sheep and monkeys (Bramlage 2009; Moss 2005; Stewart 1997).
Similarly, in the Auckland Steroid Trial and the Dutch trial a single course of antenatal betamethasone did not affect body size in childhood (Dalziel 2004; Smolders-de Haas 1990) or adulthood (Dalziel, Walker, 2005; Dessens 2000). However, adults in the Auckland trial who were exposed to betamethasone had increased lower body segment Z-scores compared to the upper body, suggesting increased growth in the appendicular skeleton relative to the axial skeleton (Dalziel, Fenwick, 2006). One possible explanation for this difference is delayed onset of puberty, as appendicular growth is more rapid than axial growth before puberty and decelerates at puberty when axial growth accelerates. Indeed, in the Dutch trial, there was a trend towards delayed pubarche in boys exposed to antenatal betamethasone (Smolders-de Haas 1990), although in the Auckland trial there was no difference between treatment groups in self-reported age of puberty onset (Dalziel, Walker, 2005). Repeat doses of antenatal glucocorticoids have not been associated with altered body size in early childhood (McKinlay 2012).

Animal data have suggested that antenatal glucocorticoids may have differential effects on the developing skeleton. For example, antenatal betamethasone reduced calcification in the axial skeleton of rat pups but enhanced it in the distal appendicular skeleton (Mosier 1981). In another rat study, antenatal dexamethasone caused a transient growth acceleration of long bones but not vertebrae in young males, while adult females had altered femoral geometry with reduced cortical thickness (Swolin-Eide 2002). Despite these effects on skeletal development, adult bone mineral density (areal and volumetric) was not altered by antenatal glucocorticoid exposure (Swolin-Eide 2002). Data from monkeys are conflicting; juvenile New World monkeys exposed to a six-day course of dexamethasone in late gestation had accelerated leg growth without any effect on body weight (Hauser 2007; Hauser 2008), whereas juvenile Old World monkeys exposed to a longer course of dexamethasone had reduced appendicular skeletal growth (de Vries 2007). In the Auckland Steroid Trial, adults exposed to betamethasone had similar bone mineral density (total skeleton, lumbar spine and femur), and femoral geometry and mechanical properties as those exposed to placebo (Dalziel, Fenwick, 2006).

1.3.7.2 Brain growth
In sheep, antenatal glucocorticoids had multiple adverse effects on fetal brain development, including reduced brain size and mass (Huang 1999), decreased myelination (Antonow-Schlorke 2009; Dunlop 1997; Huang 2001), and decreased astrocyte maturation (Huang 2001). The effects on myelination and glial cells may be reversible (Quinlivan 2002) but brain mass was permanently reduced (Moss 2005). These effects tended to be
greater after exposure to repeat doses (Huang 1999; Malaeb 2009; Moss 2005), and repeat but not single doses were associated with delayed maturation of the retina, optic nerve and peripheral nerves (Quinlivan 1999; Quinlivan, Archer, 2000; Quinlivan, Beazley, 2000; Quinlivan 2002). Weekly courses of dexamethasone also impaired neuronal apoptosis in the frontal lobe of preterm lambs (104-106 days), a process that is important for normal cortical development (Malaeb 2009). Similarly in rats, betamethasone reduced brain cell proliferation but impaired caspase-3-mediated apoptosis (Scheepens 2003).

The hippocampus may be particularly affected by antenatal glucocorticoids because of its high density of corticosteroid receptors (Matthews 2001). In rhesus monkeys, exposure to dexamethasone in the third trimester was associated with neuronal degeneration and neuron loss in the hippocampus, which resulted in selective loss of hippocampal volume (Uno 1990; Uno 1994). However, this occurred with pharmacological rather than physiological doses.

In humans, brain mass was examined at post-mortem in 39 preterm infants exposed to antenatal glucocorticoids and there was no association between brain weight and the number of doses received (Murphy 2001). However, in ten infants exposed to multiple courses of antenatal glucocorticoids, cortical convolution and brain surface area, as determined by magnetic resonance imaging, was reduced compared to term control infants, although brain volume and the medial temporal lobe were unaffected (Modi 2001).

1.3.7.3 Glucose homeostasis

1.3.7.3.1 Short-term effects

Antenatal glucocorticoids promote fetal glycogen deposition in a number of species including the rat (Klepac 1985; Manniello 1977), sheep (Barnes 1978; Franko 2007), pig (Fowden, Apatu, 1995) and monkey (Engle 1996; Epstein 1977; Johnson 1979; Johnson 1981). Glucocorticoids also induce the activity of key gluconeogenic enzymes in the fetal liver (Fowden 1993; Franko 2007). These changes may improve neonatal glucose homeostasis. For example, preterm lambs that were infused with hydrocortisone shortly before birth had augmented plasma glucose concentrations (Stein 1993), and growth-retarded rat pups that were exposed to antenatal glucocorticoids had a reduced incidence of hypoglycaemia (Manniello 1977). However, in other studies, glucose concentrations in lambs did not change with either single or repeat doses of antenatal betamethasone (Moss, Nitsos, 2003; Padbury 1995).
Human data on the effects of antenatal glucocorticoids on neonatal glycaemia have also been conflicting. An observational study found that a single dose of antenatal betamethasone did not influence neonatal glucose concentrations (Rokicki 1987), but in a randomised trial infants exposed to single or repeat doses of antenatal betamethasone had a higher incidence of hypoglycaemia compared to those not exposed to glucocorticoids (Papageorgiou 1979). However, in another trial infants exposed to a rescue dose of antenatal betamethasone compared with those exposed only to a single course had a lower incidence of hyperglycaemia with no increase in hypoglycaemia (Koivisto 2007).

1.3.7.3.2 Long-term effects
In rats, a prolonged course of antenatal dexamethasone in late pregnancy but not a short course caused fasting hyperglycaemia, reduced glucose tolerance, and insulin resistance in adult offspring (Nyirenda 1998; Nyirenda 2001; O'Regan 2004). These changes may have been due to hepatic insulin resistance, as the rate limiting gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) was upregulated in the liver, which has previously been shown to cause glucose intolerance in rodents (Rosella 1993; Valera 1994). The increased expression of PEPCK in dexamethasone-exposed rats may have been related to changes in local glucocorticoid action as the hepatic glucocorticoid receptor was upregulated (Nyirenda 1998). Similar results were observed in non-human primates, but the duration of exposure to antenatal glucocorticoids was also prolonged (de Vries 2007).

The contribution of peripheral insulin resistance to glucocorticoid-induced glucose intolerance in rats is unclear. In one study, antenatal glucocorticoid exposure did not affect overall uptake of glucose by skeletal muscle (Cleasby 2003). However, in another study, antenatal betamethasone treatment was associated with impaired intracellular signalling of the insulin receptor in fat, skeletal muscle and liver of adult offspring (O'Brien 2008).

In sheep, both single and repeat doses of antenatal betamethasone caused long-term changes in glucose regulation. However, repeat doses had more pronounced effects in adult offspring, including fasting hyperglycaemia, reduced glucose tolerance and evidence of insulin resistance (Moss 2001; Sloboda 2005). As in the rat, these effects may have been due to hepatic insulin resistance mediated by an increase in glucose-6-phosphatase (G6P) activity, which regulates hepatic production of glucose by glycogenolysis and gluconeogenesis (Moss 2005). Sheep that were exposed to repeat doses of antenatal betamethasone had a greater increase in the activity of this enzyme.
Studies in rats, sheep, and non-human primates suggest that antenatal glucocorticoid exposure may also adversely affect pancreatic development, resulting in reduced \( \beta \)-cell mass and capacity. This could contribute to the impaired glucose tolerance of adult offspring (Blondeau 2001; de Vries 2007; Gesina 2004; Shen 2003; Sloboda 2005).

In humans, an observational study of young adults born very preterm found that those who were exposed to a single course of antenatal betamethasone had lower fasting insulin concentrations and better insulin sensitivity, as determined by the homeostasis model index of insulin resistance, compared with those who were not exposed to antenatal glucocorticoids (Finken 2008). In the Auckland Steroid Trial, there were no differences in fasting plasma glucose or insulin concentrations at early adulthood between those exposed to betamethasone or placebo (Dalziel, Walker, 2005). However, betamethasone-exposed participants had slightly increased insulin responses to oral glucose challenge. There was some evidence that this effect was greater in those treated with the higher dose of betamethasone (two doses of 24 mg versus two doses of 12 mg). The clinical significance of this finding is unclear as there was no evidence of glucose intolerance, but it could represent mild insulin resistance.

1.3.7.4 Fat metabolism

1.3.7.4.1 Short-term effects
In preterm lambs, antenatal glucocorticoid exposure was associated with improved thermogenesis due to increased expression of mitochondrial uncoupling protein in brown fat (Clarke 1998; Mostyn 2003). This uncouples the electron transport chain from adenosine triphosphate synthesis, resulting in rapid generation of heat.

1.3.7.4.2 Long-term effects
In rats, dexamethasone exposure in mid-gestation was associated with increased fat deposition in adulthood (Dahlgren 2001). However, exposure in the last third of pregnancy resulted in a persistent decrease in fat depots, although visceral fat in these animals showed reduced capacity for fatty acid uptake, which could contribute to hepatic insulin resistance (Cleasby 2003). These changes were accompanied by increased expression of glucocorticoid receptors in visceral fat.

In the Auckland Steroid Trial, there were no differences in adiposity or plasma lipid concentrations at early adulthood between subjects exposed to antenatal betamethasone or placebo (Dalziel, Walker, 2005). Similar results were obtained in an observational study of
young adults born very preterm (Finken 2008). However, abdominal fat was increased in subjects who were exposed to antenatal betamethasone and were carriers of the 363S polymorphism of the glucocorticoid receptor gene, which increases glucocorticoid bioactivity (Finken 2011).

1.3.7.5 Cardiovascular system and kidneys

1.3.7.5.1 Short-term effects
Antenatal glucocorticoids caused an acute increase in mean arterial blood pressure in preterm lambs when administered shortly before delivery, due to increased cardiac output and peripheral vascular resistance (Anwar 1999; Berry 1997; Derks 1997; Ervin 2000; Padbury 1995; Segar 1998; Stein 1993; Zimmermann 2003). Even modest elevations of maternal cortisol increased fetal lamb blood pressure by 20% (Jensen, Gallahere, 2002). Similarly, in non-human primates, betamethasone caused an acute increase in fetal (Koenen 2002) and early postnatal (Smith 2004) blood pressure. However, the cardiovascular status of preterm and term lambs was unaffected by single or repeat doses of antenatal betamethasone when birth was delayed more than seven days (Smith, Ervin, 2003).

In humans, a single course of antenatal glucocorticoids increased neonatal blood pressure (Kari 1994), although this was not associated with any change in ventricular function in an observational study (Vural 2006). Repeat doses of betamethasone had an even greater effect on neonatal blood pressure in one observational study (Mildenhall 2006), but this was not confirmed in a randomised trial (Mildenhall 2009). Nevertheless, treatment with repeat dose(s) of antenatal betamethasone has been shown to reduce the need for neonatal inotropic support, suggesting improved haemodynamic stability (Crowther, Haslam, 2006; Peltoniemi 2007). In observational studies, repeat doses of antenatal glucocorticoids (Mildenhall 2009; Yunis 1999) but not a single course (de Vries 2008; Vural 2006) induced fetal cardiac hypertrophy. However, this did not occur in a randomised trial (Mildenhall 2009).

Antenatal glucocorticoids may enhance postnatal circulation through several different mechanisms. For example, glucocorticoids influence the neurohormonal regulation of the developing cardiovascular system by augmenting sympathetic nervous system activity and altering baroreflex responses (Segar 1998; Segar 2001), increasing vascular responsiveness to the vasoconstrictor angiotensin II (Roghair 2004; Tangalakis 1992), and up-regulating cardiac adenylate cyclase activity, the second messenger for β-adrenoreceptors (Stein
Several other effects in the fetal heart may contribute to improved cardiac function, including upregulation of angiotensin receptors (Segar 1995), and increased synthesis of ATP and proteins associated with cardiac contraction (Mizuno 2010). The beneficial effects of antenatal glucocorticoids on neonatal circulation occur despite attenuated postnatal surges in plasma cortisol, catecholamine and angiotensin II concentrations (Ervin 2000; Padbury 1995; Segar 1998; Stein 1993).

In sheep and rabbits, antenatal glucocorticoids improved kidney function with effects on glomerular filtration rate, urinary flow, sodium reabsorption, and acid-base balance (Baum 1991; Berry 1997; Ervin 1996; Hill 1988; Kim 2011; Stonestreet 1983). However, the benefits were transient, and were limited to preterm gestations and early postnatal ages. Maturational effects appear to be more prominent in the renal tubule than in the glomerulus (Jahnukainen 2001), but changes to intra-renal blood flow also increase glomerular filtration rate (Berry 1997). In lambs, repeat doses of antenatal glucocorticoids did not result in additional improvements in renal function compared to a single course (Smith, Ervin, 2003). Evidence from a number of observational studies suggests that antenatal glucocorticoids also improve renal function in preterm infants (al-Dahan 1987; Dimitriou 2005; Omar 1999; Omar 2000; van den Anker 1994).

### 1.3.7.5.2 Long-term effects

Several animal studies have shown that exposure to antenatal glucocorticoids can cause long-term elevations in the blood pressure of offspring. However, this effect varies according to species, glucocorticoid dose and the timing of fetal exposure. For example, in sheep exposure to a short course of dexamethasone in early gestation (0.2) (Dodic 1998; Dodic, Peers, 1999; Dodic 2001) caused permanently elevated blood pressure. However, this effect was inconsistent when glucocorticoids were administered at mid-gestation (Dodic 1998; Dodic, Wintour, 1999; Figueroa 2005; Shaltout 2010), and was not seen with prolonged low dose dexamethasone (Dodic 2003; Moritz, Butkus, 2002) or with single or repeat doses of betamethasone in late gestation (0.7 to 0.8) (Moss 2005). Similarly, in non-human primates results have been conflicting (Bramlage 2009; de Vries 2007). In contrast, adult rat offspring have consistently been shown to have increased blood pressure following antenatal glucocorticoid treatment, with both long (Benediktsson 1993; Celsi 1998; Dagan 2010; Levitt 1996; O'Regan 2004; Tang 2011) and short courses (Ortiz 2001; Ortiz 2003; Singh 2007), although the effect appears to be greatest at gestation days 15 to 16 (~0.7 gestation) (Ortiz 2001; Ortiz 2003). Repeat courses of antenatal dexamethasone...
compared with no glucocorticoid exposure were also associated with elevated blood pressure in adult male guinea pigs (Banjanin 2004).

In humans, randomised trials of single course antenatal glucocorticoids have found either no long-term effect of treatment on blood pressure (Dalziel 2004; Dalziel, Walker, 2005) or slightly reduced systolic pressure (Dessens 2000). However, these cohorts were born in the 1960s and 1970s and included few subjects born very preterm. Several observational studies have examined the long-term effects of antenatal glucocorticoid exposure in subjects born very preterm, but results for blood pressure were conflicting, with some showing no effect (de Vries 2008; Finken 2008) and others showing a small increase in systolic and diastolic pressure (Doyle, Ford, Davis, 2000). There was no effect of a single course of betamethasone on ventricular function in childhood in one observational study (de Vries 2008). Repeat doses of antenatal betamethasone were not associated with altered blood pressure in early childhood in two trials (Crowther 2007; Wapner 2007).

In rats and sheep, the association between antenatal glucocorticoid exposure and increased blood pressure may be mediated by adverse effects on renal development, including reduced nephron number (Celsi 1998; Figueroa 2005; Ortiz 2001; Ortiz 2003; Singh 2007; Wintour 2003), and changes to the intra-renal renin-angiotensin system, which affects tubule function and sodium reabsorption (Contag 2010; Dagan 2010; Massmann 2006; Moritz, Johnson, 2002). Antenatal glucocorticoids may also permanently reduce 11-BHSD2 activity in the kidney resulting in increased local corticosteroid activity (Tang 2011). Glucocorticoids appear to have the greatest effect on nephrogenesis when exposure occurs during the preglomerular stage of metanephric development. Variation in the timing of kidney development between species may thus explain some of the different responses to glucocorticoids (Moritz 2003). For example, in sheep, permanent kidneys first appear at about 0.15 gestation with nephrogenesis commencing at about 0.3 gestation and peaking by mid-gestation, whereas the rat kidney is still mostly mesenchyme by 0.7 gestation (15 days) and nephrogenesis continues throughout the first postnatal week (Lumbers 1995). Exposure of non-human primates to a week-long course of dexamethasone at 0.3 or mid-gestation did not affect blood pressure or nephron number, which may be due to the early timing of metanephric development in higher species (Bramlage 2009). In another study, preterm baboons exposed to antenatal betamethasone had a similar number of glomeruli compared with un-exposed animals, but there were more glomeruli with mature morphology (Gubhaju 2009).
In humans, the long-term effects of antenatal glucocorticoids on renal development have not been well studied. One observational study found that antenatal glucocorticoid treatment had no effect on renal volume, glomerular filtration rate and tubular function in early childhood (Carballo-Magdaleno 2011), while another study of very preterm infants found that those exposed to antenatal betamethasone had reduced glomerular filtration rate in early adulthood (Finken 2008).

Extra-renal mechanisms are also likely to play a role in mediating elevated blood pressure after antenatal glucocorticoid exposure, as the effect on nephrogenesis has not always been associated with changes in renal function (Ortiz 2003), and hypertension can occur without changes in glomerular filtration rate or nephron number (Zhang, Massmann, 2010). Other possible mechanisms include altered vascular tone (Moritz, Dodic, 2009; Pulgar 2006; Roghair 2004), resetting of baroreflexes (Dodic, Peers, 1999; Segar 2006; Shaltout 2010; Shaltout 2011), increased sympathetic activity (Segar 2006; Shaltout 2011), changes to the angiotensin system in the brain (Dodic, Abouantoun, 2002; Dodic, Hantzis, 2002; Dodic 2006), and resetting of central mineralocorticoid receptors (Banjanin 2004; Dodic, Hantzis, 2002). Increased hypothalamic-pituitary-adrenal (HPA) tone and elevated basal cortisol may also be implicated (Levitt 1996).

In adult sheep exposed to antenatal betamethasone in mid-gestation, elevated blood pressure was associated with reduced heart rate variability, but increased variability of blood pressure, suggesting autonomic imbalance (Shaltout 2010; Shaltout 2011). However, in a human observational study no such effect was seen at birth (Schaffer 2010). Synthetic and natural glucocorticoids may have different effects on long-term cardiovascular regulation (Dodic 2006; Moritz, Dodic, 2009).

1.3.7.6 Hypothalamic-pituitary-adrenal axis
1.3.7.6.1 Short-term effects
Most animal data show that the fetal HPA axis is sensitive to feedback by maternally administered synthetic glucocorticoids, resulting in an acute decrease in fetal basal and stress plasma cortisol concentrations. This has been demonstrated in sheep (Fletcher 2000; Jellyman 2004; Segar 2001), guinea pigs (Dean 1999; McCabe 2001; Owen 2003; Owen 2005), and non-human primates (Leavitt 1997; Uno 1990). Negative feedback may occur at the level of the hippocampus, hypothalamus or pituitary, and acute changes in corticosteroid expression in the hippocampus (Dean 1999; McCabe 2001; Owen 2003; Sloboda 2008) and pituitary (Sloboda 2000) have been observed. The suppression of the
HPA axis appears to be transient and may be followed by a compensatory increase in HPA axis activity (Fletcher 2004; Owen 2007; Sloboda 2000). In sheep, antenatal glucocorticoids also increased cortisol binding capacity, which could affect glucocorticoid feedback by reducing free cortisol concentrations (Berdusco 1995; Sloboda 2000). Studies in guinea pigs indicate that the effects of synthetic glucocorticoids can be sex-specific, with male fetuses being more susceptible to HPA axis suppression (Dean 1999; Owen 2003; Owen 2005). Although there is some evidence to support a dose-response relationship between glucocorticoid exposure and HPA axis suppression (Owen 2005; Uno 1990), no animal study has directly compared the effect of single and multiple courses of antenatal glucocorticoids on fetal or neonatal HPA axis function. Large doses of antenatal glucocorticoids appear to be particularly toxic to neurons in the fetal hippocampus, an important site of glucocorticoid feedback and HPA axis control, which may be due to the high density of corticosteroid receptors in this region of the brain (De Kloet 1998; Uno 1990). Prolonged suppression of the fetal HPA axis may also impair adrenal growth and maturation (Aberdeen 1998; Leavitt 1997).

In humans, randomised trial data on the effects of a single course of antenatal glucocorticoid treatment on neonatal HPA axis function are limited to two studies. One small trial found that infants exposed to a single course of antenatal betamethasone compared to those exposed to placebo had reduced cord blood cortisol concentrations if born within 48 hours of treatment, but by 24 hours of age had similar basal cortisol concentrations (Teramo 1980). There was also no significant difference between groups in plasma cortisol concentration after adrenal stimulation (adrenocorticotrophic [ACTH] hormone challenge), although the increase tended to be less in infants born within 48 hours of betamethasone exposure. In the much larger Auckland Steroid Trial, infants exposed to a single course of betamethasone did not have an increased risk of acute adrenal insufficiency compared to those exposed to placebo (Liggins 1972). The results from these trials suggest that infants exposed to a single course of antenatal glucocorticoids are still able to mount an adrenocortical response to stress, despite transient suppression of the fetal HPA axis.

This is supported by a large number of observational studies, summarised in a systematic review by Tegethoff et al (2009). Although there was considerable heterogeneity of results, such that pooled estimates of effect were not possible, most studies in the review showed that antenatal glucocorticoid exposure was associated with reduced cortisol concentrations in amniotic fluid and cord blood compared to healthy control fetuses. However, recovery
of HPA axis suppression appeared to be rapid, as more than half of the studies showed no effect of antenatal glucocorticoid exposure on basal cortisol concentrations within the first few postnatal weeks, and in most other studies basal cortisol concentrations had completely recovered by the end of the second postnatal week. One study found that fetal HPA suppression was related to reduced amplitude of secretory cortisol bursts (Arnold 1998). This is likely due to reduced central HPA axis tone, as several studies have shown that antenatal glucocorticoid exposure, including multiple doses, does not substantially alter adrenocortical reactivity to pharmacological stimulation (Ng 1997; Ng 2002; Noguchi 1978; Ohrlander 1977; Sandesh Kiran 2007; Terrone 1999). A single course of antenatal betamethasone also did not alter neonatal HPA axis response to physiological stress and illness (Ballard 1980), although responses to pain and noxious stimuli may be reduced (Davis 2004).

Two randomised trials have compared HPA axis function in infants exposed to a single course of antenatal glucocorticoids or weekly repeat doses of betamethasone. McEvoy et al (2002) found that in a subset of 11 infants, those in the repeat compared to single course group had similar basal plasma cortisol concentrations but reduced stimulated adrenal cortisol responses on day five. In the ACTORDS trial, treatment groups in one centre did not differ in cord blood cortisol concentrations but infants exposed to repeat betamethasone had a transient decrease in basal salivary cortisol concentrations at the end of the first postnatal week and decreased cortisol response to pain on day three (Ashwood 2006). However, in another centre, infants randomised to repeat betamethasone or placebo had similar pituitary and adrenal responses to metyrapone challenge (Battin 2007).

1.3.7.6.2 Long-term effects

Studies in several animal species have shown that antenatal glucocorticoid exposure, both single and repeat courses, can lead to long-term changes in HPA axis function, including rats (Levitt 1996; O'Regan 2004), sheep (Sloboda 2002; Sloboda 2007), pigs (Haussmann 2000), guinea pigs (Banjanin 2004; Dean 2001; Dunn 2010; Liu 2001) and non-human primates (Uno 1994). However, these findings have not been universal (Hauser 2008). In addition, interpretation of the data is difficult as the effects on the HPA axis are dynamic and change throughout the life course. Nevertheless, the central pattern that has emerged from animal studies is that antenatal glucocorticoid treatment seems to increase HPA axis activity in juvenile animals and in early adulthood (Dean 2001; Sloboda 2002; Uno 1994) but decrease it in later adulthood (Banjanin 2004; Liu 2001; Sloboda 2007). Furthermore, these effects appear to be greatest in males (Dean 2001; O'Regan 2004), although
investigation of females is complicated by the influence of the reproductive cycle on HPA activity (Dunn 2010).

The physiological basis for these long-term effects is unclear, although a number of studies have demonstrated changes in the expression of corticosteroid receptors in the hippocampus (Banjanin 2004; Dean 2001; Dunn 2010; Levitt 1996; Liu 2001; Sloboda 2008). However, interpretation of these data is complicated by the potential for corticosteroid receptor autoregulation. For example, increased receptor expression could be a cause (via increased negative feedback) or a consequence of reduced circulating cortisol concentrations (Levitt 1996; Sloboda 2007). In adult sheep, antenatal betamethasone exposure was also associated with increased expression of 11β-HSD2 in the hippocampus, which could lead to reduced local cortisol concentrations and negative feedback (Sloboda 2008). Another possible mechanism is altered adrenocortical responsiveness due to changes in adrenal morphology or steroidogenesis (Banjanin 2004; Haussmann 2000; Sloboda 2007). In sheep, serial HPA challenges with corticotrophin-releasing hormone and arginine vasopressin suggested that adrenal sensitivity to adrenocorticotropic hormone stimulation was increased in betamethasone exposed animals in early adulthood but decreased in later adulthood (Sloboda 2007).

Only one animal study has directly compared the long-term effects of single and repeat doses of antenatal glucocorticoids (Sloboda 2002; Sloboda 2007). Sheep exposed to a single dose of antenatal betamethasone (0.7 gestation) had increased basal and stimulated HPA axis activity compared to control animals in early adulthood but not in later adulthood. In contrast, animals exposed to repeat doses of antenatal betamethasone had no apparent differences in HPA axis function in early adulthood compared to controls but had reduced basal and stimulated cortisol concentrations with raised ACTH concentrations in later adulthood.

In the Auckland Steroid Trial, subjects exposed to a single course of antenatal betamethasone tended to have slightly higher early morning plasma cortisol concentrations in early adulthood than those exposed to placebo, although the effect was not significant in adjusted analyses (Dalziel, Walker, 2005). Stimulated or stress HPA responses were not measured.
1.4 The developmental origins of disease: role of glucocorticoids and preterm birth

An extensive body of epidemiological and animal experimental evidence has shown that small size at birth, indicative of reduced fetal growth, is strongly associated with an increased incidence of a cluster of related adult cardiovascular and metabolic diseases, including coronary artery disease, stroke, hypertension, type 2 diabetes and metabolic syndrome (McMillen 2005; Wells 2009). This has led to a developmental and life-course model of chronic disease that recognises that an individual’s physiological potential is determined not only by genotype but by environmental conditions during early growth and development (Barker 2004) (Table 1.9). Growth in early life is important because this is when the structural organisation and functional units of major organs are established, whereas after infancy growth by hyperplasia largely ceases and organ growth is due to hypertrophy (Hales 1992). Fetal life is a critical period during which environmental factors may influence later health. Intrauterine growth is largely dependent on the availability of nutrients, which are delivered along a somewhat precarious supply line involving the mother, uterus and placenta (Bloomfield 1998). Therefore, many interacting factors may contribute to impaired fetal growth, including maternal undernutrition, stress and disease, and placental dysfunction. In addition, neural and endocrine control systems that determine later homeostasis are developing and set-points may be permanently altered by the intrauterine nutritional and hormonal milieu. Nutritional restriction results in fetal metabolic adaptations that aim to conserve energy and redistribute fuels to critical tissues, such as the brain. This may result in a so-called thrifty phenotype (Hales 1992), characterised by low lean mass, reduced insulin sensitivity, decreased beta cell reserve, reduced nephron mass, arterial stiffness, higher blood pressure, heightened stress responses and a tendency to central adiposity; factors that are important in the pathogenesis of cardiovascular and metabolic disease (Wells 2011). Individuals that have poor early growth followed by accelerated weight gain in childhood or adulthood have the highest risk of cardiovascular and metabolic disease in later life (Barker 2004). This is likely to be due to the increased metabolic burden of larger body size being placed on a smaller underlying metabolic capacity and reserve (Wells 2011).

While fetal nutrition is a major determinant of fetal growth and later physiological function (Harding 2001), it has been proposed that excess exposure of the fetus to glucocorticoids may also underlie the associations between small size at birth and later risk of cardiovascular disease (Benediktsson 1993; Edwards 1993). This is supported by the known role of glucocorticoids in regulating fetal tissue maturation and growth, and the
demonstration that aspects of the thrifty phenotype can be produced by administration of antenatal glucocorticoids, as described above. Although there is normally limited placental transfer of maternal cortisol, this may increase during maternal stress when maternal plasma cortisol concentrations are elevated, or if placental 11β-HSD2 activity is impaired (Seckl 2007). There is growing evidence that this enzyme may represent a common pathway through which various early life factors can increase fetal glucocorticoid exposure and thereby influence fetal growth and development. For example, placental 11β-HSD2 activity is positively correlated with birthweight; babies with intrauterine growth restriction have low placental 11β-HSD2 activity; and individuals with homozygous deleterious mutations of 11β-HSD2 have low birthweight (McTernan 2001; Mune 1995; Murphy 2002; Stewart 1995). In rats, inhibition of 11β-HSD2 throughout pregnancy resulted in a 20% reduction in birthweight, and adult offspring developed hypertension and glucose intolerance (Lindsay, Lindsay, Edwards, 1996; Lindsay, Lindsay, Waddell, 1996). Furthermore, mild maternal protein restriction reduced placental 11β-HSD2 activity and birthweight, and induced hypertension in adult offspring, which was prevented by pharmacological blockade of maternal glucocorticoid synthesis (Langley-Evans 1996; Langley-Evans 1997). A variety of other antenatal factors have been shown to reduce placental 11β-HSD2 activity, including hypoxia, pro-inflammatory cytokines, infection, and environmental toxins (Seckl 2007). Endogenous glucocorticoids presumably influence long-term physiological function through the same mechanisms described above for exogenous glucocorticoids, though downregulation of hippocampal glucocorticoid receptors and increased activity of the hypothalamic-pituitary-adrenal axis is likely to be a dominant mechanism (Seckl 2007).

Although these data show that exposure of the fetus to inappropriate levels of endogenous glucocorticoids can affect fetal development and long-term function, it is not known to what extent this underlies the known association between reduced size at birth and adult disease. Furthermore, it is far from clear that maternal nutritional manipulations inevitably result in increased passage of maternal glucocorticoids to the fetus (Bloomfield 2006).

Preterm birth may represent another pathway of sub-optimal early growth and development leading to increased risk of later cardiovascular and metabolic disease (Hofman, Regan and Cutfield 2006). Preterm infants have immature organ systems that must undergo growth and maturation in a vastly different postnatal environment, which is commonly compromised by inadequate nutrition and acute illness. Epidemiological studies
have shown that preterm birth is associated with an increased risk of stroke and diabetes (Kaijser 2009; Koupil, Leon, 2005; Lawlor 2005; Lawlor 2006), but not coronary artery disease (Kaijser 2008; Koupil, Leon, 2005). Although these studies were based on subjects born in the first half of the 20th century, before the modern era of neonatology, similar patterns appear to be present in more recent preterm cohorts, including evidence of higher blood pressure (Norman 2010) and insulin resistance (Hofman 2004).
Table 1.9 Key components of the developmental origins of disease hypothesis.

<table>
<thead>
<tr>
<th>Indicators of sub-optimal early developmental conditions</th>
<th>Environmental &amp; maternal signals to the fetus</th>
<th>Mechanisms by which early developmental conditions alter life-course physiology</th>
<th>Postnatal factors that influence pathways to disease</th>
<th>Adult diseases that have a significant developmental component</th>
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</thead>
<tbody>
<tr>
<td>Maternal nutrition</td>
<td>Placental nutrient &amp; oxygen supply</td>
<td>Decreased cell number or functional units in organs that provide metabolic capacity:</td>
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<tr>
<td>Placental disease</td>
<td>Placental hormones and growth factors</td>
<td>• Kidney (nephrons)</td>
<td>Reduced metabolic capacity:</td>
<td>Coronary artery disease</td>
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<tr>
<td>Reduced fetal size</td>
<td>Glucocorticoids (maternal, fetal stress</td>
<td>• Pancreatic beta cells</td>
<td>• Tissue injury: infection, disease, smoking</td>
<td>Stroke</td>
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<td>Maternal stress</td>
<td>response)</td>
<td>• Skeletal muscle (glucose clearance)</td>
<td>• Poor infant growth</td>
<td>Hypertension</td>
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<td>Maternal metabolic disease, e.g. diabetes</td>
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<td>• Lung</td>
<td>• Decreased physical activity</td>
<td>Type 2 diabetes</td>
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<td>Gestation length</td>
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<td>• Liver</td>
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<td>Metabolic syndrome</td>
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<td></td>
<td>• Cardiomyocytes</td>
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<td>(obesity, dyslipidaemia, insulin resistance)</td>
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<td>Permanently altered expression of receptors, proteins and enzymes that determine the activity of hormonal axes and metabolism of tissues:</td>
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<td>• Stress systems: hypothalamic-pituitary-adrenal axis, sympatho-adrenal system</td>
<td>Increased metabolic load:</td>
<td>Osteoporosis (?)</td>
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<td>• Tissue glucocorticoid activity: corticosteroid receptors, 11-beta-hydroxysteroid dehydrogenase-2</td>
<td>• Compensatory growth (?)</td>
<td>Cancer (?)</td>
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<tr>
<td></td>
<td></td>
<td>• Rennin-angiotensin-aldosterone axis</td>
<td>• Excess weight gain</td>
<td>Depression (?)</td>
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<td>• Insulin resistance</td>
<td>• Increase relative adiposity</td>
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<td>• Adipose tissue, adipocytokines</td>
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<td>• Reproductive physiology</td>
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(Barker 2004; Glover 2011; Hofman, Regan and Cutfield 2006; Kajantie 2008; Seckl 2007; Thornburg 2010; Wells 2011)
1.5 Summary

Preterm birth is a major global health problem and is the leading cause of neonatal mortality and morbidity. Its incidence is increasing in many developed countries. Although the survival of very preterm infants has improved in recent decades, the incidence of long-term pulmonary and developmental complications remains high. In addition, there is concern that preterm birth may contribute to the developmental origins of adult cardiovascular and metabolic disease. There are currently no public health interventions that have had a major impact on reducing the incidence of preterm birth.

For women with acute risk of preterm birth, antenatal glucocorticoid treatment is one of very few interventions that improve infant outcomes. A single course of betamethasone or dexamethasone substantially decreases neonatal mortality and major morbidity, and is indicated in virtually all women at risk of preterm birth before 35 weeks’ gestation. These benefits are due to acceleration of fetal lung development as well as maturation of numerous other tissues and biochemical processes necessary for extra-uterine life. Antenatal glucocorticoid treatment also enhances the effectiveness of other neonatal treatments such as surfactant therapy and non-invasive ventilatory support.

The duration of clinical benefit is not known, but may be up to several weeks, after which fetal maturation appears to gradually revert to the basal developmental state. Current diagnostic tests for preterm birth have poor positive predictive value, and many women at apparent risk of preterm birth deliver after the optimal period of glucocorticoid exposure. Our systematic review of randomised trials showed that administering repeat doses of betamethasone to women who remain undelivered 7 or more days after an initial course of treatment and who have ongoing risk of preterm birth at <34 weeks’ gestation results in additional neonatal benefit, including reduced respiratory and other serious morbidity. These benefits are likely to be due to two factors: one, repeat treatment ensures that delivery occurs proximal to glucocorticoid exposure; and two, repeat treatment may induce a more advanced state of tissue maturation.

An important and largely unanswered question is whether repeat dose treatment is safe in the long term. A single course of antenatal glucocorticoids has not been associated with any adverse clinical effects in early adulthood. However, the demonstration in animals of a dose-response effect for many physiological outcomes, and the possible role of glucocorticoids in the developmental programming of adult disease provide grounds for caution about increasing fetal glucocorticoid exposure. There are now data to show that
treatment with repeat doses of antenatal betamethasone does not adversely affect neurodevelopment at two to three years of age. However, longer-term follow-up is needed to assess effects on higher cognitive function, lung function, and physiological outcomes relating to future risk of cardiovascular and metabolic disease.

1.6 Thesis objectives
This thesis is based on a follow-up study of children at early school age whose mothers participated in the ACTORDS trial. The primary objective is to determine whether exposure to repeat doses of antenatal betamethasone has any long-term effect on physiological risk factors for cardiovascular and metabolic disease, including:

- Growth and body composition (chapter 4)
- Glucose and insulin metabolism (chapter 5)
- Blood pressure and renal function (chapter 6)
- Hypothalamic-pituitary-adrenal axis function (chapter 7)

A secondary objective of this thesis is to determine whether there are any independent effects of gestation length and fetal growth on these outcomes at early school age (chapter 8). This is important for providing a context in which to evaluate the long-term effects of repeat antenatal betamethasone treatment and to further explore the possible contribution of these factors to the developmental origins of adult disease.
2 Methods

2.1 Summary of chapter contents
This chapter begins with an overview of the Australasian collaborative trial of repeat doses of steroids (ACTORDS). It then details the methods of the early school-age follow-up of the New Zealand ACTORDS cohort undertaken for this thesis. The final section outlines the development and validation of the salivary cortisol assay, including attempts to develop a salivary dehydroepiandrosterone (DHEA) assay, which proved unsuccessful.

2.2 ACTORDS trial
The aim of ACTORDS study was to establish whether repeat doses of glucocorticoids given to women at risk of preterm birth can reduce neonatal morbidity without harm (Crowther, Haslam, 2006). It was conducted in 23 centres in Australia and New Zealand from 1998 to 2004. Women with single, twin, or triplet pregnancy at <32 weeks’ gestation were eligible for inclusion if they had received a course of antenatal glucocorticoids 7 or more days previously and they were judged to have ongoing risk of preterm delivery by their responsible clinician. Women were excluded if they were in the second stage of labour, had chorioamnionitis needing urgent delivery, had mature fetal lung development, or if further corticosteroid treatment was considered essential or contraindicated. Following written informed consent, women were randomly assigned to an intramuscular injection of either Celestone Chronodose, containing 7.8 mg betamethasone sodium phosphate and 6 mg betamethasone acetate (Schering-Plough, Sydney, Australia) or a saline placebo. Each week, if a woman remained undelivered at <32 weeks’ gestation and was considered to have ongoing risk of preterm birth the allocated treatment was repeated. Antenatal and neonatal care was otherwise according to local protocol.

The randomisation sequence was generated by computer using variable block sizes and stratification for centre, gestational age (<28 weeks’ gestation or ≥28 weeks’ gestation), and number of fetuses (singleton, twin or triplet). Concealment of treatment allocation was ensured using a central telephone randomisation service that assigned a study number corresponding to an appropriate masked treatment pack held at the participating centre. Study packs looked identical and enclosed an opaque study-labelled syringe containing the allocated treatment. For each subsequent treatment, the telephone randomisation service assigned a further study pack from the same treatment group. All women, clinical and research staff, and investigators were blinded as to treatment group allocation.
The primary outcomes of the trial were the incidence of neonatal respiratory distress syndrome; the severity of respiratory disease present; the need for and duration of oxygen treatment and mechanical ventilation via an endotracheal tube; and weight, length, and head circumference at birth and at discharge from hospital. Secondary outcomes were chorioamnionitis requiring intrapartum antibiotics; maternal postpartum pyrexia ≥38.0°C; maternal side effects of the injection; and other measures of neonatal morbidity. Respiratory distress syndrome was defined as clinical signs of respiratory distress with a ground-glass appearance on chest radiograph. Respiratory disease was defined as the need for respiratory support or oxygen, and was further classified as:

- Mild, if the maximum mean airway pressure was <7 cm H\textsubscript{2}O, or the maximum fractional inspired oxygen was <0.4
- Moderate, if the maximum mean airway pressure was 7 to <10 cm H\textsubscript{2}O, or the maximum fractional inspired oxygen was 0.4 to 0.79
- Severe, if the maximum mean airway pressure was ≥10 cm H\textsubscript{2}O or more, or the maximum fractional inspired oxygen was ≥0.8, with need for ventilation

It was estimated that 980 women would be required to detect a 25% reduction in the risk of respiratory distress syndrome from 30% to 22.5%, with 80% power and a two-sided significance level of 5%. Nine hundred and eighty-two women were enrolled (1146 live fetuses), representing about 44% of all women who were eligible to participate in the trial during the study period. Ninety percent of women received betamethasone (2 doses of 12 mg 24 hours apart) for their initial course of steroids, while the remainder received dexamethasone. Six women in the repeat betamethasone group (1%) and nine in the placebo group (2%) did not receive their allocated treatment. Eleven women in the repeat betamethasone group (2%) and 12 in the placebo group (2%) received an open-label dose of antenatal glucocorticoids for clinical reasons.

Statistical analysis was performed on an intention-to-treat basis. It was pre-specified that analyses would be adjusted for gestational age at trial entry and for prognostic variables with imbalance, defined as an absolute difference of 5% or more. These included the frequency of antepartum haemorrhage (32% in the repeat betamethasone group versus 26% in the placebo group) and preterm prelabour rupture of membranes (31% in the repeat betamethasone group versus 37% in the placebo group) as a reason for being at risk of preterm birth. The frequency of other baseline characteristics was similar between
treatment groups. Binary outcomes were compared using risk ratios, which were estimated using log-binomial regression. Continuous variables were compared by analysis of variance if normally distributed or by non-parametric methods if not. Robust variance estimation was used to account for the non-independence of twins and triplets. The study was registered as an International Standard Randomised Controlled Trial (ISRCTN48656428) and ethical approval was obtained at each of the participating centres.

The reasons that the enrolled women were considered to be at risk of preterm birth included antepartum haemorrhage (29%), preterm prelabour rupture of membranes (34%), spontaneous preterm labour (26%), cervical incompetence (10%), pre-eclampsia (10%), and severe fetal growth restriction (7%). Ten percent of women also had a history of previous very preterm birth. One-third of women were nulliparous and 16% had multiple pregnancy. The median gestation at the time of the first course of antenatal glucocorticoids was 26 weeks and at trial entry was 28 weeks. Infants were born at a mean gestation of 32 weeks with 18% being born at term. The majority of women (42%) received only one study treatment, 35% received two or three treatments, and 22% received four or more. Women and their infants were followed to the point of primary discharge from hospital with no losses to follow-up during this period.

Infants exposed to repeat betamethasone compared to placebo had a reduced risk of respiratory distress syndrome and severity of respiratory disease, and required less oxygen therapy and mechanical ventilation (Table 2.1). Fourteen infants needed to be treated with repeat doses of antenatal betamethasone in order to prevent one case of respiratory distress syndrome (95% CI 8 to 50). Associated with this improved respiratory status, infants in the repeat group were less likely to need surfactant (RR 0.81, 95% CI 0.68 to 0.97, P=0.02) or treatment for patent ductus arteriosus (RR 0.59, 95% CI 0.40 to 0.87, P=0.01), and had reduced combined serious neonatal morbidity (RR 0.79, 95% CI 0.65 to 0.97, P=0.02). There was no difference between groups in the gestational age at birth (mean difference [MD] 0.06 weeks, 95% CI -0.4 to 0.5, P=0.79).

Although infants exposed to repeat betamethasone compared to placebo did not differ in size at birth, gestation-specific standard deviation scores (Z-scores) for weight and head circumference at birth were slightly reduced (weight Z-score MD –0.13, 95% CI –0.26 to -0.004, P=0.04; head circumference Z-score MD –0.17, 95% CI –0.32 to -0.02, P=0.03). However, these differences were not seen by the time of primary hospital discharge. Perinatal mortality did not differ between groups.
Repeat betamethasone treatment was associated with a higher overall rate of maternal side effects (RR 2.04, 95% CI 1.27 to 3.29, P=0.003) and a slightly increased rate of caesarean delivery (RR1.13, 95% CI 1.02 to 1.24, P=0.01) but had no effect on maternal infectious morbidity.

Table 2.1 Primary respiratory outcomes for live born infants in ACTORDS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Repeat betamethasone n=567</th>
<th>Placebo n=577</th>
<th>Adjusted treatment effect (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory distress syndrome</td>
<td>186 (33)</td>
<td>239 (41)</td>
<td>0.82 (0.71, 0.95)</td>
<td>0.01</td>
</tr>
<tr>
<td>Severity of respiratory disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>229 (40)</td>
<td>191 (33)</td>
<td>1.23 (1.05, 1.44)</td>
<td>0.01</td>
</tr>
<tr>
<td>Mild</td>
<td>181 (32)</td>
<td>169 (29)</td>
<td>1.09 (0.91, 1.31)</td>
<td>0.34</td>
</tr>
<tr>
<td>Moderate</td>
<td>92 (16)</td>
<td>103 (18)</td>
<td>0.91 (0.70, 1.19)</td>
<td>0.49</td>
</tr>
<tr>
<td>Severe</td>
<td>65 (12)</td>
<td>114 (20)</td>
<td>0.60 (0.46, 0.79)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Oxygen</td>
<td>317 (56)</td>
<td>361 (63)</td>
<td>0.90 (0.81, 0.99)</td>
<td>0.03</td>
</tr>
<tr>
<td>Duration of oxygen—days†</td>
<td>1 (0, 7)</td>
<td>1 (0, 8)</td>
<td></td>
<td>0.24</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>167 (30)</td>
<td>204 (35)</td>
<td>0.87 (0.75, 1.01)</td>
<td>0.08</td>
</tr>
<tr>
<td>Duration of mechanical ventilation—days†</td>
<td>0 (0,1)</td>
<td>0 (0,2)</td>
<td></td>
<td>0.01</td>
</tr>
</tbody>
</table>

ACTORDS, Australasian Collaborative Trial of Repeat Doses of corticoSteroids. CI, confidence interval. Data are number (percent) or †median (interquartile range). Adapted from Crowther et al (2006). See text for definitions of respiratory disease severity and statistical adjustments.

2.2.1 Preschool follow-up

Ninety-five percent of infants alive at randomisation survived to primary discharge from hospital. Two infants in the repeat betamethasone group and three infants in the placebo group died after discharge. Surviving infants were followed up at 12 months’ corrected age by a parental postal questionnaire (Crowther, Hiller, 2006), and at two years’ corrected age (Crowther 2007) with a formal paediatric examination and psychological assessment using the Bayleys Scales of Infant, second edition (Bayley 1993). All caregivers and assessors remained blinded as to treatment group allocation.

At 12 months’ corrected age, treatment groups did not differ in body size or in any of the developmental domains of the Ages and Stages Questionnaire-2 (Bricker 1999). However, children exposed to repeat betamethasone compared to placebo had slightly higher scores for two out of nine measures of temperament on the Toddler Temperament Scale (Sewell 1988), namely, intensity (MD 0.1, P=0.03) and negative mood (MD 0.1, P=0.03).
At two years’ corrected age, 96% of surviving children were assessed by a paediatrician, 92% underwent psychometric testing, and 98% of caregivers completed questionnaires on their child’s health, behaviour, and emotions. Children treated with repeat betamethasone compared to placebo did not differ in the primary outcome of survival free of major neurosensory disability (RR 1.04, 95% CI 0.98 to 1.10, P=0.20). This was defined as a developmental index score equal to or greater than two standard deviations below the mean, being ambulant, and the absence of cerebral palsy, blindness, or deafness. There were also no differences between groups in the rates of combined neurosensory disability, individual disabilities or developmental delay, the severity of neurosensory disability, developmental index scores, body size, rates of health service utilisation or asthma, and mean behavioural scores. However, children in the repeat betamethasone group were nearly twice as likely to score within the clinical range (>97.5th percentile) for attention problems compared to those in the placebo group (RR 1.87, 95% CI 1.03 to 3.42, P=0.04).

### 2.3 ACTORDS early school-age follow-up study

#### 2.3.1 Aims and overview

At 6 to 8 years’ corrected age, children throughout Australia and New Zealand underwent a common developmental and general health assessment that consisted of a paediatric neurological examination, detailed psychometric and motor function testing, auxology, spirometry, and parental and teacher questionnaires regarding emotions, behaviour, learning and general health. This is referred to as the neurological and health study (Table 2.2). Its aim was to determine whether there are additional health gains, without adverse effects, at 6 to 8 years’ corrected age following exposure to repeat doses of antenatal betamethasone. The primary outcome was survival free of neurosensory disability, defined as the absence of cerebral palsy, deafness, or blindness, and an intelligence quotient of greater than or equal to one standard deviation below the population mean. These results will be analysed for the whole ACTORDS cohort and so are not reported in this thesis.

In addition, participating New Zealand children who assented to further testing underwent a more detailed investigation of cardiovascular, metabolic and endocrine function, termed the physiological study. The aim of this study, which was conducted in parallel to the neurological and health study, was to investigate whether exposure to repeat doses of antenatal betamethasone has any long-term adverse effects at 6 to 8 years’ corrected age on key physiological systems that determine cardiovascular and metabolic health in later life. This included assessment of glucose and insulin metabolism, ambulatory blood pressure,
growth and body composition, cortisol concentrations, and renal function. For logistical reasons, children in the Australian arm of the early school-age follow-up study did not undergo the additional physiological tests.

This thesis is based on data from the physiological study and auxology measurements from the New Zealand neurological and health study. The author completed all paediatric medical examinations in the New Zealand neurological health study, including auxology, and performed all physiological investigations, except for dual-energy X-ray absorptiometry on children outside the Auckland region.

2.3.2 Physiological Study hypothesis
The hypothesis of the physiological study was that administration of repeat doses of antenatal betamethasone to women at risk of preterm birth at <32 weeks’ gestation 7 or more days after an initial course of antenatal glucocorticoids does not alter the following outcomes in their children at 6 to 8 years’ corrected age:

- Body size and growth
- Body composition (fat and lean tissue mass, and bone mineral content), as assessed by total body dual energy X-ray absorptiometry
- Insulin sensitivity and secretion, as assessed by minimal model analysis of a frequently sampled intravenous glucose tolerance test
- 24-hour ambulatory blood pressure
- Hypothalamic-pituitary-adrenal function, as assessed by basal diurnal salivary cortisol concentrations and plasma cortisol response to insulin challenge during the modified intravenous glucose tolerance test
- Renal function, as assessed by estimated glomerular filtration rate
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuromotor and sensory function</td>
<td>Neurological examination and gross motor function grade (1997)</td>
</tr>
<tr>
<td></td>
<td>Visual acuity and otological examination</td>
</tr>
<tr>
<td></td>
<td>Movement Assessment Battery for Children (Movement ABC-2) (Henderson 2007)</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Average of three seated oscillometric recordings</td>
</tr>
<tr>
<td>Lung function</td>
<td>Portable flow spirometry (EasyOne 2001, NDD Technologies, Zurich, Switzerland)</td>
</tr>
<tr>
<td>General health</td>
<td>Medical history by paediatrician</td>
</tr>
<tr>
<td></td>
<td>Australian Authorised Adaptation of the Child Health Questionnaire to assess psychosocial health, physical health and well-being (Waters 2000)</td>
</tr>
<tr>
<td></td>
<td>Multi-attribute Health Status questionnaire to assess health-related quality of life (Saigal 1994)</td>
</tr>
<tr>
<td>General cognitive ability</td>
<td>Wechsler Abbreviated Scale of Intelligence (1999)</td>
</tr>
<tr>
<td>Attention and executive function</td>
<td>Subtests from the Test of Everyday Attention for Children (TEACh) including Sky Search (selective attention), Score (sustained attention), Sky Search Dual Task (divided attention) and Creature Counting (shifting attention) (Manly 1999)</td>
</tr>
<tr>
<td></td>
<td>Fruit Stroop task to assess impulse control (Archibald 1999)</td>
</tr>
<tr>
<td></td>
<td>Rey Complex Figure to assess spatial organisation and strategic decision-making (Rey 1993)</td>
</tr>
<tr>
<td></td>
<td>Behaviour Rating Inventory of Executive Function (BRIEF) questionnaire for parents and teachers to assess behavioural manifestations of inattention and executive function (Gioia 2000)</td>
</tr>
<tr>
<td>Memory and learning</td>
<td>Rey Auditory Verbal Learning Test (RAVLT) to assess verbal memory and learning, including proactive inhibition, retention, encoding versus retrieval, and subjective organisation (Rey 1964)</td>
</tr>
<tr>
<td>Visual-perceptual skills</td>
<td>Visual-spatial relations, visual figure ground, and visual closure subtests of the Test of Visual-Perceptual Skills, 3rd edition (TVPS-3), to assess visual-perceptual skills (Martin 2006)</td>
</tr>
<tr>
<td>Educational progress</td>
<td>Word reading, spelling, and arithmetic subtests of the Wide Range Achievement Test (WRAT-4) (Wilkinson 2005)</td>
</tr>
<tr>
<td>Behaviour</td>
<td>Strengths and Difficulties Questionnaire (SDQ) for parents and teachers to assess overall behaviour problems, emotional symptoms, hyperactivity / inattention, peer relationship problems, and pro-social behaviour (Goodman 1997)</td>
</tr>
<tr>
<td></td>
<td>Conners’ Rating Scales (2000)</td>
</tr>
</tbody>
</table>
2.3.3 Inclusion and exclusion criteria
All children whose mothers took part in the ACTORDS trial and who were residing in New Zealand were eligible for inclusion in the New Zealand early school-age follow-up study from the beginning of their 6th year of corrected age. We aimed to assess children before the beginning of their 9th year of corrected age, although children who were seen after this time were not excluded. There were no exclusion criteria for the neurological and health study. However, children with known severe neurosensory disability did not undergo formal developmental testing. These children were also excluded from the physiological study because it was considered unethical to subject them to invasive procedures without being able to determine their assent to testing. Triplets were excluded from the frequently sampled intravenous glucose tolerance test for logistical reasons, as were children with known diabetes mellitus. Blood samples for plasma cortisol and creatinine were taken only from children who underwent the frequently sampled intravenous glucose tolerance test. All other children were eligible for the physiological study, including those with chronic medical conditions. Children were encouraged to complete all of the physiological tests, but were not excluded from the physiological study if consent or assent was given for only some of the tests.

2.3.4 Contact Tracing
Since commencement of the ACTORDS trial, a database of participant contact details has been maintained at the main study centre at The University of Adelaide. At the two-year follow-up, caregivers were asked to provide contact details for two family members or close friends, usually grandparents. In subsequent years, regular contact was maintained with participants by means of newsletters and cards, and they were encouraged to forward any change of address to the main study centre.

For the present follow-up, the first step in contact tracing was to send an information sheet to the most recently recorded residential address, which was followed by a phone call from the author or study research nurse within two weeks. If contact tracing was unsuccessful then secondary contacts were phoned and asked to provide a current address or phone number for the participant. Most children were successfully traced by these means. Some families were traced through a search of the national telephone directory.

2.3.5 Ethical approval and informed consent
Ethical approval for the New Zealand early school-age follow-up study was provided by the Multi-region Ethics Committee, Ministry of Health, Wellington (reference number
Before children were recruited to the follow-up study, caregivers were provided with written information about the assessments, including potential benefits and risks. They were also given the opportunity to discuss the study with a paediatrician or research nurse prior to attending for follow-up. The child’s legal guardian provided written consent for each of the physiological investigations and assent was sought from the child before each procedure.

2.3.6 Sample size and power calculations
The sample size for this study was limited by the number of mothers and babies recruited to the ACTORDS trial. Power calculations for the physiological study were based on the number of children alive and residing in New Zealand at two years’ corrected age (total 308). For continuous and normally distributed physiological outcome variables, it was estimated that with 90% follow-up the New Zealand cohort would have 80% power to detect differences as small as 0.32 of a standard deviation (two-sided significance level of 5%). With 70% follow-up it would have 80% power to detect differences of 0.36 of a standard deviation, and with 50% follow-up (sample size 171) differences of 0.43 of a standard deviation. For logarithmically transformed data, these differences equated to an increase in the geometric mean of 38%, 43%, and 53%, or a decrease of 27%, 30% and 35%, respectively.

2.3.7 Auxology and pubertal status
Height or stature was measured to the nearest 1 mm by fixed wall (Holtain, Crymych, UK) or portable spirit level (Harpenden, London, England) stadiometer. A non-stretch technique was used with the head positioned in the Frankfort plane (Norton 1996). Sitting height was measured using a wooden measuring box (40 cm high and 30 cm deep) placed directly beneath the stadiometer. Children were seated on the box so that their back was pressed firmly against the wall. Sitting height (upper segment length) was calculated as the stadiometer reading less 40 cm, and the subischial leg length (lower segment length) was calculated as stature less sitting height. Weight was measured to the nearest 100 g using a digital bathroom scale with children wearing light clothing. Head circumference was measured to the nearest 1 mm by placing a paper tape measure above the supra-orbital ridges at the most prominent part of the frontal bulge, and over the occiput. The largest of three measurements was recorded. Arm circumference was measured to the nearest 1 mm at the level of the mid-acromiale-radiale with the arm in a relaxed and extended position by the side of the body (Norton 1996).
Standard deviation scores specific for sex and corrected age were calculated for stature, sitting height, subischial leg length, weight, body mass index (kg/m\(^2\)), and head circumference using the British Growth Reference 1990, revised 1996 (Cole, Freeman, 1995; Cole 1998; Dangour 2002; Freeman 1995). This was performed in LMSGrowth software (v2.29, 2010, Tim Cole and Huiqi Pan).

Pubertal status was determined by breast examination in girls and estimation of testicular size via a Prader orchidometer in boys, using the classification of Tanner and Marshall (1969, 1970). Puberty onset was defined as breast stage \(\geq 2\) in girls or testicular volume \(\geq 4\) mL in boys.

### 2.3.8 Total body dual energy X-ray absorptiometry

Body composition and bone mass were measured by total body dual energy X-ray absorptiometry (DXA). Because DXA results can vary between different manufacturers and models, this study was restricted to a single type of machine, the Lunar Prodigy (GE Healthcare, Wisconsin, USA). This was available in Auckland (Maurice and Nessie Paykel Clinical Research Unit, Liggins Institute) and Christchurch (St Georges Radiology, Merivale). All children attending these centres were eligible for body composition assessment.

The Lunar Prodigy was introduced in 2000 and utilises a narrow fan-beam (4.5°) parallel to the longitudinal axis of the body (Mazess 2000). The effective radiation dose of a total body scan in children has not been measured for the Lunar Prodigy. However, studies using a variety of other scanners have shown the radiation dose is very low and less than or equivalent to the daily background environmental effective radiation dose of approximately 5 \(\mu\)Sv (Bezakova 1997; Blake 2006; Njeh 1997).

#### 2.3.8.1 Background

##### 2.3.8.1.1 Body composition model

The DXA approach to body composition assumes that the body contains three components that are distinguishable by their photon attenuation properties: bone mineral, fat, and residual or lean soft tissue (water, proteins, glycogen, and minerals). DXA scanners generate photons from an X-ray tube at two designated energies. The Prodigy utilises an 80 kVp X-ray source below the patient bed in conjunction with a cerium K-edge filter to produce 38 and 70 keV congruent X-ray beam energies. The low-energy photons penetrate only the soft tissue surrounding bone, whereas the high-energy photons penetrate both soft
tissue and bone. A detector located above the patient measures the photons exiting from the scan area and determines the ratio of high and low energy photons, also known as the R value. This ratio reflects the relative attenuation of the two photons through different tissues. The R value is converted to body composition by computer using complex mathematical algorithms derived from various calibration materials (Pietrobelli 1996). In Lunar densitometers bone mineral content is based on the ashing of bone samples containing marrow fat (Mazess 1991).

The first stage of the computer analysis consists of dividing the body into a series of pixels and computing the R value for each pixel. Pixels that contain bone are identified by their high R value and image processing methods are used to delineate the skeletal boundaries. In pixels where bone is not present, suitable calibration allows fat and lean fractions of soft tissue to be resolved. Lean mass includes extracellular fluid, muscle, organs and connective tissue. The soft-tissue composition of bone-containing pixels is estimated based on the composition of adjacent non-bone pixels. The pixels in the region of interest are combined to give the following results: bone mineral content (g), projected bone area (cm²), non-bone lean mass (kg), fat mass (kg), and total mass (kg).

Skeletal muscle is a large and physiologically important component of lean mass. Magnetic resonant imaging studies in children have shown that more than half of skeletal muscle is contained within the limbs (Kim 2006) and that approximately 75% of DXA determined lower limb lean mass represents skeletal muscle (Shih 2000). Strong correlations have been demonstrated between lean mass and muscle mass in the lower limbs (Bridge 2009; Shih 2000), and between combined upper and lower limb lean mass and total body muscle mass (Kim 2006). DXA limb lean mass explained more than 98% of the variance in total muscle mass among children and adolescents. Thus, DXA appendicular lean mass appears to be a good proxy for skeletal muscle mass.

DXA has high short-term precision, which has been demonstrated in children using the Lunar Prodigy (Margulies 2005). DXA is highly accurate for measurement of bone mineral content but is less accurate for soft tissue composition when compared to the gold-standard four-compartment method. Williams et al (2006) found that the Lunar Prodigy, on average, underestimated fat mass in lean boys by 0.5 kg but overestimated it in obese boys by 0.9 kg, though no significant bias was seen in girls. The overall limits of agreement between the two methods for fat mass and lean mass were ± 1 to 2 kg.
2.3.8.1.2 Interpretation of bone mineral content

The investigation of bone health in children involves assessment of bone mineral accretion and bone architecture, both of which contribute to overall bone strength. Bone mineral accretion is measured by bone mineral density, which is the amount of mineral (g) per unit volume of bone. Bone volume may be determined in several ways, resulting in different measures of density. Compartment density is the mineral content of a particular bone region divided by the volume of the trabecular or cortical compartments in that region. Total density is the mineral content divided by the volume enclosed by the periosteal bone surface. Bone strength is more closely correlated with compartment density than total density, especially in long bones (Leonard 2004). Bone geometry, particularly bone diameter and cortical thickness, is also an important determinant of bone strength.

DXA is the most commonly used technique for measurement of bone mineralisation because of availability, relatively low cost, and ease of use. However, an important limitation of densitometers is that bone size can be measured in only two dimensions (projected area). Thus, bone mineral content is assessed in relation to bone area rather than bone volume. In adults, DXA areal density provides a useful index of bone mineralisation because bone dimensions are stable. However, in children, changing bone geometry complicates the interpretation of areal density. For example, the areal density of cancellous bone increases as cubic dimensions increase, even if there is no change in volumetric density (Carter 1992). Conversely, the areal density of a hollow long bone decreases if the periosteal and endosteal diameters increase proportionately, even though bone strength is increased (Leonard 2004).

Despite these limitations, total body DXA provides useful insights into overall bone mass and structure. Because cortical bone accounts for 80% of skeletal bone mass, total body bone mineral content primarily reflects mineral accretion into cortical bone, the main function of which is mechanical strength. Sometimes total body mineral content is analysed minus the head component to provide a more direct assessment of the appendicular skeleton. Total body measurement of bone mineral is preferred to regional densitometry in children due to its greater precision (Margulies 2005).

The net effect of changing bone geometry with linear growth in childhood is that total body areal bone mineral density increases steadily with age until the late teens, even though volumetric density changes little until late puberty (Neu 2001). Therefore, paediatric areal bone mineral density results are commonly presented as standard scores for
age and sex. However, the biomechanical significance of bone mineral content relative to bone area across the whole skeleton is uncertain. For example, when total body DXA was compared to tibial peripheral quantitative computed tomography, which can measure compartmental density, areal density for age was only weakly correlated with an index of bone strength. Bone area for height and bone mineral content for height were more closely correlated with bone strength (Leonard 2004). Although lean tissue mass, which reflects muscle mass, has been proposed as an important determinant of bone development (Hogler 2003), neither total body bone mineral content for lean tissue mass nor bone area for lean tissue mass were correlated with bone strength. The authors concluded that total body bone area and bone mineral content adjusted for height were the best indicators of bone health, at least for cortical bone (Leonard 2004).

2.3.8.2 Scan protocol

One operator in each centre carried out total body scans using an identical protocol. Children were scanned in light clothing with shoes and metal objects removed. They were positioned supine with legs together and arms pronated. The scan mode, which determines scan depth, was set automatically by the scanner computer depending on the thickness of the child, as estimated from their weight and height. Both scanners were operated by EnCore software using the paediatric module (GE Healthcare, Wisconsin, USA). Scans for the first 24 children were acquired using either version 8.1 or 8.8, after which both centres upgraded to version 11.4. Image data for all children were processed using version 11.4. Scan reports provided total and regional bone mineral content, bone area, fat mass, and non-bone lean mass. Body regions were determined using computer generated and manually confirmed default lines on anterior view planogram as follows:

- The head cut was positioned immediately below the chin.
- The arm cuts were positioned as close to the body as possible, ensuring separation of upper limbs from the trunk, and passing through the shoulder joint.
- The spine cuts were positioned as close to the spine as possible without including the rib cage.
- The upper pelvis cut was positioned immediately above the iliac crests and the lateral pelvis cuts were positioned to pass through the femoral necks without touching the pelvis.
• The centre leg cut was positioned on the child’s midline to separate the legs and the outer leg cuts were positioned to separate the upper and lower limbs.

• The lower boundary of the android region was the pelvis cut. The upper boundary was set at 20% of the distance between the pelvis and neck cuts. The lateral boundaries were the arm cuts.

• The upper boundary of the gynoid region was formed by a line below the pelvic cut at a distance equal to 1.5 times the height of the android region. The height of the gynoid region was set at two times the height of the android region. The lateral boundaries were the outer legs.

2.3.8.3 Calibration
Daily quality assurance procedures were performed using the manufacturer’s block phantom, containing three bone and three soft tissue simulation standards. Stable calibration was maintained throughout the study period.

2.3.8.4 Data analysis
For parameters relating to bone mass, sex-specific standard deviation scores for healthy Australian children were obtained courtesy of Julie Briody at the Institute of Endocrinology and Diabetes, Department of Nuclear Medicine, The Children's Hospital at Westmead, Sydney, Australia (Hogler 2003). These included:

• Areal bone mineral density for age
• Areal bone mineral density for height
• Bone area for height
• Bone mineral content for age
• Bone mineral content for height
• Bone mineral content for lean tissue mass

2.3.9 Glucose and insulin metabolism
Glucose and insulin metabolism was assessed using Bergman’s minimal model with a 90-minute insulin-modified frequently sampled intravenous glucose tolerance test, which has been validated in children (Cutfield 1990). The minimal model is recommended by the
American Diabetes Association as one of two gold standard measures of insulin sensitivity (1998).

2.3.9.1 Minimal model background

Insulin sensitivity is a measure of the activity of insulin on whole-body glucose metabolism, and it can be assessed in several ways. The most direct measure of whole-body insulin sensitivity is the hyperinsulinaemic euglycaemic clamp. This involves infusion of insulin at a constant rate while glucose is infused at a variable rate so as to maintain euglycemia. (DeFronzo 1979). At steady state, assuming suppression of hepatic glucose output, the glucose infusion rate (M value) reflects the net effect of insulin on glucose uptake and utilisation, principally in skeletal muscle. Because of its complexity, the clamp is not suitable for large cohorts and is difficult to perform in children. It also may not accurately reflect insulin action under dynamic physiological conditions (Muniyappa 2008). An alternative method for the measurement of insulin sensitivity is Bergman’s minimal model, which uses mathematical modelling to characterise the glucose and insulin dynamics observed during a frequently sampled intravenous glucose tolerance test (Bergman 1979; Bergman 1981; Boston 2003; Pacini 1986). An important advantage of this approach is that the closed-loop relationship between glucose and insulin is maintained, thereby allowing investigation of several different aspects of glucose homeostasis during a single test, including insulin sensitivity, insulin secretion, glucose effectiveness, and glucose tolerance.

Minimal modelling refers to a rigorous strategy for selecting models from among a series of rival models of variable complexity, and it is particularly suitable for studying homeostatic physiological systems (Pacini 1986). The development of Bergman’s model has been described in detail (Bergman 1979). His approach was to uncouple the glucose and insulin feedback loop into two subsystems: a) the effect of insulin to accelerate glucose uptake; and b) the effect of glucose to enhance insulin secretion from the pancreas. Modelling the pancreatic islet cells proved to be highly complex and beta cell function was not amenable to representation in simple mathematical terms. However, it was possible to model the effect of secreted insulin on the disappearance of glucose as an open-loop system and gain substantial information regarding blood glucose regulation, even though the closed-loop relationship between glucose and insulin remains intact during the test (Bergman 2005).
In order for the model to accurately reflect the effect of secreted insulin on glucose dynamics it has to account for two additional factors: a) that glucose mediates its own disposal in proportion to its concentration in plasma, also known as glucose effectiveness (Sg) (Ader 1985; Bergman 1989; Best 1981; Galante 1995); and b) that insulin exerts its effects in a compartment remote from blood, namely, the interstitium (Bergman 1990). Therefore, Bergman’s minimal model consists of two differential equations: one that relates glucose disappearance to glucose effect, catalysed by insulin (1); and a second that describes the kinetics of insulin movement from blood to the active compartment (2).

\[
\text{(1) Glucose restoration rate} = -([P1 + \text{remote insulin effect}] \times \text{plasma glucose concentration}) + P4
\]

\[
\text{(2) Rate of increase in remote insulin effect} = (P2 \times \text{elevation of plasma insulin above basal concentration}) - (P3 \times \text{remote insulin effect})
\]

\[
P1 = \text{glucose effectiveness (Sg)} \\
P2 = \text{rate of movement of circulating insulin to interstitial space} \\
P3 = \text{rate of removal of insulin from interstitial space} \\
P4 = \text{glucose production at basal insulin}
\]

To carry out minimal model analysis, the insulin and glucose time curves obtained from a frequently sampled intravenous glucose tolerance test are submitted to a computer programme (MinMod), which automatically adjusts the values of the parameters until the glucose pattern is fitted, based on known insulin, using a least squares method (Boston 2003; Pacini 1986). The variables of clinical interest are derived from the fitted parameters; glucose effectiveness (Sg) (min\(^{-1}\)) equals P1, whereas the insulin sensitivity index (Si) (min\(^{-1}\).mIU\(^{-1}\).L\(^{-1}\)) is the ratio of parameter P3 to P2. The insulin sensitivity index expresses the increase in the fractional disappearance of blood glucose per unit increase of blood insulin and reflects the action of insulin on both peripheral glucose utilisation, especially skeletal muscle, and the suppression of hepatic glucose output. It is independent of body size and is largely independent of the levels of glycaemia and insulinaemia at which it is determined (Pacini 1986). The computer programme also calculates the acute insulin response to glucose (AIRg) (mIU.L\(^{-1}\).min), which is defined as the area under the plasma insulin curve from 0 to 10 min and reflects first phase pancreatic insulin secretion.

The frequently sampled intravenous glucose tolerance test has been standardised based on a dextrose dose of 0.3 g/kg (Bergman 1979). This results in rapid hyperglycaemia and stimulation of endogenous insulin release. The test is usually modified to achieve a second insulin response after 20 min. This is because the minimal model requires a substantial
effect of insulin after the post-glucose mixing phase (0 to 8 min) to estimate insulin sensitivity precisely (Yang 1987). This is achieved by administration of either intravenous tolbutamide, which stimulates endogenous insulin secretion, or a small intravenous dose of exogenous short acting insulin (usual adult dose 0.03 IU/kg) (Saad, Steil, Kades, 1997). The insulin-modified protocol is now more common because tolbutamide has become difficult to obtain and because the addition of exogenous insulin improves the assessment of diabetic patients (Welch 1990). In the modified test, plasma glucose concentration typically returns to baseline after about 50 min, undershoots, and then returns to baseline again at around 90 to 120 min. In adults, the total duration of the test is usually 3 hours, during which 30 paired glucose and insulin samples are collected. However, a shortened 90-min, 27 sample protocol has been validated in children (Cutfield 1990) and was used in this study. Insulin was administered by intravenous bolus, as this has been shown to be equivalent to a short infusion (Saad, Steil, Riad-Gabriel, 1997).

The minimal model estimate of Si has been extensively validated against the hyperinsulinaemic euglycaemic clamp (Ader 1985; Beard 1986; Bergman 1987; Henderson 2011; Saad 1994; Saad, Steil, Kades, 1997). Bergman et al (1987) found that in healthy subjects tolbutamide-modified model Si was highly correlated with clamp Si ($r=0.89$, $P<0.001$), with a slope not different from unity and an intercept not different from zero. Furthermore, both measures were very similar when converted to the same units, suggesting that they quantify the same physiological process (Bergman 1987). Similar results were observed when repeated a decade later (Saad, Steil, Kades, 1997). Tolbutamide-modified model Si has good intra-subject reproducibility, with a coefficient of variation on repeated testing of between 14% and 17% (Abbate 1993; Ferrari 1991).

Insulin-modified and tolbutamide-modified model Si are highly correlated in non-diabetic subjects, although insulin-modified Si is 16% to 29% lower than tolbutamide Si (Saad, Steil, Kades, 1997; Welch 1990). This difference may be due to the more prolonged time course of insulin action after tolbutamide than with an insulin bolus (Saad, Steil, Kades, 1997). When insulin and tolbutamide-modified model Si were compared to euglycaemic clamp Si in the same individuals, similar correlations were observed (insulin modified model Si versus clamp Si $r=0.70$, tolbutamide modified model Si versus clamp Si $r=0.71$, $P< 0.0001$ for both) (Saad, Steil, Kades, 1997). However, insulin-modified model Si was 44% lower, on average, than clamp Si. Thus, it is likely that insulin-modified model Si underestimates insulin sensitivity and hence it is regarded as an index rather than a direct measure. The correlation between insulin-modified model Si and clamp Si is weaker in
diabetic subjects (Saad 1994). The reproducibility of insulin-modified model Si is not known, but it is expected to be similar to that of the tolbutamide-modified test (Saad 1994).

Another parameter that is obtained from the minimal model is the dimensionless disposition index, which is the product of Si and AIRg. In normal subjects, a decrease in insulin sensitivity leads to a compensatory increase in early beta cell insulin secretion, mediated by glucose feedback and secretion of intestinal hormones, such as glucagon-like peptide-1 (Bergman 2002). Cross-sectionally, the relationship between Si and AIRg in the minimal model is hyperbolic, such that their product is constant for a given level of glucose tolerance (Kahn 1993). In subjects with reduced insulin sensitivity, the development of impaired glucose tolerance is characterised by inadequate compensatory insulin secretion for the degree of insulin resistance. This results in a declining disposition index, even though changes in insulin secretion may be minimal early on. Thus, the disposition index reflects an individual’s overall capacity to clear a glucose load and is more sensitive for beta cell dysfunction than AIRg alone (Bergman 2002). Individuals with low DI are at high risk for subsequent development of type 2 diabetes (Lorenzo 2010; Weyer 1999).

A low Sg is also an independent risk factor for future diabetes (Goldfine 2003; Lorenzo 2010; Martin 1992), although its longitudinal relationship with Si in the natural course of diabetes is not yet clear. However, unlike Si it does not appear to be modifiable by exercise (Kahn 1990) or weight loss (Escalante-Pulido 2003).

2.3.9.2 Frequently sampled intravenous glucose tolerance test

Children attended the clinic in the morning after an overnight fast, commencing at 22:00. Following application of topical anaesthetic (EMLA cream, AstraZeneca), a single 22-gauge cannula was inserted into an antecubital fossa vein and connected to a 3-way tap. Catheter patency was maintained by a slow infusion of heparinised 0.9% saline (1 U/mL). Three blood samples for fasting glucose and insulin were collected at times –20, -10 and –1 min via the 3-way tap. Intravenous glucose 0.3 g/kg, as 25% dextrose, was given at time 0 and short-acting insulin 0.015 IU/kg (Actrapid, NovoNordisk) was given at time 20 min, both by slow push over 1 min. Although the insulin dose was lower that the standard adult dose of 0.03 IU/kg, previous experience in our institution has shown that this achieves satisfactory minimal modelling in children and that higher doses risk causing severe hypoglycaemia. A 10 mL flush of 0.9% saline was administered after glucose and insulin to prevent contamination during blood collection. Blood samples (1.5 to 3 mL) for paired
glucose and insulin measurements were taken at time 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80 and 90 min. Blood was placed in 4 mL heparinised plastic tubes (Vacutainer, BD Diagnostics) and stored on ice. At the completion of the test tubes were centrifuged at the bedside (1300 x g for 12 minutes at room temperature, Heraeus Labofuge 200, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and plasma was transferred to microtubes (1.5 mL polypropylene, Sarstedt, Numbrecht, Germany) in divided aliquots for frozen storage at -20 °C.

2.3.9.3 Glucose and insulin assays
Microtubes of plasma were thawed on ice, vortexed, and then centrifuged at 1100 x g for 3 min at 4 °C to remove particulate matter. A 150 µL volume of plasma was then transferred to an appropriate test tube.

Plasma insulin concentration was measured using a microparticle enzyme immunoassay on an AxSYM analyser (Abbott, Abbot Park, Illinois, USA). This assay has a between-day coefficient of variation of between 4% to 5%, and a lower limit of detection of 1 mU/L (manufacturer’s data). The assay is specific for human insulin with no detectable cross-reactivity with C-peptide or glucagon. The cross-reactivity with proinsulin is low at 0.016%. A daily calibration check was performed using Abbott insulin control solutions (low, medium, high). If the plasma insulin concentration exceeded the upper limit of the calibration curve (300 mU/L), the sample was repeated after a 1:5 dilution with a buffer solution (0.01 M phosphate buffered saline pH 7.6, 0.37% sodium ethylenediaminetetraacetic acid, and 0.5% bovine serum albumin).

Plasma glucose concentration was measured using a Roche glucose oxidase colorimetric assay (Indianapolis, Indiana, USA) on a Hitachi 902 auto-analyser (Ibaraki, Japan). This assay has a between day coefficient of variation of 1.8% for human serum, and a lower limit of detection of 0.11 mmol/L (manufacturer’s data). A daily calibration check was performed using Roche control solutions (precinorm U, precipath U).

2.3.9.4 Data analysis
Minimal model analysis was performed using Millenium MinMod software (version 6.02) (Boston 2003). The mean glucose and insulin concentration of the three baseline samples was submitted for the time 0 and 180 min values. Glucose samples from 0 to 8 min were assigned a weight of zero, as per the default settings of the programme, to allow for mixing effects. Glucose samples that deviated markedly from the fitted line were also weighted out.
of the model. Children were excluded from analysis if their test was terminated before 70 min.

In addition to minimal model analysis, glucose tolerance was estimated by the glucose disappearance constant (Kg) (min\(^{-1}\)). This was calculated as the slope of the linear least-square regression line to the natural logarithm of the glucose concentration versus time from 10 to 19 minutes after the glucose bolus. Kg is determined by both DI and Sg, and values >1.5 \times 10^{-2} \text{ min}^{-1} represent normal glucose tolerance (Bergman 1981).

### 2.3.10 Ambulatory blood pressure
Ambulatory blood pressure monitoring has a number of advantages over casual clinic blood pressure measurement including detection of ‘white coat effect’, which is common in children, and provision of additional information on circadian rhythm and blood pressure load. Ambulatory blood pressure is more predictive of sustained hypertension and cardiovascular morbidity and mortality than clinic blood pressure (Pickering 2006).

Ambulatory blood pressure monitoring was performed using Spacelabs 90217 monitors (Issaquah, Washington, USA) with Spacelabs reporting software (version 2.0.9). The Spacelabs 90217 is an oscillometric device that operates in a similar manner to other oscillometric blood pressure machines, except that inflation and pressure detection occur via a single tube. Four monitors were used on a rotational basis throughout the course of this study.

#### 2.3.10.1 Monitoring protocol
Children were instructed to wear the monitor for any 24-hour period on a routine day, removing it only for washing and dressing. Cuffs were fitted on the non-dominant arm, with cuff size determined by mid-arm circumference (child 12 to 20 cm; small adult 17 to 26 cm; large adult 24 to 32 cm). If a child was between cuff sizes then the larger was used. Caregivers were given a demonstration on the use of the equipment and written instructions were provided. A diary card was used to record awake and sleep periods and any nocturnal disturbance.

The monitors were programmed to inflate half hourly from 08:00 to 20:00, hourly from 20:00 to 00:00, half hourly from 00:00 to 0:300, and hourly from 0:300 to 8:00. Measurements were automatically excluded if any of the following occurred: systolic pressure <60 or >200 mmHg; diastolic pressure <40 or >150 mmHg; mean arterial pressure <40 or >200 mmHg; pulse pressure difference <20 or >150 mmHg; or heart rate
<40 or >200 beats per minute. When interference or error in the reading occurred, the process was automatically repeated after 2 min while retaining the pre-established sequence.

2.3.10.2 Calibration
A manual calibration check was performed every six months by connecting the cuff tubing to a mercury manometer via a T-piece. A standard adult cuff was then inflated to maximum pressure around a solid cylinder and then deflated. Monitors were deemed accurate if the pressure readings were within 2 mmHg of the mercury manometer throughout the deflation cycle. All monitors had stable calibration throughout the study period.

2.3.10.3 Data analysis
Children who had fewer than seven daytime or fewer than seven night-time measurements were excluded from analysis. Ambulatory monitoring data were processed in four ways. First, mean systolic and diastolic pressure and heart rate were calculated for the entire monitoring period (designated as 24-hour value). Second, mean systolic and diastolic pressure and heart rate were calculated for daytime and night-time periods according to the child’s own sleep-wake cycle. From these values, percentage diurnal drop in blood pressure was determined. Third, standard deviation scores (Z-scores) for sex and height were calculated using the central European reference data (Soergel 1997; Wuhl 2002) with the LMS method (Cole 1992). LMS values were interpolated for height from 115 to 185 cm. Children with height <115 cm had standard deviation scores calculated using reference data at 115 cm. To be compatible with the reference data, this analysis was based on fixed-clock intervals from 8:00 to 20:00 and 00:00 to 06:00, with exclusion of recordings from the intervening transition periods. Fourth, daytime and night-time blood pressure load (percentage of elevated recordings), based on the child’s own diurnal pattern, was calculated using the European reference data 95th and 90th percentiles for sex and height.

2.3.11 Renal function
For children who underwent the intravenous glucose tolerance test, plasma creatinine concentration was measured in the first fasting blood sample using the Roche creatinine plus assay (Indianapolis, Indiana, USA) on a Hitachi 902 auto-analyser (Ibaraki, Japan). This assay has a between day coefficient of variation of 2.1% for human serum, and a lower limit of detection of 0.27 μmol/L (manufacturer’s data). A daily calibration check was performed using Roche control solutions (precinorm U, precipath U).
Glomerular filtration rate (mL/min/1.73m²) was estimated from height using the following prediction equation, which was derived from measured glomerular filtration rate using the iohexal plasma disappearance technique (Schwartz 1976; Schwartz 2009):

\[
\text{Estimated glomerular filtration rate} = \frac{(k \times H)}{PCr}
\]

\[
H = \text{height in cm}
\]

\[
PCr = \text{plasma creatinine concentration in mg/dL}
\]

\[
k = 0.41
\]

2.3.12 Salivary cortisol

Basal HPA axis function was assessed by measuring salivary cortisol with timed, home-based diurnal sampling. Cortisol concentrations were determined using an in-house tandem mass spectrometry assay that was developed for this study.

2.3.12.1 Background

Cortisol, the main glucocorticoid in humans, is released from the adrenal cortex into the bloodstream under the influence of pituitary ACTH. ACTH secretion is in turn regulated by hypothalamic corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) (Papadimitriou 2009). CRH secreting cells are responsive to the actions of various neurotransmitters and higher brain centres, as well as negative feedback exerted by cortisol. In normal adults, ACTH is secreted in frequent pulses during the second half of nocturnal sleep, resulting in an early morning cortisol rise. Basal levels then decline steadily throughout the day, although under stressful conditions large increases in cortisol can occur (Kirschbaum 1989). In addition to this circadian rhythm, most adults have a pronounced release of cortisol within the first 30 min after waking, which increases peak salivary cortisol concentration by 50% to 100% (Dockray 2007; Federenko 2004; Pruessner 1997). The cortisol awakening response represents a discrete part of the diurnal cycle that has different regulatory influences to the basal circadian rhythm (Clow 2004; Edwards 2001; Wust 2000). The physiological role of the cortisol awakening response is unclear, although it may have a regulatory effect on the immune system (Hucklebridge 1999; Petrovsky 1997). The magnitude and dynamics of the response are relatively stable over weeks or months, but they can be altered by chronic stress (Schulz 1998). An important practical consequence of the cortisol awakening response is that morning cortisol concentrations show greater variability if samples are collected at a fixed time rather than in relation to the time of waking (Dockray 2007; Kudielka 2003).

Diurnal variation in cortisol secretion usually emerges at one to two months of age (Rivkees 2003; Spangler 1991), but it may take several months for the diurnal pattern to
become stable (de Weerth 2003; Kiess 1995). The circadian rhythm continues to develop throughout infancy and mature adult-like patterns generally do not emerge until daytime napping has ceased (Watamura 2004). A cortisol awakening response has been demonstrated in children, but it appears to be less marked than in adults and has not been found in all studies (O'Connor 2005; Pruessner 1997; Rosmalen 2005).

Measurement of cortisol in saliva has a number of advantages compared to plasma. First, unlike venesection, sampling is stress-free and can be performed in the home environment under normal conditions. Second, salivary cortisol reflects the unbound fraction of blood cortisol and hence is less affected by changes in protein binding and may be more closely related to biological activity (Levine 2007; Vining 1983). Most circulating cortisol (~95%) is bound to plasma proteins, especially cortisol binding globulin, and measurement of free plasma cortisol is difficult with standard techniques. Cortisol diffuses readily through the salivary epithelium into salivary fluid due to its low molecular size and lipophilic nature but only the unbound fraction can enter salivary glands (Dunn 1981; Kirschbaum 1989). Because cortisol protein binding in saliva is low (~10 to 15%) (Chu 1988), salivary cortisol concentrations are only 2% to 5% of total plasma cortisol concentrations. However, salivary cortisol may be up to 30% to 50% lower than free plasma cortisol concentration due to the conversion of cortisol to corticosterone by 11β-HSD2, which is highly expressed in salivary epithelium (Edwards 1988; Meulenberg 1987; Morineau 1997; Smith 1996).

Nevertheless, salivary cortisol and free plasma cortisol concentrations are highly correlated in both basal and dynamic conditions, and this relationship is linear throughout the range of free plasma cortisol concentrations (Meulenberg 1987; Peters, Walker, 1982; Umeda 1981; Vining 1983). Salivary cortisol concentration also shows modest correlation with total plasma cortisol concentration but the association is weaker at higher plasma concentrations (Goodyer 1996). This may be partly due to saturation of protein binding at high concentrations leading to a non-linear relationship between total plasma and salivary cortisol concentrations (Aardal 1995; Perogamvrosa 2009; Vining 1983). However, salivary and plasma cortisol diurnal patterns are synchronous (Dorn 2007).

Salivary cortisol concentrations are not appreciably affected by salivary flow rate but, like plasma cortisol, are increased after exercise, meals and stress (Kirschbaum 1989; Vining 1983). Blood contamination can also falsely elevate cortisol levels but this appears to be uncommon in children (Granger 2007; Groschl 2001). Another limitation of measuring salivary compared to plasma cortisol is greater variability on repeat testing (Reynolds
1998). However, the degree of concordance between salivary and free plasma cortisol concentration over time may be subject-specific (Levine 2007).

2.3.12.2 Saliva sampling protocol
To assess the cortisol circadian rhythm in detail, multiple timed saliva samples must be collected throughout a 24-hour period, including several morning samples to capture the cortisol awakening response. Sampling should be repeated over several days to determine an individual’s typical physiological state, as intra-subject variation can be considerable, especially in the afternoon and evening (Kirschbaum 1990). On the other hand, compliance with complex sampling protocols may be poor, which can invalidate results (Kudielka 2003). Given the target age of children in this study, and the fact that they were undergoing multiple investigations, it was decided that a simplified sampling schedule, involving morning and evening samples over three days, was more likely to be adhered to.

Children were asked to collect the morning saliva samples immediately on waking, before dressing or eating. This time was chosen to avoid any possible confounding effect with the cortisol awakening response, which could not be adequately characterised with the simplified sampling protocol. Evening samples were collected at 5 pm or before dinner, whichever occurred sooner. Children were requested to collect samples on typical days that did not involve any major sporting events or other stressful activity. School days were encouraged on the assumption that they would follow a more consistent routine. The three sampling days did not have to be consecutive, although children were asked to collect samples within a one-week period. Samples were not to be taken within half an hour of eating or brushing teeth, or on days when the child was unwell (Hanrahan 2006). Written instructions were provided to caregivers and colour-coded tubes, labelled with day and time, were used to facilitate compliance. Caregivers recorded date, time of waking and time of sample collection on a collection card.

Initially, it was envisaged that saliva would be collected using cotton Salivettes. However, validation studies (section 2.4.4) showed that hormone recovery from the swabs was not consistent. Therefore, saliva was collected by passive drool, using cut-off straws, directly into Salivette tubes that had had the swab insert removed (Sarstedt, Mawson Lakes, South Australia). Children were asked to collect at least 1 mL of saliva, the volume of which was marked on the side of the tube.

Caregivers were instructed to place samples in a freezer immediately after collection. Once completed, samples were returned by post using a pre-paid bubble pack (Clements 1998).
The postmark was used to determine the number of days that samples were in transit. On arrival, the Salivette tubes were transferred to frozen storage at –20 °C until assayed.

### 2.3.12.3 Salivary cortisol assay protocol

Salivary cortisol concentration was measured by liquid chromatography and tandem mass spectrometry, using cortisol-D₄ as an internal standard (Pointe-Claire, Quebec, Canada). Salivettes were thawed on ice, vortexed, and centrifuged at 3000 x g for 10 min at 4°C. A 300 µL volume of saliva was combined in a 6 mL glass tube with 100 µL of internal standard solution (cortisol-D₄ 27.3 nmol/L). Cortisol was extracted by adding 1.5 mL of ethyl acetate to the test tube, followed by vortexing, and centrifugation for 5 min (Hetovac VR-1, Heto Lab Equipment, Denmark). The organic supernatant was then transferred to a new glass tube and the solvent was evaporated using a vacuum concentrator (Hetovac VR-1 connected to a Heto cooling trap CT110). The dried sample was reconstituted in 60 µL of chromatographic mobile phase and transferred to an autosampler microvial. Quality control samples at three different concentrations (low, medium, high) were included at four different positions within each run. Seven cortisol standards (0.14, 0.28, 0.79, 2.76, 8.28, 27.59, 82.76 nmol/L) were prepared by serial dilution of cortisol stock solution in methanol (Sigma, St Louis, USA). Quality control samples and standards were prepared and extracted as per the saliva samples.

A 15 µL sample volume was injected onto a high pressure liquid chromatography (HPLC) mass spectrometer system consisting of a Waters Alliance 2690 Separations Module followed by an Ion Max atmospheric pressure chemical ionisation (APCI) source on a Finnigan TSQ Quantum Ultra AM triple-quadrapole mass spectrometer, controlled by Finnigan Xcaliber software (Thermo Electron Corp., San Jose, California, USA). Chromatography was carried out in reversed-phase on a Luna 3µC18(2) 100A 100 x 3 mm column (Phenomenex, Auckland, New Zealand) using an isocratic mobile phase (65% methanol, 35% water) at a flow rate of 0.3 mL/min and a temperature of 40 °C. The retention time for cortisol was 3.52 min. Ionisation was in positive mode and the mass spectrometer was operated in selective reaction monitoring (SRM) mode with 1.2 mTorr of argon at quadrapole two. The mass to charge ratio (m/z) transitions followed for cortisol and cortisol-D₄ were 363.2 to 121.15 and 367.2 to 121.15, respectively, both at a collision energy of 28 V. Results were analysed according to the peak area ratio of cortisol to the internal standard. The calibration curve was determined by weighted (1/x) least squares
linear regression of the spiked cortisol concentration and measured peak area ratio of the standards.

Assays were clustered in batches to minimise variation in experimental conditions. For each batch, concentrated stock solutions of the standards and internal standard were prepared in methanol. Before each assay run, working solutions of the standards and internal standard were prepared by diluting the methanol stock solutions 1:100 in water. All samples from each individual child were assayed in the same run to minimise intra-subject variation.

A single set of quality control samples was used for all batches and runs to monitor inter-run and intra-run precision, respectively. Quality control samples were prepared from pooled saliva from healthy adult volunteers, collected in the evening. The low control consisted of the un-spiked saliva pool. The medium and high controls were prepared by spiking the pooled saliva with cortisol 5.5 nmol/L and 55 nmol/L, respectively. The controls were divided into aliquots and stored at –20 °C until required.

### 2.3.13 Plasma cortisol and modified insulin stress test

Although it was not possible to include a formal HPA stress test in the follow-up study, the conditions of the frequently sampled intravenous glucose tolerance test provided an opportunity to assess HPA axis response to physiological stress (section 2.3.9.2), as mild hypoglycaemia commonly occurred after the intravenous insulin dose (0.015 U/kg). The cortisol response to hypoglycaemia on insulin challenge is recognised as a reliable test of the integrity of the entire HPA axis (Chrousos 2009). Although a formal insulin tolerance test uses a larger dose of insulin (0.15 U/kg) and aims to achieve marked hypoglycaemia (<2.2 mmol/L), pilot data from 10 children showed that even mild hypoglycaemia (<3 mmol/L) was associated with a rise in plasma cortisol concentration (Table 2.3). Thus, this part of the intravenous glucose tolerance test was regarded as a modified insulin stress test. The cortisol response was measured in plasma because of the availability of these samples as part of the test procedure.

Pilot data showed that the glucose nadir occurred at around 20 to 30 min and that plasma cortisol concentration increased from 30 to 60 min after the insulin bolus (Table 2.3). Therefore, plasma cortisol was measured immediately before insulin (time 19 min of the frequently sampled intravenous glucose tolerance test) and after 60 min. Plasma was not available to test the cortisol response beyond 60 min. All children had plasma glucose
concentrations measured at least every 10 min after the insulin bolus, allowing the approximate timing and level of the glucose nadir to be determined.

Of note, pilot data showed that plasma cortisol concentrations decreased over the 40 min period from the insertion of the intravenous cannula to the intravenous insulin bolus (Table 2.3). This may have been due to an anticipatory stress response associated with venepuncture or to the normal decline in cortisol with the diurnal rhythm. Subsequent blood sampling from the cannula was pain-free and unlikely to elicit a stress response.

Table 2.3: Cortisol response to insulin bolus (pilot data).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Plasma concentration —nmol/L</th>
<th>Time from insulin bolus—min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-40</td>
<td>-20</td>
</tr>
<tr>
<td>All children (n=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>261</td>
<td>251</td>
</tr>
<tr>
<td>(64)</td>
<td>(56)</td>
<td>(91)</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.8</td>
<td>4.9</td>
</tr>
<tr>
<td>(0.4)</td>
<td>(0.4)</td>
<td>(1.5)</td>
</tr>
<tr>
<td>Children with glucose nadir &lt;3 mmol/L (n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>234</td>
<td>233</td>
</tr>
<tr>
<td>(38)</td>
<td>(57)</td>
<td>(107)</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.8</td>
<td>4.9</td>
</tr>
<tr>
<td>(0.3)</td>
<td>(0.4)</td>
<td>(2.1)</td>
</tr>
<tr>
<td>Children with glucose nadir &gt;3 mmol/L (n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>284</td>
<td>269</td>
</tr>
<tr>
<td>(80)</td>
<td>(55)</td>
<td>(73)</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.9</td>
<td>4.8</td>
</tr>
<tr>
<td>(0.5)</td>
<td>(0.4)</td>
<td>(0.5)</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation)

2.3.13.1 Plasma cortisol assay

Plasma cortisol concentrations were measured using a fluorescence polarisation immunoassay on an AxSYM analyser (Abbott, Abbott Park, Illinois, USA). Microtubes of plasma were thawed on ice, vortexed, and then centrifuged at 1100 x g for 3 min at 4 °C to remove particulate matter. A 150 µL volume of plasma was then transferred to an analyser test tube. A daily calibration check was performed using Abbott cortisol control solutions (low, medium, high). The assay has a between day coefficient of variation of 14% at 130 nmol/L and 7% at 418 nmol/L, and a lower limit of detection of 30 nmol/L (manufacturer’s data).

2.3.14 Structure of assessments

In Auckland, most children were seen in the Maurice and Nessie Paykel Clinical Research Unit at the Liggins Institute, which has a DXA scanner and all necessary laboratory
services on site. Children outside the Auckland region were assessed at the nearest public hospital or other suitable medical facility. Some children who participated in only the neurological and health study were assessed at school.

Children who assented to blood testing attended in the morning after an overnight fast and commenced their assessment with measurement of weight and height, followed by the frequently sampled intravenous glucose tolerance test. They were then given breakfast, after which they completed the paediatric examination and, if available, underwent DXA scanning. Children who did not undergo the intravenous glucose tolerance test generally had their psychological testing before the paediatric examination and DXA scan, and were seen in either the morning or the afternoon. In Christchurch, most DXA scans were performed on a separate day because the machine was located off-site. The ambulatory blood pressure and saliva collection were performed at home under the supervision of child’s caregiver.

2.3.15 Data management
All clinical data were recorded by hand on study data forms containing the child’s name and study number. Ambulatory blood pressure, dual energy X-ray absorptiometry and laboratory data were obtained from computer printouts. Data forms were checked for errors and completeness before data was entered into a computer database. Distributions were examined for outlier points (outside the 95% range), which were assessed for possible recording or transcription errors. Data were also checked for anomalies during analyses, including visual inspection of residual plots.

2.3.16 General statistical methods
Data were analysed using SAS JMP software (v 8.0.2, SAS Institute, Cary, North Carolina, USA). For the comparison of categorical data, two-sided P values were calculated using Fisher’s Exact Test if variables were dichotomous or Pearson’s Chi-square Test if variables had more than two levels. The effect of treatment on dichotomous outcomes was assessed by the risk ratio, and 95% confidence intervals were calculated using the Agresti-Coull method. For the comparison of continuous data that were Normally distributed, two-sided P values were calculated using Student’s t-test. The effect of treatment was assessed by the mean difference between groups, and 95% confidence intervals were determined from the standard error of the difference. Continuous outcome variables with a Lognormal distribution were analysed after natural logarithmic transformation, and the effect of treatment was assessed by the ratio of geometric means. This was calculated as the
exponential of the mean difference of the transformed data. Confidence intervals (95%) for the ratio of geometric means were determined from the exponential of the confidence interval for the mean difference of the transformed data. Similar to the interpretation of confidence intervals for risk ratios, if the confidence interval for the ratio of geometric means included one, then the geometric means were not considered significantly different. For baseline continuous variables that did not have a Normal distribution or outcome continuous variables that did not have a Normal or Lognormal distribution, comparisons were made using Wilcoxon’s rank sum test.

Primary statistical analyses were performed by comparing the repeat betamethasone group versus the placebo group. Two-sided P values <0.05 were considered statistically significant, and no adjustment was made for multiple comparisons. Analyses were conducted on an intention to treat basis.

Secondary statistical analyses adjusted for confounding by the baseline variables of gestational age at trial entry and prognostic categorical variables with imbalance between treatment groups, defined as an absolute risk difference of 5% or more. This was assessed in all participants of the New Zealand neurological and health study, and variables with imbalance that were judged to have prognostic importance were included as covariates in all secondary analyses of the New Zealand follow-up study, even for outcomes that included only a subset of participants. In addition, for each of the physiological tests, the confounding effect of any further baseline variables with imbalance was explored using the change-in-estimates technique at the 10% level (Greenland 1989).

For comparisons of dichotomous data, adjusted risk ratios and 95% confidence intervals were estimated using log-binomial regression (Barros 2003). The adjusted values were determined from the exponential of the parameter for the group factor in the model, and its confidence limits. For comparisons of continuous data with a Normal or Lognormal distribution, adjusted mean differences and 95% confidence intervals were calculated using mixed linear regression. In these adjusted analyses, the mother was included as a random effect to account for the non-independence of fetuses from multiple pregnancy (Shaffer 2009). It was not possible to make this adjustment for dichotomous data as JMP does not support the use of random effects in the logistic regression or the generalized linear model platforms. For dichotomous outcomes, data from fetuses from multiple pregnancy were assumed to be uncorrelated, as this is preferable to other analysis structures that use pregnancy as the denominator unit (Gates 2004).
All research staff involved in tracing, recruitment and assessment of children, and most caregivers were unaware of treatment group allocation. After the two-year follow-up, caregivers were given the option of being told their child’s treatment group or remaining blinded for future studies; most chose to remain blinded, and those that did not were asked not to reveal their treatment group at the time of assessment.

Funding bodies had no role in the study design, data collection, data analysis and interpretation, the writing of manuscripts, or in the decision to submit manuscripts for publication.

2.4 Development of the salivary cortisol test

2.4.1 Salivary cortisol assay development
Salivary cortisol concentration was measured using HPLC and tandem mass spectrometry, as described above (section 2.3.12.3). This method was chosen because of its specificity and capacity for high-throughput. The assay was based on a method for measuring cortisol in sheep plasma (Rumball 2008) but several modifications were made to enhance performance for saliva. To improve sensitivity, sample volume was increased from 200 µL to 300 µL and the reconstitution volume was decreased from 100 µL to 60 µL. To compensate for the larger sample volume, 1.5 mL instead of 1 mL of ethyl acetate was used for the steroid extraction. The column size was decreased from 250 x 4.6 mm to 100 x 3 mm to reduce the run time. However, this required that the aqueous content of the mobile phase be increased from 20% to 35% to achieve adequate separation of the cortisol peak. To compensate for the smaller column size, the flow rate of the mobile phase was reduced from 0.6 mL/min to 0.3 mL/min, and the injection volume was reduced from 25 µL to 15 µL. The other chromatographic and instrument parameters remained unchanged.

Standards were prepared in water instead of blank saliva matrix because saliva was viscous and difficult to pipette. It also avoided the possibility of a variable matrix effect if different saliva pools were required to provide enough matrix for multiple batches (Matuszewski 2003).

2.4.1.1 Assay performance

2.4.1.1.1 Lower limit of quantification
This was defined as a signal to noise ratio of ≥10 (peak height to baseline), and was determined by replicate (n=5) extraction and analysis of steroid-stripped saliva spiked with
varying amounts of cortisol using standard curve stock solutions (dilution 1:100) (Table 2.4). A matrix solution that contained no detectable endogenous cortisol was prepared by mixing pooled saliva with charcoal 120 mg/mL for 12 h. At a nominal value of 0.14 nmol/L, the mean signal to noise ratio was 11, whereas at 0.28 nmol/L the mean signal to noise ratio was 20. Thus, 0.14 nmol/L was regarded as the lower limit of quantification for the salivary cortisol assay and was the lowest point included in the standard curve.

Table 2.4: Signal to noise ratio of spiked steroid-stripped saliva.

<table>
<thead>
<tr>
<th>Determination</th>
<th>Matrix + spiked cortisol 0.14 nmol/L</th>
<th>Matrix + spiked cortisol 0.28 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured cortisol concentration—nmol/L</td>
<td>Signal to noise ratio</td>
</tr>
<tr>
<td>1</td>
<td>0.17</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>0.11</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>0.14</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>0.16</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>0.18</td>
<td>20</td>
</tr>
<tr>
<td>Mean</td>
<td>0.15</td>
<td>11</td>
</tr>
</tbody>
</table>

2.4.1.1.2 Linearity

The assay response was linear throughout the expected clinical salivary cortisol range, as determined from the peak area ratios plotted against the nominal concentration of steroid-stripped saliva samples spiked with varying amounts of cortisol using the seven standard curve stock solutions (cortisol concentration after 1:100 dilution from 0.14 to 82.76 nmol/L) (Figure 1).
2.4.1.1.3 Accuracy and precision

The accuracy and precision of the assay were determined from five determinations (replicate extraction and analysis) of quality control solutions at three different cortisol concentrations (low, medium, high). These were prepared by spiking pooled adult saliva (collected from 5 healthy volunteers in the evening) with cortisol using three of the standard curve stock solutions (spiked cortisol concentration after 1:100 dilution 0.28, 8.28, and 82.76 nmol/L). Because of endogenous cortisol in the saliva pool (mean concentration in triplicate assay 1.33 nmol/L), the nominal cortisol concentrations of the low, medium and high quality control solutions were of 1.61, 9.61 and 84.10 nmol/L, respectively. The accuracy and precision of the assay were ≤10% and ≤8%, respectively (Table 2.5) (Shah 2000). The inter-run precision for all three quality controls was ≤9%, determined from three runs over a fortnight period with four determinations of each control sample per run (runs were performed with the same standard curve stock solutions that were used to spike the quality control samples).
Table 2.5: Accuracy and precision of salivary cortisol assay.

<table>
<thead>
<tr>
<th>Quality control</th>
<th>Nominal cortisol concentration —nmol/L*</th>
<th>Measured cortisol concentration—nmol/L</th>
<th>Accuracy —%</th>
<th>Precision —%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Determination</td>
<td>1  2  3  4  5  Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.61</td>
<td>1.43  1.69  1.47  1.41  1.58  1.52</td>
<td>5.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Medium</td>
<td>9.61</td>
<td>8.56  9.00  8.83  8.87  10.05  9.06</td>
<td>5.7</td>
<td>6.3</td>
</tr>
<tr>
<td>High</td>
<td>84.10</td>
<td>92.55  93.21  89.71  93.68  94.23  92.67</td>
<td>10.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*Spiked cortisol (0.28, 8.28, or 82.76 nmol/L) plus measured endogenous cortisol of pool (1.33 nmol/L). Accuracy is deviation of the mean value from nominal value. Precision is the coefficient of variation.

2.4.2 Stability of salivary cortisol

Several studies have shown that cortisol is stable in saliva at ambient temperature for up to five to seven days (Aardal 1995; Clements 1998; Groschl 2001). Therefore, it is common practice to return salivary cortisol samples by post. To validate this, pooled saliva spiked with cortisol was either frozen immediately or exposed to different environmental conditions to simulate a postal journey. Saliva was stored in closed Salivette tubes without the swab or insert chamber removed. After five days, all samples were assayed for cortisol as above (section 2.4.1), and cortisol recovery was determined with reference to the samples that were frozen immediately. For each intervention, samples were prepared and assayed in triplicate, at two different spiked concentrations. The different experimental conditions were as follows:

- Frozen at –20 °C for 5 days
- Three freeze-thaw cycles. Samples were thawed at room temperature and then frozen for 24 to 48 hours
- Storage at room temperature (21 °C to 23 °C) on windowsill for 5 days
- Storage at room temperature (21 °C to 23 °C) on windowsill for 3 days, followed by frozen storage at –20 °C for 2 days
- Storage at room temperature (21 °C to 23 °C) for 3 days covered with aluminium foil, followed by frozen storage at –20 °C for 2 days

Compared to samples that were frozen immediately, storage at room temperature for up to five days or exposure to three freeze-thaw cycles did not substantially affect salivary...
cortisol concentration (Table 2.6). Therefore, it was concluded that returning samples by post, using sealed bubble packs, would not adversely affect salivary cortisol recovery.

Table 2.6: Effect of temperature, light and freezing on cortisol stability.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Exposure</th>
<th>Measured cortisol concentration after 5 days—nmol/L</th>
<th>Recovery—%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva pool + spiked cortisol 41.4 nmol/L</td>
<td>Frozen immediately</td>
<td>49.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 Freeze-thaw cycles</td>
<td>51.9</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>Room temperature in light 5 days</td>
<td>50.0</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Room temperature in light 3 days, frozen 2 days</td>
<td>49.1</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Room temperature in dark 3 days, frozen 2 days</td>
<td>48.9</td>
<td>99</td>
</tr>
<tr>
<td>Saliva pool + spiked cortisol 5.5 nmol/L</td>
<td>Frozen immediately</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 Freeze-thaw cycles</td>
<td>11.9</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>Room temperature in light 5 days</td>
<td>11.8</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>Room temperature in light 3 days, frozen 2 days</td>
<td>11.8</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>Room temperature in dark 3 days, frozen 2 days</td>
<td>10.5</td>
<td>101</td>
</tr>
</tbody>
</table>

Data are mean values of triplicate assay. Recovery compared to samples that were frozen immediately and assayed after 5 days.

2.4.3 Salivary dehydroepiandrosterone assay development

2.4.3.1 Background

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are major secretory products of the human adrenal cortex and are under hypothalamic-pituitary control (Parker 1996). DHEAS is hydrophilic and is the main circulating form, whereas DHEA is lipophilic and is taken up into cells and is converted to androgens and oestrogens (Kroboth 1999). The physiological role of DHEA(S) is not yet fully understood but it has neuroprotective, antioxidant, and anti-inflammatory effects. It has been associated with longevity, emotional well-being, and resistance to cancer and cardiovascular disease (Allolio 2002; Kroboth 1999; Maninger 2009). DHEA(S) appears to modulate the actions of cortisol and may have anti-glucocorticoid protective effects (Goodyer 2001). Thus, the molar ratio of salivary cortisol to DHEA may be a better marker of stress than the cortisol concentration alone, and there is increasing evidence that these two hormones have an integrative role. For example, in a study of adolescents with major depression, the ratio of
the two hormones was a better predictor of recovery than cortisol concentrations alone (Goodyer 1998).

An advantage of mass spectrometry is that multiple analytes can be measured in the same assay. Initially, it was envisaged that salivary DHEA would be assayed along with cortisol. However, salivary DHEA concentrations are even lower than cortisol before adrenarche (mean AM concentration 0.3 to 0.4 nmol/L; PM 0.1 to 0.2 nmol/L), so assays must be highly sensitive (Granger 1999). Unfortunately, DHEA did not ionise and fragment as easily as cortisol, so it was difficult to achieve adequate detection in the clinical range. Despite various attempts to improve the performance of the mass spectrometry system for DHEA, as described below, an adequate lower limit of quantification could not be achieved. Therefore, saliva samples were assayed only for cortisol.

2.4.3.2 Instrument tuning and sensitivity
Selective reaction monitoring mode tuning for DHEA was performed by infusing a 10 µg/mL solution of DHEA in methanol (Steraloids, Newport, Rhode Island, USA) at 0.01 mL/min with 0.2 mL/min of mobile phase (65% methanol, 35% water) with an APCI source in positive mode. The best signal response was achieved by monitoring the transition of the precursor ion \( m/z \) 271.25 to two daughter ions \( m/Z \) 213.1 and 197.2, at a collision energy of 18 V and with 1 mTorr of argon at quadrupole 2. It was planned that DHEA-D\(_2\) (Pointe-Claire, Quebec, Canada) would be used as an internal standard for DHEA. However, during tuning no daughter ions were identified for DHEA-D\(_2\), so an SRM method was not established. Therefore, corticosterone-D\(_8\) or testosterone-D\(_3\) were used as an internal standard for DHEA.

Extraction and analysis of water standards and pooled saliva containing varying amounts of spiked DHEA showed that the assay was not sufficiently sensitive in the clinical target range. The lower limit of detection was approximately 0.3 nmol/L and of quantification was 0.7 nmol/L. The sensitivity did not improve with modification of the chromatic conditions, including altering the aqueous component of the mobile phase, using different columns, or the addition of 0.1% formic acid to the mobile phase. Ionisation by electrospray also did not improve the performance of the assay.

2.4.3.3 Derivatisation
Given that poor ionisation efficiency was the likely cause of the low sensitivity, charged derivatisation was attempted, as this has been shown to improve the detection of steroids by mass spectrometry (Higashi 2004). Higashi et al (2007) reported a lower limit of
quantification for DHEA of <0.1 nmol/L by derivatising DHEA with the polar moiety 2-hydrazino-1-methylpyridine (HMP). We attempted to replicate this method using HMP synthesised by the Auckland Cancer Society Research Centre, University of Auckland.

Standard solutions of DHEA in water (range 0.03 nmol/L to 20 nmol/L), pooled saliva, and pooled saliva spiked with DHEA 0.17 nmol/L or 2 nmol/L were combined with internal standard (testosterone-D3 68.6 nmol/L, Steraloids, Newport, Rhode Island, USA), using 200 µL and 100 µL volumes, respectively. Samples were extracted using 1 mL of ethyl acetate, and after drying of the organic phase, were reconstituted with 40 µL of ethanol and 50 µL of HMP 0.2 ng/mL in ethanol with 1% trifluoroacetic acid. Samples were incubated for 1 h at 60°C, dried, and reconstituted with 60 µL of mobile phase (50% methanol, 50% 10 mM ammonium formate).

A new instrument method was required for the derivatised hormones. Tuning solutions of DHEA-HMP and testosterone-D3-HMP were prepared by derivatising 30 µL of DHEA or testosterone-D3 10 µg/mL with 50 µL of HMP solution as above. The dried sample was reconstituted in 500 µL of the mobile phase. Tuning was performed with electrospray ionisation in positive mode and maximal SRM signal responses were obtained with the following transitions at a collision energy of 32V: DHEA-HMP m/z 394.35 to 109.15; and testosterone-D3-HMP m/z 397.35 to 109.15. A 15 µL volume was injected onto a Lunar 3µC18(2) 100A 100 x 3 mm column with mobile phase flowing at 0.2 mL/min.

Derivatisation improved the sensitivity of the assay slightly, reducing the lower limit of detection of DHEA to around 0.1 nmol/L. While this experiment showed that DHEA could be successfully derivatised with HMP, which improved the assay performance a little, it was still necessary to combine the cortisol and DHEA assays into a single method, because of the possibility of low sample volumes from children. However, further testing showed that cortisol ionised poorly with electrospray and it did not derivatise with HMP. Conversely, the signal intensity for derivatised DHEA decreased about 10-fold with APCI. Therefore, a combined assay seemed impractical and was not developed further.

2.4.4 Effect of swab on salivary hormone recovery
Cotton swabs are commonly used to collect saliva. However, several studies have shown that recovery of cortisol using this collection method is reduced by 20% to 30% (Groschl 2006; Poll 2007; Shirtcliff 2001; Strazdins 2005). Cotton swabs appear to cause even greater interference with DHEA measurements, either reducing or falsely elevating
recovery (Gallagher 2006; Shirtcliff 2001). Polyester swabs may perform better, but were not available at the commencement of the study (Groschl 2006). In view of these reports, the recovery of cortisol and DHEA from cotton swabs was investigated in the following experiments.

2.4.4.1 Analytical conditions
Cortisol and DHEA were assayed using the method described above (section 2.4.1), although several of the assay parameters were different, as the final method had not been established at this stage. A 200 µL volume of sample, combined with 100 µL of internal standard, was extracted using 1 mL of ethyl acetate. Residues were reconstituted in 80 µL of mobile phase (80% methanol, 20% water) and 25 µL was injected onto a Lunar 3µC18(2) 100A 250 x 4.6 mm column. The mobile phase was isocratic at 0.6 mL/min. Corticosterone-D$_8$ 56.4 nmol/L was used as the internal standard for both cortisol and DHEA (CDN Isotopes, Quebec, Canada). APCI was used in positive mode. The SRM transitions were as follows: cortisol $m/z$ 363.3 to 121.2, and corticosterone $m/z$ 355.3 to 125.2 at 28V; and DHEA $m/z$ 271.15 to 213.1 and 197, at 18 V. The following retention times were achieved: corticosterone-D$_8$ 6.17 min, cortisol 7.01 min, and DHEA 9.58 min. The calibration curve for cortisol consisted of eight standards ranging from 0.14 nmol/L to 275.88 nmol/L, and for DHEA, six standards from 0.35 nmol/L to 34.67 nmol/L.

2.4.4.2 Sample preparation
Cotton swab inserts from Salivettes (Sarstedt, Mawson Lakes, South Australia) were used to test the effect of swabs on hormone recovery. Samples of pooled saliva, spiked with varying amounts of cortisol or DHEA, or water blanks, were absorbed onto cotton swabs by slowly dripping a measured volume (0.5 mL, 1 mL, or 2 mL) from a pipette. After 5 min, the swabs were centrifuged in the Salivettes at 2000 x g for 10 min at 4°C, and the volume of the eluate was determined by weight. Each sample was prepared and assayed in triplicate, both directly and after being absorbed onto swabs. Steroid recovery was calculated from the observed concentration in the swab eluate as a percentage of the observed concentration in the sample when assayed directly.

2.4.4.3 Swab impurity
Sample volumes of 1 mL of milli-Q water were absorbed onto cotton swabs. Analysis of the eluate produced a signal response equivalent to 1.3 nmol/L (n=3) for the SRM transition for DHEA at its retention time. Thus, an organic compound with similar chromatographic, ionisation and molecular fragmentation properties to DHEA was eluted.
from the cotton swab, possibly a plant sterol. No such interference was seen at the retention time for cortisol.

**2.4.4.4 DHEA recovery**

When pooled saliva spiked with DHEA was absorbed onto the swab, the interference from this impurity resulted in falsely elevated steroid recovery (Table 2.7). This interference decreased as the sample volume increased, presumably due to a decrease in the amount of interfering substance relative to the eluted volume. Adjusting the chromatic conditions did not separate the DHEA peak from the impurity, including changing the aqueous component of the mobile phase, using a shorter column, or a mobile phase gradient. Therefore, it was concluded that cotton swabs are not suitable for measurement of salivary DHEA.

Table 2.7: DHEA recovery through swab.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured DHEA concentration—nmol/L</th>
<th>Volume applied to swab—mL</th>
<th>Measured DHEA concentration in swab elute—nmol/L</th>
<th>DHEA recovery—%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva pool + spiked DHEA 1.7 nmol/L</td>
<td>2.1</td>
<td>0.5</td>
<td>6.3</td>
<td>305</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>5.7</td>
<td>273</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>4.7</td>
<td>225</td>
</tr>
</tbody>
</table>

Data are mean values of triplicate assay.

**2.4.4.5 Cortisol recovery**

At low sample volume (0.5 mL), the recovery of cortisol through the swab was only around 60%, regardless of the amount of spiked cortisol (Table 2.8). However, cortisol recovery improved at higher sample volumes (1 mL to 2 mL). In order to avoid a possible confounding effect of sample volume, children were asked to collect saliva by passive drool rather than by swab.
Table 2.8: Cortisol recovery through swab.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured cortisol concentration—nmol/L</th>
<th>Volume applied to swab—mL</th>
<th>Cortisol recovery—%</th>
<th>Volume recovery—%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva pool + spiked cortisol 0.7 nmol/L</td>
<td>5.1</td>
<td>0.5</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Saliva pool + spiked cortisol 8.2 nmol/L</td>
<td>12.9</td>
<td>0.5</td>
<td>65</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>92</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>108</td>
<td>89</td>
</tr>
<tr>
<td>Saliva pool + spiked cortisol 82.8 nmol/L</td>
<td>90.5</td>
<td>0.5</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean values of triplicate assay.
3 The New Zealand ACTORDS early school-age follow-up cohort

3.1 Summary of chapter contents
This chapter outlines the recruitment of children into the New Zealand ACTORDS follow-up study at 6 to 8 years’ corrected age. It reports baseline characteristics and key perinatal and infant outcomes of participating and non-participating New Zealand children, as well as baseline characteristics of participants exposed to repeat betamethasone or placebo.

3.2 Introduction
Randomised trials, including ACTORDS, have shown that administration of repeat doses of antenatal glucocorticoids to women at risk of preterm birth, 7 or more days after an initial course, is associated with short-term benefits for infants, especially reduced respiratory morbidity (McKinlay 2012). Follow-up of children at two to three years of age in ACTORDS and three other trials did not demonstrate any adverse effects of repeat dose treatment on neurodevelopment or general health. However, longer-term outcomes are currently unknown and concern remains regarding possible effects of repeat doses on higher cognitive function, growth, and cardiovascular and metabolic physiology. The ACTORDS early school-age follow-up study was established to help address this evidence gap. The New Zealand arm consisted of two follow-up studies, a neurological and health study and a physiological study, which were conducted in parallel.

3.3 Chapter aim and hypothesis
The aims of the chapter are to:

- Determine whether participants in the New Zealand 6 to 8 year follow-up study are representative of the New Zealand ACTORDS cohort as a whole
- Determine whether baseline characteristics with prognostic significance are balanced between participants exposed to repeat betamethasone or placebo

The chapter hypotheses are:

- That there are no significant differences between participants and non-participants in the New Zealand ACTORDS follow-up study in baseline characteristics and perinatal and infant neurodevelopmental outcomes
• That baseline characteristics of participants in the neurological and health study are similar in those exposed to repeat betamethasone or placebo

3.4 Additional statistical methods used in chapter analyses
Participants and non-participants in both the neurological and health study and physiological study were compared for baseline obstetric and demographic characteristics and key perinatal and two-year neurodevelopmental outcomes using appropriate two-tailed hypothesis tests. Similarly, baseline characteristics of participants in the neurological and health study exposed to repeat betamethasone or placebo were compared. Although baseline variables of randomised treatment groups should not normally be compared by hypothesis tests (Senn 1994), P values are presented because the follow-up cohort represents a non-random sample of the original trial. However, imbalance between treatment groups was assessed according to absolute risk difference, at the 5% level, rather than by P values.

Although ethnicity data were collected during the early school-age follow-up, and are therefore not available for the whole New Zealand cohort, ethnicity was treated as a pre-randomisation variable.

3.5 Recruitment

3.5.1 Participant flow to follow-up
A total of 290 women and 352 live fetuses were enrolled in the ACTORDS trial in New Zealand. Eighteen of these infants emigrated to Australia after birth and were transferred to the Australian arm of the study. All of these infants participated in the Australian early school-age neurological follow-up study but their results are not included in this thesis. Five Australian-born infants immigrated to New Zealand and have been included in the analyses for the New Zealand cohort. Thus, the present New Zealand ACTORDS cohort consists of 339 infants alive at randomisation (165 repeat betamethasone group, 174 placebo group) (Figure 3.1). Of these, eight died in the neonatal period (three repeat betamethasone group, five placebo group) and two died shortly after primary hospital discharge (one each group). In addition, one child in the placebo group was known to have died at age three years from severe haemorrhage after tonsillectomy.

Therefore, 328 children in the New Zealand ACTORDS cohort were presumed to be alive at 6 to 8 years’ corrected age and potentially available for follow-up (161 repeat betamethasone group, 167 placebo group). However, five of these children were
withdrawn from the trial by caregivers at two years of age and so were not approached further (three repeat betamethasone group, two placebo group). Of the remaining 323 children, two were living overseas and did not return to New Zealand during the follow-up period (both placebo group), five could not be traced in either New Zealand or Australia (three repeat betamethasone group, two placebo group), and eight declined to participate in the early school-age follow-up (seven repeat betamethasone group, one placebo group). Thus, a total of 20 (6%) New Zealand children were lost to follow-up (13 [8%] repeat betamethasone group, seven [4%] placebo group, P=0.17).

Of the 308 children recruited to the New Zealand neurological and health study, eight had known severe neurosensory disability and were unable to undergo formal neurodevelopmental testing (three repeat betamethasone group, five placebo group). These children were also not considered eligible for the physiological study. Of the remaining 300 New Zealand early school-age participants, 264 (88%) completed at least one of the physiological study investigations (125 [86%] repeat betamethasone group, 139 [90%] placebo group) and 102 (34%) completed the entire physiological study protocol (48 [33%] repeat betamethasone group, 54 [35%] placebo group). Follow-up rates for the individual physiological tests varied, but were similar in both treatment groups (Figure 3.1). Of all 328 New Zealand ACTORDS children presumed to be alive at 6 to 8 years’ corrected age, rates of non-participation in the physiological study were not significantly different between treatment groups (repeat betamethasone 36 [22%], placebo group 28 [17%], P=0.22).

3.5.2 Timing of follow-up
The New Zealand early school-age follow-up study commenced in January 2008 and children were assessed approximately in order of corrected age. Because New Zealand mothers were recruited to the ACTORDS trial over a six-year period and children were not eligible for assessment until six years’ corrected age, the early school-age follow-up study took three years to complete. The last child was assessed in November 2010.
Figure 3.1 Participant flow to early school-age follow-up.

ACTORDS trial: 982 Women / 1146 Infants alive at randomisation

290 Women / 352 Infants recruited in New Zealand
18 Infants moved to Australia
5 Infants moved to New Zealand
692 Women / 704 Infants recruited in Australia

339 Infants remaining in the New Zealand study cohort

165 Infants allocated to repeat antenatal betamethasone (100% received intervention)
0 Fetal death
3 Neonatal deaths
1 Infant death

161 Infants presumed alive in New Zealand at 6-8 years' corrected age
13 Lost to follow-up:
• 10 Declined
• 3 Not traced

Neurological and health study
148 Children assessed:
• 3 Known severe neurosensory disability
• 145 Underwent neurodevelopmental examination
152 / 165 (92%) infants alive at randomisation had primary outcome data at 6-8 years' corrected age

174 Infants allocated to placebo (98% received intervention)
0 Fetal death
5 Neonatal deaths
2 Infant deaths

167 Infants presumed alive in New Zealand at 6-8 years' corrected age
7 Lost to follow-up:
• 3 Declined
• 4 Not traced

Neurological and health study
160 Children assessed:
• 5 Known severe neurosensory disability
• 155 Underwent neurodevelopmental examination
167 / 174 (96%) infants alive at randomisation had primary outcome data at 6-8 years' corrected age

Physiological study (n=125)

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Eligible children*</th>
<th>Test completed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-insulin metabolism</td>
<td>136</td>
<td>84 (62)</td>
</tr>
<tr>
<td>Ambulatory blood pressure</td>
<td>145</td>
<td>106 (73)</td>
</tr>
<tr>
<td>Body composition</td>
<td>103</td>
<td>91 (88)</td>
</tr>
<tr>
<td>Salivary cortisol</td>
<td>145</td>
<td>102 (70)</td>
</tr>
<tr>
<td>Plasma cortisol</td>
<td>136</td>
<td>84 (62)</td>
</tr>
<tr>
<td>Renal function</td>
<td>136</td>
<td>89 (65)</td>
</tr>
</tbody>
</table>

Physiological study (n=139)

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Eligible children*</th>
<th>Test completed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-insulin metabolism</td>
<td>148</td>
<td>98 (66)</td>
</tr>
<tr>
<td>Ambulatory blood pressure</td>
<td>155</td>
<td>116 (75)</td>
</tr>
<tr>
<td>Body composition</td>
<td>103</td>
<td>94 (91)</td>
</tr>
<tr>
<td>Salivary cortisol</td>
<td>155</td>
<td>110 (71)</td>
</tr>
<tr>
<td>Plasma cortisol</td>
<td>148</td>
<td>98 (66)</td>
</tr>
<tr>
<td>Renal function</td>
<td>148</td>
<td>100 (68)</td>
</tr>
</tbody>
</table>

* See text for eligibility criteria
3.5.3 Geographical location of participants

Nearly two-thirds of the children in the neurological and health study were born to mothers who were recruited to the ACTORDS trial in Auckland (National Women’s Hospital and Middlemore Hospital) (Figure 3.2). However, at 6 to 8 years’ corrected age only half of the participants were living in the wider Auckland region, and nearly one-third had moved from the centre in which they were born (Figure 3.3). In many cases, this was because mothers had been transferred antenatally for tertiary obstetric care. Most follow-up assessments were performed in the child’s home town, although 13 children travelled to Auckland from Tauranga, the Waikato or Palmerston North. Recruitment rates to the physiological study were high in the South Island but were more variable in the North Island, being lowest in Taupo, Hawke’s Bay, and Wanganui (Figure 3.4).

Figure 3.2: Original recruitment centre of New Zealand-born participants in the neurology and health study [N=303].
Figure 3.3: Location of participants in the neurological and health study (percentage of total participants) \([N=308]\).
Figure 3.4: Location of participants in the physiological follow-up (percent of eligible participants in each area) [N=300].
3.6 Comparison of participants and non-participants

There were no significant differences between participants and non-participants in the neurological and health study for baseline demographic and obstetric characteristics (Table 3.1), or key perinatal and two-year neurodevelopmental outcomes (Table 3.2). There were also no significant differences between participants and non-participants in the physiological study for baseline demographic and obstetric characteristics (Table 3.1). However, non-participants in the physiological study were born, on average, one week later than participants, experienced significantly less respiratory distress syndrome, and at two years’ corrected age had significantly lower cognitive scores, higher rates of moderate or severe disability and higher rates of cerebral palsy (Table 3.2). Even when the eight children with known severe neurosensory disability were excluded from analysis, non-participants in the physiological study compared with participants had significantly lower cognitive scores (mental developmental index [SD] 85 [13] versus 91 [14], P=0.008) and higher rates of mild disability (41% versus 25%, P=0.04).

3.7 Comparison of participants by treatment group

3.7.1 Baseline characteristics

In the neurological and health study, there was imbalance between participants exposed to repeat betamethasone and those exposed to placebo for the following baseline variables: maternal parity and smoking, preterm prelabour rupture of membranes and spontaneous preterm labour as reasons for preterm birth, and European ethnicity (Table 3.3). However, unlike the whole Australasian ACTORDS cohort, there was no imbalance in the rate of antepartum haemorrhage as a reason for being at risk of preterm birth (Crowther, Haslam, 2006). Although there was also imbalance in the proportion of women judged to be at risk of preterm birth due to multiple pregnancy, actual rates of multiple pregnancy did not differ between treatment groups.

3.7.2 Age and pubertal status

In the neurological and health study, there was no significant difference in corrected age at follow-up between participants exposed to repeat betamethasone or placebo (median years [interquartile range] 7.2 [6.4, 7.9] versus 6.8 [6.3, 7.9], P=0.37). Eight children were seen at the beginning of their ninth corrected year of age (three repeat betamethasone group, five placebo group). Five children had entered puberty but were only Tanner stage 2 (four repeat betamethasone group, one placebo group).
Table 3.1: Baseline characteristics of children, and their mothers, presumed alive in New Zealand at 6 to 8 years’ corrected age.

<table>
<thead>
<tr>
<th></th>
<th>Neurological and health study</th>
<th></th>
<th>Physiological study</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Participants</td>
<td>Non-participants</td>
<td>P</td>
<td>Participants</td>
</tr>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of women</td>
<td>258</td>
<td>17</td>
<td>0.46</td>
<td>220</td>
</tr>
<tr>
<td>Allocated to repeat betamethasone</td>
<td>126 (49)</td>
<td>10 (59)</td>
<td></td>
<td>107 (49)</td>
</tr>
<tr>
<td>Maternal age—yr</td>
<td>30.6 (5.6)</td>
<td>31.6 (5.0)</td>
<td>0.44</td>
<td>30.6 (5.5)</td>
</tr>
<tr>
<td>Parity—no. (%)</td>
<td></td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>93 (36)</td>
<td>5 (29)</td>
<td></td>
<td>81 (37)</td>
</tr>
<tr>
<td>1 to 3</td>
<td>132 (51)</td>
<td>10 (59)</td>
<td></td>
<td>115 (52)</td>
</tr>
<tr>
<td>≥ 4</td>
<td>33 (13)</td>
<td>2 (12)</td>
<td></td>
<td>24 (11)</td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>80 (32)</td>
<td>9 (53)</td>
<td>0.11</td>
<td>67 (31)</td>
</tr>
<tr>
<td>Gestational age at first glucocorticoids—wk†</td>
<td>26.9 (24.7, 28.7)</td>
<td>27.5 (25.5, 29.8)</td>
<td>0.24</td>
<td>26.9 (24.7, 28.8)</td>
</tr>
<tr>
<td>Gestational age at trial entry—wk†</td>
<td>28.6 (26.3, 30.3)</td>
<td>29.4 (27.9, 30.9)</td>
<td>0.12</td>
<td>28.6 (26.2, 30.3)</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>53 (21)</td>
<td>4 (24)</td>
<td>0.76</td>
<td>46 (21)</td>
</tr>
<tr>
<td><strong>Main reasons for risk of preterm birth§</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm prelabour rupture of membranes</td>
<td>72 (28)</td>
<td>4 (24)</td>
<td>0.79</td>
<td>65 (30)</td>
</tr>
<tr>
<td>Preterm labour</td>
<td>49 (19)</td>
<td>3 (18)</td>
<td>1.00</td>
<td>37 (17)</td>
</tr>
<tr>
<td>Severe fetal growth restriction</td>
<td>34 (13)</td>
<td>1 (6)</td>
<td>0.71</td>
<td>30 (14)</td>
</tr>
<tr>
<td>Preeclampsia or eclampsia</td>
<td>53 (21)</td>
<td>1 (6)</td>
<td>0.21</td>
<td>175 (80)</td>
</tr>
<tr>
<td>Cervical incompetence</td>
<td>21 (8)</td>
<td>0 (0)</td>
<td>0.38</td>
<td>18 (8)</td>
</tr>
<tr>
<td></td>
<td>Neurological and health study</td>
<td></td>
<td>Physiological study</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>------------------------------</td>
<td>------------------</td>
<td>---------------------</td>
<td>------------------</td>
</tr>
<tr>
<td></td>
<td>Participants</td>
<td>Non-participants</td>
<td>P</td>
<td>Participants</td>
</tr>
<tr>
<td>Antepartum haemorrhage</td>
<td>58 (22)</td>
<td>7 (35)</td>
<td>0.14</td>
<td>52 (24)</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>20 (8)</td>
<td>2 (12)</td>
<td>0.63</td>
<td>17 (8)</td>
</tr>
<tr>
<td>Other</td>
<td>11 (4)</td>
<td>0 (0)</td>
<td>1.00</td>
<td>9 (4)</td>
</tr>
</tbody>
</table>

**Child Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Number of children</th>
<th>Allocated to repeat betamethasone</th>
<th>Sex—female</th>
<th>Ethnicity‡</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>308</td>
<td>148 (48)</td>
<td>142 (46)</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>13 (65)</td>
<td>8 (40)</td>
<td>88 (29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Ethnicity‡**

<table>
<thead>
<tr>
<th></th>
<th>Participants</th>
<th>Non-participants</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maori</td>
<td>88 (29)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Pacific Peoples</td>
<td>36 (12)</td>
<td>30 (11)</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Other</td>
<td>18 (58)</td>
<td>16 (6)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>European</td>
<td>166 (54)</td>
<td>20 (55)</td>
<td>20 (45)</td>
</tr>
</tbody>
</table>

Data are number (percent), mean (standard deviation) or †median (interquartile range). Physiological study includes children who completed one or more of the physiological tests. P values are for the comparison between participants and non-participants (two-tailed). § Not mutually exclusive. ‡ Ethnicity prioritised in order of Maori, Pacific Peoples, Other, and European. Ethnicity data were not available (NA) for the 20 non-participants in the neurological and health study who were lost to follow-up.
Table 3.2: Perinatal and two-year neurodevelopmental outcomes of children presumed alive in New Zealand at 6 to 8 years’ corrected age.

<table>
<thead>
<tr>
<th></th>
<th>Neurological and health study</th>
<th>Physiological study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Participants (n=308)</td>
<td>Non-participants (n=20)</td>
</tr>
<tr>
<td>Trial treatments given</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>1</td>
<td>138 (45)</td>
<td>10 (50)</td>
</tr>
<tr>
<td>2 or 3</td>
<td>111 (36)</td>
<td>10 (50)</td>
</tr>
<tr>
<td>≥4</td>
<td>56 (18)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gestational age at birth—wk</td>
<td>31.9 (3.6)</td>
<td>31.8 (2.7)</td>
</tr>
<tr>
<td>Birthweight—g†</td>
<td>1575 (1146, 2170)</td>
<td>1715 (1189, 1950)</td>
</tr>
<tr>
<td>Birthweight Z-score</td>
<td>-0.40 (1.09)</td>
<td>-0.26 (1.11)</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
<td>124 (40)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Severe neonatal lung disease</td>
<td>39 (13)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>86 (28)</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Neonatal oxygen use</td>
<td>179 (58)</td>
<td>11 (55)</td>
</tr>
<tr>
<td>Intraventricular haemorrhage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>17 (6)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Grade 3 or 4</td>
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<td>0 (0)</td>
</tr>
<tr>
<td>Periventricular leukomalacia</td>
<td>3 (1)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Neurological and health study</td>
<td>Physiological study</td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Participants (n=308)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-participants (n=20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants (n=264)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-participants* (n=64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurosensory disability§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number assessed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate or severe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Development index score¶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number assessed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychomotor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral palsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number assessed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Data are number (percent), mean (standard deviation) or †median (interquartile range). Physiological study includes children who completed one or more of the physiological tests. P values are for the comparison between participants and non-participants (two-tailed). * Includes eight children with severe disability who participated in the neurological and health study but were excluded from the physiological study. § See methods chapter for definitions. ¶ Bayley Scales of Infant Development, second edition. ‡ Five children were withdrawn before two years of age.
Table 3.3: Baseline characteristics of children, and their mothers, in the neurological and health study exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of women</td>
<td>126</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Maternal age—yr</td>
<td>30.2 (5.6)</td>
<td>30.9 (5.6)</td>
<td>0.31</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>0</td>
<td>52 (41)</td>
<td>41 (31)</td>
<td></td>
</tr>
<tr>
<td>1 to 3</td>
<td>60 (48)</td>
<td>72 (55)</td>
<td></td>
</tr>
<tr>
<td>≥ 4</td>
<td>14 (11)</td>
<td>19 (14)</td>
<td></td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>35 (29)</td>
<td>45 (35)</td>
<td>0.28</td>
</tr>
<tr>
<td>Gestational age at first glucocorticoids—wk†</td>
<td>26.9 (24.9, 28.7)</td>
<td>27.0 (24.5, 28.8)</td>
<td>0.91</td>
</tr>
<tr>
<td>Gestational age at trial entry—wk†</td>
<td>28.5 (26.4, 30.3)</td>
<td>28.6 (26.0, 30.3)</td>
<td>0.87</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>24 (19)</td>
<td>29 (22)</td>
<td>0.64</td>
</tr>
<tr>
<td>Main reasons for risk of preterm birth§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm prelabour rupture of membranes</td>
<td>32 (25)</td>
<td>40 (30)</td>
<td>0.41</td>
</tr>
<tr>
<td>Preterm labour</td>
<td>28 (22)</td>
<td>21 (16)</td>
<td>0.21</td>
</tr>
<tr>
<td>Severe fetal growth restriction</td>
<td>18 (14)</td>
<td>16 (12)</td>
<td>0.71</td>
</tr>
<tr>
<td>Preeclampsia or eclampsia</td>
<td>26 (21)</td>
<td>27 (20)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cervical incompetence</td>
<td>12 (10)</td>
<td>9 (7)</td>
<td>0.50</td>
</tr>
<tr>
<td>Antepartum haemorrhage</td>
<td>30 (24)</td>
<td>28 (21)</td>
<td>0.66</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>13 (10)</td>
<td>7 (5)</td>
<td>0.16</td>
</tr>
<tr>
<td>Other</td>
<td>3 (2)</td>
<td>8 (6)</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Infant characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of infants</td>
<td>148</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>Sex—female</td>
<td>77 (52)</td>
<td>89 (56)</td>
<td>0.57</td>
</tr>
<tr>
<td>Ethnicity‡</td>
<td></td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>Maori</td>
<td>43 (29)</td>
<td>45 (28)</td>
<td></td>
</tr>
<tr>
<td>Pacific Peoples</td>
<td>20 (14)</td>
<td>16 (10)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>12 (8)</td>
<td>6 (4)</td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>73 (49)</td>
<td>93 (58)</td>
<td></td>
</tr>
</tbody>
</table>

Data are number (percent), mean (standard deviation) or †median (interquartile range). ‡ Ethnicity prioritised in order of Maori, Pacific Peoples, Other, and European. § Not mutually exclusive.
3.8 Discussion

ACTORDS is the first randomised trial of repeat dose(s) of antenatal glucocorticoids to follow up children at school age, and, in the New Zealand arm, to study physiological outcomes in detail. The results of the New Zealand neurological and health study can be regarded as being representative of the New Zealand ACTORDS cohort as a whole because of the high follow-up rate (94% of surviving children) and because there were no significant differences between participants and non-participants for baseline characteristics or for key perinatal and two-year neurodevelopmental outcomes. However, there were some differences between participants and non-participants in the physiological study. In particular, non-participants had a reduced incidence of neonatal lung disease, which was probably due to their higher gestational age at birth. Despite being apparently more mature, non-participants in the physiological study were more likely to have neurosensory disability at two years’ corrected age than participants. Even when children with severe disability were excluded, non-participants in the physiological study had lower cognitive scores at two years’ corrected age. Such children would be expected to have higher rates of learning disability or other developmental problems at early school age and this may have influenced caregivers’ decisions not to consent to their children undergoing physiological investigation.

It is possible that children with poorer neurodevelopment have worse physiological outcomes, in which case the results of participants in the physiological study may be more favourable than for the New Zealand ACTORDS cohort as a whole. On the other hand, participants in the physiological study were born at an earlier gestational age than non-participants, and gestation length is known to be inversely associated with risk of cardiovascular and metabolic disease (Kaijser 2009; Koupil, Leon, 2005; Lawlor 2005; Lawlor 2006). However, these factors are unlikely to bias the results of the trial, as the rate of non-participation in the physiological study was similar between treatment groups.

In the New Zealand neurological and health study, treatment groups were quite similar in baseline characteristics. This is not surprising given the high follow-up rate and the fact that in the ACTORDS trial randomisation was stratified by study centre (Crowther, Haslam, 2006). However, there were small differences in the absolute risk of some baseline variables, the most important of which are prelabour preterm rupture of membranes and European ethnicity; preterm prelabour rupture of membranes because of its association with fetal infection and inflammation (Romero 2007), and ethnicity because of its association with disparate perinatal outcomes in New Zealand (Craig, Mantell, 2004).
Therefore, it was decided that all secondary analyses in the New Zealand early school age follow-up study would be adjusted for these factors. Ethnicity was treated as a single level factor (European versus non-European) because ethnicity was prioritised and hence all categories other than European could potentially include a combination of ethnic origins. Although there was imbalance for spontaneous preterm labour as a reason for being at risk of preterm birth, this was not included as a covariate in secondary analyses, as it was expected that this would be accounted for by adjustment for preterm prelabour rupture of membranes, given the apparent reciprocal relationship between these two factors. Although gestational age at trial entry was not different between participants in the neurological and health study exposed to repeat betamethasone or placebo, this was included as a covariate in secondary analysis because this adjustment was made in the original trial analysis (Crowther, Haslam, 2006).

3.9 Summary
Participants in the neurological and health study are representative of the New Zealand ACTORDS cohort as a whole. There were some differences between participants and non-participants in the physiological study. However, these differences are unlikely to bias the results of the physiological study, as participation was similar between treatment groups. Secondary analysis of early school-age outcomes will include adjustment for prelabour preterm rupture of membranes, European ethnicity, and gestational age at trial entry.
4 Body size and composition

4.1 Summary of chapter contents
This chapter reports the body size of participants in the New Zealand ACTORDS early school-age neurological and health study, and the body composition of participants in the physiological follow-up study who underwent total body dual energy X-ray absorptiometry (DXA).

4.2 Introduction
Numerous animal studies have demonstrated a dose-dependent reduction in fetal growth with exposure to synthetic antenatal glucocorticoids (Aghajafari, Murphy, Matthews, 2002; Fowden 2009). This appears to be due to premature activation of normal physiological processes that occur in late gestation, whereby rising endogenous fetal glucocorticoid levels stimulate widespread maturation of fetal tissues and promote storage of fuel reserves (Fowden 1998). These changes are critical for successful transition to extra-uterine life and ensure that the newborn is adapted to continue growing in the altered postnatal nutritional environment, but are also associated with slowing of fetal somatic growth. This is primarily due to the effect of glucocorticoids on the cell cycle as tissue maturation and differentiation is associated with decreased DNA synthesis and cell proliferation (Ballard 1986). However, the effect of glucocorticoids on fetal growth appears to differ among species. For example, brief exposure exposure to glucocorticoids at clinically relevant doses commonly reduces birthweight in sheep (Jobe, Wada, 1998) but not in non-human primates (Bramlage 2009; Engle 1996; Hauser 2007; Uno 1990). In humans, a single course of antenatal glucocorticoids has little effect on fetal growth (Roberts 2006), but the effect of repeat doses is less clear.

Meta-analysis of nine randomised human trials found that repeat doses of antenatal glucocorticoids were associated with a small reduction in size at birth, including weight, length and head circumference (McKinlay 2012). However, in three of the trials that showed the largest differences, infants in the repeat dose group had slightly shorter gestation length, which may have explained their smaller size at birth size (Guinn 2001; Murphy 2008; Peltoniemi 2007). In the few studies that corrected for gestational age, there were no differences between groups for birthweight Z-scores or multiples of the median birthweight (McKinlay 2012). However, in the ACTORDS trial, infants exposed to repeat doses of betamethasone compared to those exposed to placebo (single course) had similar
birthweight but slightly reduced standard deviation scores for weight and head circumference (Crowther, Haslam, 2006). Thus, until neonatal body size data that are adjusted for gestational age become available from all trials, the effect of repeat dose treatment on size at birth remains uncertain.

Most animal and human data show that when glucocorticoid-induced fetal growth restriction does occur, there is relatively rapid subsequent catch-up growth (Battin 2011; Crowther, Haslam, 2006; Gatford, Owens, 2008; Moss 2001; Stewart 1998). However, there is also concern that fluctuations in the rate of growth during early development may be associated with an increased risk of obesity and adult metabolic disease (Dulloo 2006; Wells 2007). Furthermore, some data suggest that antenatal glucocorticoids may affect postnatal growth (de Vries 2007) or skeletal development (Swolin-Eide 2002) without any changes in size at birth. Thus, it is important that the long-term effects of antenatal glucocorticoid treatment on growth and body composition are examined, regardless of whether or not there is evidence of fetal growth restriction. Although human randomised trials have shown that a single course of antenatal glucocorticoids does not affect body size or composition into early adulthood (Dalziel, Walker, 2005; Dalziel, Fenwick, 2006; Dessens 2000), there are currently no data from randomised trials on the long-term effects of repeat doses on growth beyond two to three years of age and no data on body composition.

4.3 Chapter hypothesis
That administration of repeat doses of antenatal betamethasone to women at risk of preterm birth at <32 weeks’ gestation, 7 or more days after an initial course of antenatal glucocorticoids, has no effect on body size, soft tissue composition and bone mass in their children at 6 to 8 years’ corrected age.

4.4 Additional statistical methods used in the chapter analyses
The primary aim of the analyses in this chapter was to compare body size and soft tissue composition and bone mass between children exposed to repeat doses of antenatal betamethasone and those exposed to placebo (single course). The analysis of body size was conducted in all participants of the New Zealand neurological follow-up study, whereas body composition analysis was limited to children in the physiological study who underwent total body DXA. Anthropometric data were converted to standard deviation scores (Z-scores), specific for sex and corrected age, using the 1990 British growth reference (Cole 1998). In this dataset, norms for neonatal anthropometry before term are
derived from cross-sectional birth data and hence are more reflective of _in utero_ fetal growth than postnatal growth in preterm infants. Total body DXA variables relating to bone mass were also converted to sex-specific standard deviation scores in relation to height, bone area, or lean mass as appropriate (Hogler 2003).

Primary analyses were unadjusted. Secondary analyses used mixed linear regression to adjust for the following factors, decided _a priori_: confounding by baseline variables of gestational age at trial entry, maternal preterm prelabour rupture of membranes as a reason for being at risk of preterm birth and European ethnicity (fixed effects); and the non-independence of data from twins (random effect). Further confounding by baseline variables with imbalance was explored using the change in estimates technique at the 10% level (Greenland 1989).

Additional multivariate analyses of DXA soft tissue masses were performed with adjustment for height to account for body size (Cole 2008; Wells 2002). Total fat mass was also analysed with adjustment for lean mass as this may provide a better assessment of the metabolic load or risk associated with body fat than in relation to height (Wells and Victora 2005). Central fatness was determined from the android (abdominal) fat mass, which was similarly adjusted for height and lean mass. Although central fatness is often analysed as the ratio of abdominal to peripheral fat, this may underestimate the metabolic risk associated with central fat (Wells and Victora 2005). However, the effect of treatment on fat distribution was determined by adjusting the android fat mass for gynoid fat mass. In addition to total body lean mass, regional analysis of limb lean mass was performed as this is more indicative of skeletal muscle mass (Bridge 2009; Kim 2006; Shih 2000).

Bone mass in cancellous bone was determined by regional analysis of the spine from the total body DXA scan, delineated by the spinal, head and pelvis cuts. Because normative data were not available to calculate Z-scores for this region of interest, differences in body size were accounted for adjusting bone mineral content and bone area for height. In addition, volumetric density was calculated by dividing vertebral bone mineral content by vertebral column volume, estimated assuming cubic dimensions (antero-posterior projected area raised to the power of 3/2). This is also known as the spinal bone mineral apparent density (Carter 1992).

Subgroup analysis, using a test of interaction, was performed for singletons and twins. Although this was a _post hoc_ analysis, randomisation was stratified according to the number of fetuses in each pregnancy.
A secondary aim of the analyses in this chapter was to assess the effect of repeat betamethasone treatment on postnatal growth in the neurological and health cohort. This was determined using anthropometric measurements that were previously obtained as part of the ACTORDS trial at birth, primary hospital discharge and two years’ corrected age. Only children with data at each time point were included. Growth was assessed by two methods. First, cross-sectional Z-scores for anthropometric measurements, calculated from the 1990 British growth reference (Cole 1998), were compared between treatment groups at each time point. For time points after birth, additional analyses were performed that adjusted for the previous Z-score to compare the effect of treatment on the change in Z-score over the intervening period. A limitation of using cross-sectional reference data for longitudinal analysis is that there may be confounding due to regression to the mean, although this would be expected to occur equally in randomly allocated groups when measurements are made at similar ages and time intervals (Barnett 2005).

Second, growth between two years’ and 6 to 8 years’ corrected age was assessed using conditional Z-scores for weight and height. These were calculated in LGROW software (Huiqi Pan, Harvey Goldstein and Jon Rasbash, v. 2.03). Conditional growth reflects the deviation in an individual’s growth from what is expected from his or her prior measurements based on growth patterns observed in longitudinal population data. Unlike cross-sectional reference data, this method accounts for regression to the mean and provides quantitative assessment of growth (Argyle 2003; Cole 1995; Pan 1997). Unfortunately, conditional growth before two years of age could not be determined, as neonatal discharge measurements were often performed before term corrected age, which was outside the age range of the reference data.

4.5 Results: Body size

4.5.1 Recruitment and characteristics of participants
Anthropometric measurements were attempted in all children in the neurological and health study but were obtained in only 301 children (145 repeat betamethasone group, 156 placebo group), as seven children with severe neurosensory disability could not be measured (three repeat betamethasone group, four placebo group). The baseline demographic and obstetric characteristics of participants in the neurological and health study are described in chapter three. There were no statistically significant differences between children exposed to repeat betamethasone or placebo.
Three children (one repeat betamethasone group, two placebo group) had recognised short stature, and five had cerebral palsy, including one case of spastic quadriplegia (repeat betamethasone group), two of spastic diplegia (one in each group), and two of hemiplegia (one in each group). In addition, eight children had chronic medical conditions that could affect growth. In the repeat betamethasone group, two children had celiac disease, one had lymphoblastic lymphoma and one had congenital multicystic kidneys requiring renal transplant. In the placebo group, one child each had cystic fibrosis, idiopathic bronchiectasis, Crohn’s disease and steroid-resistant nephrotic syndrome. The child with Crohn’s disease also had intellectual disability. Four girls in the repeat betamethasone group and one boy in the placebo group had commenced puberty and were Tanner stage 2.

4.5.2 Primary analyses
At early school-age, measures of body size did not differ between children exposed to repeat antenatal betamethasone and those exposed to placebo, including weight, height and head circumference, and their associated Z-scores (Table 4.1). There were also no differences between groups in variables relating to adiposity including body mass index and Z-score, and mid-arm circumference. Repeat betamethasone exposure did not affect body segment proportions, as assessed by sitting height, subischial leg length, the ratio of upper to lower body segments, and Z-scores for sitting height and subischial leg length.

4.5.3 Secondary analyses
Adjustment for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity, and clustering of fetuses in multiple gestations did not alter the results for body size at early school age (Table 4.1).

In sensitivity analysis, exclusion of children who had commenced puberty or those with recognised short stature, cerebral palsy, or chronic medical illness also did not alter the results.

In subgroup analysis, the effect of treatment on Z-scores for weight, height, head circumference and body mass index did not differ between singletons and twins.
Table 4.1: Anthropometry of children in the neurological and health study exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Repeat betamethasone (n=145)</th>
<th>Placebo (n=156)</th>
<th>Treatment effect: mean difference, ratio of geometric means, or risk ratio (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: mean difference or ratio of geometric means (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height—cm</td>
<td>123.9 (8.4)</td>
<td>123.3 (9.6)</td>
<td>0.7 (-1.3, 2.8)</td>
<td>0.48</td>
<td>0.4 (-1.9, 2.6)</td>
<td>0.76</td>
</tr>
<tr>
<td>Weight—kg†</td>
<td>23.9 (20.2, 28.3)</td>
<td>24.0 (20.4, 28.8)</td>
<td>1.00 (0.94, 1.06)</td>
<td>1.00</td>
<td>0.98 (0.92, 1.04)</td>
<td>0.52</td>
</tr>
<tr>
<td>Head circumference—cm</td>
<td>52.6 (2.0)</td>
<td>52.9 (1.9)</td>
<td>-0.3 (-0.7, 0.2)</td>
<td>0.22</td>
<td>0.3 (-0.8, 0.2)</td>
<td>0.20</td>
</tr>
<tr>
<td>Body mass index†</td>
<td>15.7 (14.6, 17.3)</td>
<td>15.9 (14.9, 17.6)</td>
<td>0.99 (0.95, 1.02)</td>
<td>0.42</td>
<td>0.97 (0.94, 1.01)</td>
<td>0.13</td>
</tr>
<tr>
<td>Mid-arm circumference—cm†</td>
<td>19.1 (17.5, 20.5)</td>
<td>19.1 (17.6, 21)</td>
<td>0.99 (0.96, 1.02)</td>
<td>0.55</td>
<td>0.98 (0.95, 1.01)</td>
<td>0.18</td>
</tr>
<tr>
<td>Sitting height—cm</td>
<td>68.1 (4.0)</td>
<td>67.7 (5.0)</td>
<td>0.5 (-0.6, 1.6)</td>
<td>0.35</td>
<td>0.2 (-1.0, 1.3)</td>
<td>0.79</td>
</tr>
<tr>
<td>Subischial leg length—cm</td>
<td>55.8 (5.2)</td>
<td>55.3 (5.2)</td>
<td>0.6 (-0.6, 1.8)</td>
<td>0.36</td>
<td>0.5 (-0.8, 1.8)</td>
<td>0.48</td>
</tr>
<tr>
<td>Upper to lower segment ratio</td>
<td>0.55 (0.02)</td>
<td>0.55 (0.01)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.91</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.81</td>
</tr>
<tr>
<td>Z-score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.18 (1.15)</td>
<td>0.11 (1.24)</td>
<td>0.07 (-0.20, 0.35)</td>
<td>0.59</td>
<td>0.04 (-0.25, 0.34)</td>
<td>0.76</td>
</tr>
<tr>
<td>Weight</td>
<td>0.18 (1.30)</td>
<td>0.21 (1.34)</td>
<td>-0.03 (-0.33, 0.27)</td>
<td>0.83</td>
<td>-0.11 (-0.44, 0.21)</td>
<td>0.48</td>
</tr>
<tr>
<td>Head circumference</td>
<td>-0.36 (1.37)</td>
<td>-0.15 (1.29)</td>
<td>-0.21 (-0.51, 0.09)</td>
<td>0.17</td>
<td>-0.24 (-0.56, 0.09)</td>
<td>0.15</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.11 (1.27)</td>
<td>0.26 (1.19)</td>
<td>-0.14 (-0.42, 0.13)</td>
<td>0.31</td>
<td>-0.24 (-0.54, 0.05)</td>
<td>0.11</td>
</tr>
<tr>
<td>Sitting height</td>
<td>0.66 (1.05)</td>
<td>0.58 (1.21)</td>
<td>0.08 (-0.18, 0.34)</td>
<td>0.55</td>
<td>-0.00 (-0.29, 0.29)</td>
<td>0.98</td>
</tr>
<tr>
<td>Subischial leg length</td>
<td>-0.32 (1.21)</td>
<td>-0.37 (1.14)</td>
<td>0.05 (-0.21, 0.33)</td>
<td>0.67</td>
<td>0.07 (-0.23, 0.37)</td>
<td>0.65</td>
</tr>
<tr>
<td>Pubarche¶</td>
<td>4 (3)</td>
<td>1 (1)</td>
<td>4.3 (0.5, 38.1)</td>
<td>0.20</td>
<td>0.5 (0.2, 1.6)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation), number (percent) or median (interquartile range). CI, confidence interval. Body mass index is the weight in kilograms divided by the square of the height in metres. Results exclude data for seven children in the neurological and health study who had severe neurosensory disability and did not have anthropometric measurements. For skewed data† the estimate of treatment effect is the ratio of geometric means. ‡Analyses adjusted for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity (fixed effect), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model. ¶All pubertal children were Tanner stage 2.
4.6 Results: Dual energy X-ray absorptiometry

4.6.1 Recruitment and characteristics of participants

DXA was available only in Auckland and Christchurch. After exclusion of six children in these centres with severe neurosensory disability (two repeat betamethasone group, four placebo group), 206 children were eligible for investigation (103 repeat betamethasone group, 103 placebo group), of whom 185 completed DXA scanning (91 repeat betamethasone group, 94 placebo group). A total of 149 scans were obtained in Auckland (Maurice and Nessie Paykel Clinical Research Unit, Liggins Institute) and 36 in Christchurch (St George’s Radiology, Merivale). All total body scans were of adequate quality without movement artifact.

There tended to be a greater proportion of DXA participants in the repeat betamethasone group with non-European ethnicity than in the placebo group (Table 4.2). There were no other statistically significant differences between groups for baseline demographic and obstetric characteristics. Body size measurements were also similar between DXA participants exposed to repeat betamethasone or placebo (Table 4.3).

Of children who completed DXA, seven children in the repeat betamethasone group had medical conditions that could affect body composition, including two with celiac disease, two with cerebral palsy, one with lymphoblastic lymphoma and one with congenital multicystic kidney disease requiring renal transplant. In the placebo group two children had recognised short stature, two had cerebral palsy, and one had steroid-resistant nephrotic syndrome. Three children in the repeat betamethasone group had entered puberty and were Tanner stage 2.

4.6.2 Total body soft tissue composition

At early school-age, there were no differences between children exposed to repeat antenatal betamethasone and those exposed to placebo in total body, gynoid and android fat mass, and in total body and limb lean mass (Table 4.4).

4.6.3 Total body bone mass

At early school-age, children exposed to repeat antenatal betamethasone and those exposed to placebo had similar total body bone mineral content and bone area, and associated Z-scores for height (Table 4.5). There was also no difference between groups in Z-scores for total body bone mineral content for lean tissue mass. Although there was a trend to reduced areal bone mineral density in children exposed to repeat betamethasone (mean difference –
0.01, 95%CI –0.03 to 0.00, P=0.09), this was no longer significant after subtraction of the head component (Table 4.5). Furthermore, there was no significant difference between groups in Z-scores for areal bone mineral density for age (Table 4.5).

In sub-regional analysis of the spine, repeat antenatal betamethasone exposure did not affect vertebral bone mineral content and area, areal mineral density, or estimated volumetric mineral density (bone mineral apparent density) (Table 4.5).

The risk of fracture, as determined by parental report, was not different between treatment groups. Fractures occurred predominantly in the upper limb (Table 4.5).

**4.6.4 Secondary analyses**
Adjustment for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity, and clustering of fetuses in multiple gestations did not alter the results for any of the DXA variables relating to soft tissue composition or bone mass.

There was also imbalance between treatment groups for the baseline variables of parity, pre-eclampsia or eclampsia as a reason for being at risk of preterm birth, and sex. However, inclusion of these variables as additional covariates in the secondary analysis of total body fat mass, lean mass and bone mineral content did not alter the estimate of treatment effect, using the change-in-estimates technique at the 10% level (Greenland 1989). Although there was imbalance in the frequency of multiple pregnancy as a reason for being at risk of preterm birth, actual rates of multiple pregnancy were not different between groups.

Adjustment of total body and regional soft tissue masses for height did not alter results (Table 4.4). Similarly, adjustment of total body and android fat mass for lean mass did not affect results. There was no evidence of altered fat distribution as determined from analysis of android fat mass adjusted for gynoid fat mass. Results for spinal bone mineral content or area were not altered by adjustment for height (Table 4.5).

In sensitivity analysis, exclusion of children who had commenced puberty or those with short stature or chronic medical conditions did not alter the results for soft tissue composition or bone mass.

In subgroup analysis, the effect of treatment on total body fat, lean mass, and bone mineral content did not differ between singletons and twins.
Table 4.2: Baseline characteristics of children exposed to repeat betamethasone or placebo who underwent total body dual-energy X-ray absorptiometry, and of their mothers.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of women</td>
<td>77</td>
<td>78</td>
<td>0.12</td>
</tr>
<tr>
<td>Maternal age—yr</td>
<td>30.0 (5.4)</td>
<td>31.3 (5.4)</td>
<td>0.12</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>0</td>
<td>33 (43)</td>
<td>30 (38)</td>
<td></td>
</tr>
<tr>
<td>1 to 3</td>
<td>41 (53)</td>
<td>40 (51)</td>
<td></td>
</tr>
<tr>
<td>≥ 4</td>
<td>3 (4)</td>
<td>8 (10)</td>
<td></td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>18 (24)</td>
<td>21 (27)</td>
<td>0.64</td>
</tr>
<tr>
<td>Gestational age at first glucocorticoids—wk†</td>
<td>26.8 (24.9, 28.9)</td>
<td>27.3 (24.8, 29.1)</td>
<td>0.90</td>
</tr>
<tr>
<td>Gestational age at trial entry—wk†</td>
<td>28.9 (26.4, 30.5)</td>
<td>28.7 (26.6, 30.2)</td>
<td>0.91</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>13 (17)</td>
<td>15 (19)</td>
<td>0.84</td>
</tr>
<tr>
<td>Main reasons for risk of preterm birth§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm prelabour rupture of membranes</td>
<td>25 (32)</td>
<td>18 (23)</td>
<td>0.21</td>
</tr>
<tr>
<td>Preterm labour</td>
<td>15 (19)</td>
<td>11 (14)</td>
<td>0.40</td>
</tr>
<tr>
<td>Severe fetal growth restriction</td>
<td>9 (12)</td>
<td>10 (13)</td>
<td>1.00</td>
</tr>
<tr>
<td>Preeclampsia or eclampsia</td>
<td>13 (17)</td>
<td>22 (28)</td>
<td>0.12</td>
</tr>
<tr>
<td>Cervical incompetence</td>
<td>5 (6)</td>
<td>6 (8)</td>
<td>1.00</td>
</tr>
<tr>
<td>Antepartum haemorrhage</td>
<td>18 (23)</td>
<td>20 (26)</td>
<td>0.85</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>9 (12)</td>
<td>4 (5)</td>
<td>0.16</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1)</td>
<td>6 (8)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Infant characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of children</td>
<td>91</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Sex of child—female</td>
<td>42 (46)</td>
<td>39 (41)</td>
<td>0.52</td>
</tr>
<tr>
<td>Ethnicity of child‡</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Maori</td>
<td>24 (26)</td>
<td>13 (14)</td>
<td></td>
</tr>
<tr>
<td>Pacific Peoples</td>
<td>12 (13)</td>
<td>14 (15)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>10 (11)</td>
<td>5 (5)</td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>45 (49)</td>
<td>62 (66)</td>
<td></td>
</tr>
</tbody>
</table>

Data are number (percent), mean (standard deviation) or †median (interquartile range). § Not mutually exclusive. ‡ Ethnicity prioritised in order of Maori, Pacific Peoples, Other, and European.
Table 4.3: Anthropometry of children exposed to repeat betamethasone or placebo who underwent total body dual-energy X-ray absorptiometry.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone (n=91)</th>
<th>Placebo (n=94)</th>
<th>Treatment effect: mean difference, ratio of geometric means, or risk ratio (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: mean difference or ratio of geometric means (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height—cm</td>
<td>124.4 (9.0)</td>
<td>124.4 (9.3)</td>
<td>0.0 (-2.7, 2.7)</td>
<td>1.00</td>
<td>-0.2 (-2.9, 2.4)</td>
<td>0.88</td>
</tr>
<tr>
<td>Weight—kg†</td>
<td>24.5 (20.0, 29.0)</td>
<td>24.2 (20.9, 30.3)</td>
<td>0.99 (0.92, 1.08)</td>
<td>0.89</td>
<td>0.98 (0.90, 1.07)</td>
<td>0.67</td>
</tr>
<tr>
<td>Head circumference—cm</td>
<td>52.7 (2.1)</td>
<td>53.0 (2.1)</td>
<td>-0.3 (-0.9, 0.3)</td>
<td>0.35</td>
<td>-0.3 (-0.9, 0.4)</td>
<td>0.41</td>
</tr>
<tr>
<td>Body mass index—kg/m²</td>
<td>15.7 (14.6, 12.3)</td>
<td>15.9 (14.8, 18.0)</td>
<td>0.99 (0.95, 1.04)</td>
<td>0.71</td>
<td>0.98 (0.93, 1.03)</td>
<td>0.44</td>
</tr>
<tr>
<td>Mid-arm circumference—cm†</td>
<td>19.1 (17.4, 20.5)</td>
<td>19.4 (17.4, 21.1)</td>
<td>0.99 (0.95, 1.03)</td>
<td>0.68</td>
<td>0.98 (0.94, 1.03)</td>
<td>0.38</td>
</tr>
<tr>
<td>Sitting height—cm</td>
<td>68.6 (4.1)</td>
<td>68.3 (4.6)</td>
<td>0.3 (-1.0, 1.6)</td>
<td>0.64</td>
<td>0.2 (-1.1, 1.4)</td>
<td>0.82</td>
</tr>
<tr>
<td>Subischial leg length—cm</td>
<td>55.7 (5.7)</td>
<td>55.5 (5.2)</td>
<td>0.2 (-1.5, 1.8)</td>
<td>0.85</td>
<td>0.0 (-1.8, 1.8)</td>
<td>0.99</td>
</tr>
<tr>
<td>Upper to lower segment ratio</td>
<td>0.55 (0.02)</td>
<td>0.55 (0.01)</td>
<td>0.00 (0.00, 0.01)</td>
<td>0.54</td>
<td>0.00 (0.00, 0.01)</td>
<td>0.50</td>
</tr>
<tr>
<td>Z-score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.28 (1.22)</td>
<td>0.27 (1.42)</td>
<td>0.02 (-0.34, 0.37)</td>
<td>0.92</td>
<td>0.00 (-0.39, 0.39)</td>
<td>1.00</td>
</tr>
<tr>
<td>Weight</td>
<td>0.25 (1.40)</td>
<td>0.29 (1.42)</td>
<td>-0.04 (-0.45, 0.36)</td>
<td>0.83</td>
<td>-0.08 (-0.54, 0.36)</td>
<td>0.70</td>
</tr>
<tr>
<td>Head circumference</td>
<td>-0.29 (1.45)</td>
<td>-0.10 (1.37)</td>
<td>-0.18 (-0.59, 0.22)</td>
<td>0.37</td>
<td>-0.17 (-0.62, 0.27)</td>
<td>0.44</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.14 (1.34)</td>
<td>0.26 (1.24)</td>
<td>-0.11 (-0.49, 0.27)</td>
<td>0.57</td>
<td>-0.16 (-0.57, 0.24)</td>
<td>0.44</td>
</tr>
<tr>
<td>Sitting height</td>
<td>0.85 (1.04)</td>
<td>0.77 (1.12)</td>
<td>0.08 (-0.25, 0.40)</td>
<td>0.63</td>
<td>0.01 (-0.35, 0.36)</td>
<td>0.97</td>
</tr>
<tr>
<td>Subischial leg length</td>
<td>-0.34 (1.32)</td>
<td>-0.30 (.121)</td>
<td>-0.05 (-0.43, 0.33)</td>
<td>0.80</td>
<td>-0.05 (-0.47, 0.37)</td>
<td>0.80</td>
</tr>
<tr>
<td>Pubarche¶</td>
<td>3 (3)</td>
<td>0 (0)</td>
<td>Non-convergence</td>
<td>0.12</td>
<td>Non-convergence</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean (standard deviation), number (percent) or †median (interquartile range). CI, confidence interval. Body mass index is the weight in kilograms divided by the square of the height in metres. For skewed data† the estimate of treatment effect is the ratio of geometric means. ‡Analyses adjusted for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity (fixed effect), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model. ¶All pubertal children were Tanner stage 2.
Table 4.4: Dual-energy X-ray absorptiometry soft tissue composition of children exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone (n=91)</th>
<th>Placebo (n=94)</th>
<th>Treatment effect: ratio of geometric means (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: ratio of geometric means (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lean mass—kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body</td>
<td>19.3 (16.4, 22.2)</td>
<td>19.1 (17.0, 22.4)</td>
<td>0.98 (0.93, 1.04)</td>
<td>0.50</td>
<td>0.98 (0.92, 1.04)</td>
<td>0.52</td>
</tr>
<tr>
<td>adjusted for height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper and lower limbs</td>
<td>7.1 (5.8, 8.6)</td>
<td>7.3 (5.9, 8.9)</td>
<td>0.98 (0.91, 1.06)</td>
<td>0.63</td>
<td>0.97 (0.90, 1.05)</td>
<td>0.51</td>
</tr>
<tr>
<td>adjusted for height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limbs</td>
<td>5.5 (4.4, 6.5)</td>
<td>5.5 (4.6, 6.8)</td>
<td>0.98 (0.91, 1.06)</td>
<td>0.67</td>
<td>0.98 (0.91, 1.07)</td>
<td>0.69</td>
</tr>
<tr>
<td>adjusted for height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted for lean mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fat mass—kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body</td>
<td>3.7 (2.6, 6.3)</td>
<td>3.7 (2.5, 6.2)</td>
<td>1.01 (0.82, 1.24)</td>
<td>0.91</td>
<td>0.96 (0.76, 1.20)</td>
<td>0.70</td>
</tr>
<tr>
<td>adjusted for height</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>adjusted for lean mass</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gynoid (thigh)</td>
<td>1.0 (0.7, 1.4)</td>
<td>0.9 (0.7, 1.4)</td>
<td>1.02 (0.86, 1.21)</td>
<td>0.80</td>
<td>0.97 (0.81, 1.17)</td>
<td>0.77</td>
</tr>
<tr>
<td>adjusted for height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted for lean mass</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Android (abdominal)</td>
<td>0.2 (0.2, 0.4)</td>
<td>0.2 (0.2, 0.5)</td>
<td>1.00 (0.79, 1.26)</td>
<td>1.00</td>
<td>0.94 (0.72, 1.21)</td>
<td>0.62</td>
</tr>
<tr>
<td>adjusted for height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted for lean mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted for log (gynoid fat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are median (interquartile range). CI, confidence interval. ‡ Analyses adjusted for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity (fixed effect), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model.
Table 4.5: Total body dual-energy X-ray absorptiometry bone mass and fracture incidence of children exposed to repeat betamethasone and placebo.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone (n=91)</th>
<th>Placebo (n=94)</th>
<th>Treatment effect: mean difference, ratio of geometric means, or risk ratio (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: mean difference, ratio of geometric means, or risk ratio (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineral content—g†</td>
<td>860 (702, 1025)</td>
<td>869 (734, 1077)</td>
<td>0.97 (0.90, 1.05)</td>
<td>0.49</td>
<td>0.97 (0.89, 1.05)</td>
<td>0.42</td>
</tr>
<tr>
<td>Bone area—cm²‡</td>
<td>1041 (894, 1172)</td>
<td>1041 (911, 1241)</td>
<td>0.99 (0.93, 1.05)</td>
<td>0.75</td>
<td>0.98 (0.92, 1.05)</td>
<td>0.60</td>
</tr>
<tr>
<td>Areal bone mineral density—g/cm²</td>
<td>0.83 (0.05)</td>
<td>0.85 (0.06)</td>
<td>-0.01 (-0.03, 0.00)</td>
<td>0.09</td>
<td>-0.01 (-0.03, 0.00)</td>
<td>0.13</td>
</tr>
<tr>
<td>Areal bone mineral density less head—g/cm²</td>
<td>0.68 (0.07)</td>
<td>0.69 (0.07)</td>
<td>0.00 (-0.03, 0.01)</td>
<td>0.41</td>
<td>-0.01 (-0.04, 0.01)</td>
<td>0.35</td>
</tr>
<tr>
<td>Z-score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral content for height</td>
<td>0.32 (0.85)</td>
<td>0.44 (0.80)</td>
<td>-0.12 (-0.35, 0.12)</td>
<td>0.34</td>
<td>-0.16 (-0.43, 0.11)</td>
<td>0.24</td>
</tr>
<tr>
<td>Bone area for height</td>
<td>0.17 (1.01)</td>
<td>0.22 (0.90)</td>
<td>-0.05 (-0.33, 0.23)</td>
<td>0.72</td>
<td>-0.13 (-0.45, 0.18)</td>
<td>0.39</td>
</tr>
<tr>
<td>Bone mineral content for lean tissue mass</td>
<td>0.19 (0.94)</td>
<td>0.24 (1.01)</td>
<td>-0.05 (-0.32, 0.24)</td>
<td>0.75</td>
<td>-0.08 (-0.40, 0.23)</td>
<td>0.60</td>
</tr>
<tr>
<td>Areal bone mineral density for age</td>
<td>0.37 (0.88)</td>
<td>0.57 (0.92)</td>
<td>-0.19 (-0.45, 0.07)</td>
<td>0.15</td>
<td>-0.20 (-0.49, 0.10)</td>
<td>0.19</td>
</tr>
<tr>
<td>Spine region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone mineral content—g†</td>
<td>70 (61, 91)</td>
<td>71 (61, 90)</td>
<td>0.99 (0.90, 1.08)</td>
<td>0.75</td>
<td>0.96 (0.87, 1.06)</td>
<td>0.41</td>
</tr>
<tr>
<td>adjusted for height</td>
<td>10 (98, 129)</td>
<td>111 (100, 130)</td>
<td>0.99 (0.93, 1.05)</td>
<td>0.72</td>
<td>0.97 (0.91, 1.04)</td>
<td>0.42</td>
</tr>
<tr>
<td>Bone area—cm²‡</td>
<td>110 (98, 129)</td>
<td>111 (100, 130)</td>
<td>0.99 (0.93, 1.05)</td>
<td>0.72</td>
<td>0.97 (0.91, 1.04)</td>
<td>0.42</td>
</tr>
<tr>
<td>adjusted for height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Repeat betamethasone (n=91)</td>
<td>Placebo (n=94)</td>
<td>Treatment effect: mean difference, ratio of geometric means, or risk ratio (95% CI)</td>
<td>P</td>
<td>Adjusted treatment effect: mean difference, ratio of geometric means, or risk ratio (95% CI)‡</td>
<td>Adjusted P‡</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------------------------</td>
<td>----------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>-----------</td>
<td>------------------------------------------------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Areal bone mineral density—g/cm²</td>
<td>0.65 (0.07)</td>
<td>0.66 (0.07)</td>
<td>0.00 (-0.02, 0.02)</td>
<td>0.86</td>
<td>-0.01 (-0.03, 0.01)</td>
<td>0.51</td>
</tr>
<tr>
<td>Bone mineral apparent density—g/cm³</td>
<td>0.06 (0.005)</td>
<td>0.06 (0.005)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.78</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.86</td>
</tr>
<tr>
<td>Any childhood fracture</td>
<td>12 (13)</td>
<td>10 (11)</td>
<td>1.2 (0.6, 2.7)</td>
<td>0.65</td>
<td>1.00 (0.94, 1.05)</td>
<td>0.86</td>
</tr>
<tr>
<td>Upper limb</td>
<td>11 (12)</td>
<td>7 (7)</td>
<td>3 (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean (standard deviation), number (percent) or †median (interquartile range). CI, confidence interval. For skewed data† the estimate of treatment effect is the ratio of geometric means. ‡Analyses for continuous variables adjusted for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity (fixed effect), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model. Analyses for categorical data adjusted for gestational age and preterm prelabour rupture of membranes at trial entry, and European ethnicity using log binomial regression.
4.7 Results: Growth

Of the 301 children in the neurological and health study with anthropometric data at early school age, 300 had had weight measured at birth, primary neonatal discharge, and two years’ corrected age. Similarly, length / height and head circumference were measured at all four time points in 264 and 289 children, respectively.

There were no significant differences between children exposed to repeat antenatal betamethasone and those exposed to placebo in cross-sectional Z-scores for weight, length / height, and head circumference at birth, primary neonatal discharge, two years’ corrected age, and 6 to 8 years’ corrected age (Table 4.6). After adjustment for previous Z-score, treatment with repeat antenatal betamethasone compared to placebo tended to have a positive effect on weight gain at primary neonatal discharge (Table 4.6). However, this effect was abolished after further adjustment for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity, and clustering of fetuses in multiple gestations (Table 4.6).

Conversely, after adjustment for previous Z-score, treatment with repeat antenatal betamethasone compared with placebo tended to have a negative effect on head growth at early school-age, which was statistically significant after further adjustment for potential confounders (Table 4.6). However, in a sensitivity analysis, exclusion of children with short stature or chronic medical conditions reduced the significance of the effect of treatment on head circumference Z-score, adjusted for previous Z-score (repeat betamethasone versus placebo mean difference –0.14, 95% CI –0.32, 0.04, P=0.14; fully adjusted P=0.07 [n=274]).

Conditional growth in weight and height between two and 6 to 8 years’ corrected age did not differ between children exposed to repeat betamethasone and those exposed to placebo (Table 4.7).
Table 4.6: Growth of children in the neurological and health study exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th>Z-score</th>
<th>Repeat betamethasone</th>
<th>Placebo</th>
<th>Treatment effect: mean difference (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: mean difference (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>-0.44 (1.13)</td>
<td>-0.32 (1.06)</td>
<td>-0.11 (-0.36, 0.13)</td>
<td>0.37</td>
<td>-0.11 (-0.36, 0.14)</td>
<td>0.40</td>
</tr>
<tr>
<td>Neonatal discharge</td>
<td>-0.97 (1.10)</td>
<td>-1.04 (0.94)</td>
<td>0.08 (-0.16, 0.31)</td>
<td>0.52</td>
<td>0.00 (-0.24, 0.25)</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Adjusted for previous Z-score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.15 (-0.01, 0.32)</td>
<td>0.06</td>
<td>0.07 (-0.09, 0.24)</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 years’ corrected age</td>
<td>0.02 (1.17)</td>
<td>0.01 (1.29)</td>
<td>0.01 (-0.27, 0.29)</td>
<td>0.94</td>
<td>-0.02 (-0.33, 0.28)</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Adjusted for previous Z-score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.03 (-0.28, 0.22)</td>
<td>0.81</td>
<td>-0.02 (-0.30, 0.26)</td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 to 8 years’ corrected age</td>
<td>0.18 (1.30)</td>
<td>0.21 (1.34)</td>
<td>-0.03 (-0.33, 0.27)</td>
<td>0.85</td>
<td>-0.11 (-0.44, 0.21)</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Adjusted for previous Z-score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.04 (-0.25, 0.17)</td>
<td>0.71</td>
<td>-0.09 (-0.31, 0.12)</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Length / height</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>-0.32 (1.24)</td>
<td>-0.33 (1.11)</td>
<td>0.02 (-0.27, 0.30)</td>
<td>0.90</td>
<td>0.01 (-0.29, 0.31)</td>
<td>0.95</td>
</tr>
<tr>
<td>Neonatal discharge</td>
<td>-1.03 (1.77)</td>
<td>-1.24 (1.95)</td>
<td>0.21 (-0.25, 0.66)</td>
<td>0.37</td>
<td>0.08 (-0.40, 0.56)</td>
<td>0.73</td>
</tr>
<tr>
<td><strong>Adjusted for previous Z-score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.19 (-0.16, 0.54)</td>
<td>0.29</td>
<td>0.07 (-0.31, 0.44)</td>
<td>0.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 years’ corrected age</td>
<td>0.13 (1.11)</td>
<td>-0.05 (1.26)</td>
<td>0.18 (-0.11, 0.47)</td>
<td>0.22</td>
<td>0.18 (-0.14, 0.51)</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Adjusted for previous Z-score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.13 (-0.14, 0.40)</td>
<td>0.33</td>
<td>0.17 (-0.13, 0.47)</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 to 8 years’ corrected age</td>
<td>0.19 (1.19)</td>
<td>0.09 (1.24)</td>
<td>0.10 (-0.20, 0.40)</td>
<td>0.50</td>
<td>0.06 (-0.27, 0.38)</td>
<td>0.74</td>
</tr>
<tr>
<td><strong>Adjusted for previous Z-score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.04 (-0.24, 0.16)</td>
<td>0.71</td>
<td>-0.07 (-0.29, 0.14)</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Head circumference</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>-0.15 (0.07)</td>
<td>-0.16 (0.06)</td>
<td>0.01 (-0.01, 0.02)</td>
<td>0.39</td>
<td>0.00 (-0.01, 0.02)</td>
<td>0.70</td>
</tr>
<tr>
<td>Z-score</td>
<td>Repeat betamethasone</td>
<td>Placebo</td>
<td>Treatment effect: mean difference (95% CI)</td>
<td>P</td>
<td>Adjusted treatment effect: mean difference (95% CI) ‡</td>
<td>Adjusted P‡</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>----------------------</td>
<td>---------</td>
<td>-----------------------------------------------</td>
<td>------------</td>
<td>------------------------------------------------------</td>
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</tr>
<tr>
<td>neonatal discharge</td>
<td>-0.08 (1.22)</td>
<td>-0.17 (1.16)</td>
<td>0.09 (-0.19, 0.37)</td>
<td>0.53</td>
<td>0.04 (-0.26, 0.34)</td>
<td>0.79</td>
</tr>
<tr>
<td>Adjusted for previous Z-score</td>
<td>-0.10 (-0.21, 0.33)</td>
<td>0.06</td>
<td>0.06 (0.09, -0.17)</td>
<td>0.66</td>
<td>0.02 (-0.27, 0.31)</td>
<td>0.89</td>
</tr>
<tr>
<td>2 years’ corrected age</td>
<td>-0.56 (1.40)</td>
<td>-0.51 (1.36)</td>
<td>-0.05 (-0.37, 0.27)</td>
<td>0.75</td>
<td>-0.02 (-0.36, 0.32)</td>
<td>0.89</td>
</tr>
<tr>
<td>Adjusted for previous Z-score</td>
<td>-0.10 (-0.38, 0.19)</td>
<td>-0.10</td>
<td>0.10 (0.00, -0.20)</td>
<td>0.50</td>
<td>-0.04 (-0.35, 0.26)</td>
<td>0.77</td>
</tr>
<tr>
<td>6 to 8 years’ corrected age</td>
<td>-0.37 (1.38)</td>
<td>-0.15 (1.31)</td>
<td>-0.21 (-0.52, 0.10)</td>
<td>0.18</td>
<td>-0.24 (-0.57, 0.10)</td>
<td>0.17</td>
</tr>
<tr>
<td>Adjusted for previous Z-score</td>
<td>-0.17 (-0.35, 0.01)</td>
<td>-0.17</td>
<td>0.17 (0.08, -0.26)</td>
<td>0.06</td>
<td>-0.21 (-0.40, -0.03)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation). CI, confidence interval. Only children with data at each time point are included. ‡ Analyses adjusted for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity (fixed effect), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model.

Table 4.7: Conditional growth between two and 6 to 8 years’ corrected age of children in the neurological and health study exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th>Conditional Z-score</th>
<th>Repeat betamethasone</th>
<th>Placebo</th>
<th>Treatment effect: mean difference (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: mean difference (95% CI) ‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight conditional on previous weight</td>
<td>0.57 (1.33)</td>
<td>144</td>
<td>0.63 (1.33)</td>
<td>155</td>
<td>-0.06 (-0.36, 0.24)</td>
<td>0.69</td>
</tr>
<tr>
<td>Height conditional on previous height</td>
<td>0.80 (1.42)</td>
<td>143</td>
<td>0.97 (1.64)</td>
<td>153</td>
<td>-0.18 (-0.53, 0.17)</td>
<td>0.32</td>
</tr>
<tr>
<td>Weight conditional on current height and previous weight</td>
<td>0.35 (1.38)</td>
<td>143</td>
<td>0.50 (1.21)</td>
<td>153</td>
<td>-0.15 (-0.44, 0.15)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation). CI, confidence interval. ‡Analyses adjusted for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity (fixed effect), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model.
4.8 Discussion

This chapter reports body size and composition of 301 and 185 New Zealand children at early school age, respectively, whose mothers participated in the ACTORDS randomised trial. At 6 to 8 years’ corrected age there were no significant differences in body size, soft tissue composition or bone mass between children exposed to repeat doses of antenatal betamethasone at <32 weeks’ gestation and those not so exposed, 7 or more days after an initial course of antenatal glucocorticoids. Additional analysis of growth suggested that repeat antenatal betamethasone treatment may have a positive effect on weight gain in the neonatal period, but it may have a negative effect on head circumference growth in later childhood. However, these results were inconclusive.

Childhood growth is important not only as a marker of current health but also because it influences adult body size and composition, and risk of chronic disease. For example, accelerated linear growth or weight gain, and early maturation are associated with an increased risk of obesity in adulthood (Baird 2005; Eriksson 2001; Parsons 1999; Parsons 2001; Singh 2008), which in turn is a major risk factor for metabolic syndrome and cardiovascular disease. Conversely, poor growth in early life can permanently reduce lean tissue mass, especially skeletal muscle (Eriksson 2002; Sayer 2008), leading to decreased fitness and longevity (Rolland 2008; Yliharsila 2007). There are also associations between childhood body size and growth, and risk of cancer (Davis 2011; Fuemmeler 2009; Li 2010; Okasha 2002).

Furthermore, certain patterns of prenatal and postnatal growth appear to be particularly important in determining propensity to chronic disease. For example, poor fetal and infant growth followed by accelerated childhood (compensatory or catch-up) growth has been associated with a substantially increased risk of diabetes and cardiovascular disease, even if adult body size remains within the average range (Barker 2005; Barker 2002; Osmond 1993). An important pathogenic pathway that may explain these associations, especially in males, is persisting high relative fat mass (Eriksson 1999; Parsons 2001). In view of these data and the fact that in some animal studies antenatal glucocorticoid treatment has been shown to have similar effects on early growth, albeit often at higher doses than are used clinically, careful assessment of the long-term effects of antenatal glucocorticoid treatment on growth and body composition is important.

Although animal studies have identified several potential mechanisms by which antenatal glucocorticoids could influence postnatal growth trajectories, including effects on the
somatotrophic axis (Gatford, Owens, 2008), fat metabolism (Cleasby 2003), and glucose homeostasis (Moss 2001; Nyirenda 1998; Sloboda 2005), examination of the long-term effects on growth and body composition has been limited, particularly after repeat doses. Of the few animal studies that have assessed long-term outcomes, most have not shown any adverse effects on postnatal growth or adult body size once any initial neonatal catch-up growth has occurred (Bramlage 2009; Liu 2001; Moss 2005; Stewart 1997). Some animal studies did demonstrate reduced long-term weight gain and linear growth after repeat antenatal glucocorticoid treatment (Cleasby 2003; de Vries 2007), but the duration of drug exposure was quite long relative to the length of gestation, and so it is unlikely that these data apply to the clinical situation. Indeed, two human randomised trials have shown that a single course of antenatal glucocorticoids has no effect on child (Dalziel 2004; Smolders-de Haas 1990) or adult (Dalziel, Walker, 2005; Dessens 2000) body size and no effect on adult anthropometric variables relating to adiposity, including skin fold thickness and the ratio of waist and hip circumferences (Dalziel, Walker, 2005).

Some trials, including ACTORDS, have shown that treatment with repeat antenatal glucocorticoids can have a small negative effect on size at birth, although combined results from all trials remain inconclusive (McKinlay 2012). This was not evident in subgroup analysis of the New Zealand cohort but the mean difference between groups for birthweight Z-scores was of a similar magnitude to that seen in the whole ACTORDS trial (Crowther, Haslam, 2006). These differences are no longer apparent by the time of primary hospital discharge, indicating that catch-up growth must occur (Crowther, Haslam, 2006; McEvoy 2002). This is supported by a previous subgroup analysis of infants in the ACTORDS trial born in Auckland who underwent detailed neonatal growth assessment, including knemometry (Battin 2011). The repeat antenatal betamethasone group had greater growth velocity than the placebo group for weight, head circumference and limb length from three to five weeks after birth. The current finding in the whole New Zealand cohort of a possible positive effect of repeat antenatal betamethasone treatment on weight gain from birth to discharge, as demonstrated by relative change in Z-scores, is consistent with this more detailed analysis. Catch-up growth is a widely observed phenomenon in biology and refers to the ability of an organism to grow at an accelerated rate following a period of retarded growth due to undernutrition (Mangel 2005; Metcalfe 2001). In animals, this is achieved primarily through hyperphagia (Metcalfe 2001), but this is unlikely to occur in preterm infants where feed volumes are largely externally regulated. Instead, accelerated postnatal growth after antenatal glucocorticoid exposure may be due to the
maturational effect of glucocorticoids on organs and physiological processes related to nutrient absorption and utilisation. For example, antenatal glucocorticoids promote growth of the intestinal mucosa, increase secretion of gastric acid and digestive enzymes, upregulate gluconeogenic enzymes in the liver and enhance glycogen storage (Ballard 1995; Fowden 1998). These effects are likely to be greater in infants exposed to repeat antenatal glucocorticoids. In addition, the reduced incidence and severity of neonatal lung disease in infants exposed to repeat antenatal glucocorticoids may contribute to better nutrition and growth, especially through earlier establishment of enteral feeding.

Despite a possible influence on early growth, exposure to repeat antenatal glucocorticoids does not appear to affect subsequent growth in infancy. There is evidence from four trials, including ACTORDS, that body size at two to three years of age is not different between children exposed to a single course of antenatal glucocorticoids and those exposed to repeat doses (McKinlay 2012). Our study now extends this evidence to early school age, with no differences observed between treatment groups for any of the measures of body size. Furthermore, longitudinal analysis of both cross-sectional and conditional Z-scores demonstrated that the groups are following similar growth trajectories for weight and height. Therefore, there is no evidence of altered somatic growth in childhood after exposure to repeat antenatal betamethasone.

Nevertheless, accelerated postnatal growth after a period of fetal growth restriction has been strongly linked in epidemiological studies of historical European cohorts to increased risk of adult cardiovascular and metabolic disease (Barker 2005; Barker 2002; Osmond 1993). Similar associations appear to be present in recent prospective cohorts in industrialized countries (Ibanez 2006; Ong, Ahmed, 2000; Ong 2004) and possibly developing countries undergoing nutritional transition (Adair 2009; Victora 2008). A possible explanation for these observations is that catch-up growth is a marker of an imbalance between long-term metabolic capacity and load (Hales 1992; Wells 2011). It is proposed that poor growth during early development, especially fetal life, leads to permanent reductions in pancreatic beta-cell mass and insulin secretory capacity, nephron number, liver volume, lung function, and skeletal muscle mass. These traits show a broadly continuous association with birth weight (Chang 2006; Chomtho 2008; Dezateux 2004; Gatford, Mohammad, 2008; Hancox 2009; Luyckx 2010; Ong 2004; Singhal, Wells, 2003), and they tend to track into later life because growth by hyperplasia largely ceases after early infancy (Hales 1992). However, accelerated weight gain during the hypertrophic growth phase results in larger tissue masses and increased metabolic demand relative to
functional capacity, potentially contributing to various aspects of metabolic syndrome (Wells 2011). Metabolic load may be further exacerbated by sedentary lifestyle, which reduces metabolic flexibility, and poor quality diet (Wells 2011).

However, catch-up growth is not invariably detrimental and it seems unlikely that repeat antenatal glucocorticoid treatment poses any substantial risk of long-term metabolic disease by virtue of effects on early growth, for several reasons. First, the effect of repeat antenatal betamethasone on fetal growth, if present, is small with birthweight being reduced by only about 80 g or a tenth of a standard deviation (McKinlay 2012). This is considerably less than the growth restrictive effect of preterm birth per se, which is due to various factors such as recurrent severe illness, inadequate nutrition, and immaturity of the somatotrophic axis (Cooke 2004; Hay 2008; Kajantie 2003). For example, by the time of primary hospital discharge the mean weight and length of both groups in the New Zealand ACTORDS cohort was approximately one standard deviation below the reference population mean. While the accelerated postnatal growth of infants exposed to repeat antenatal glucocorticoids can be viewed as compensatory catch-up growth, it may also act to limit the inevitable growth faltering that occurs after preterm birth, which may itself be a risk factor for future metabolic disease (Hofman, Regan and Cutfield 2006).

Second, catch-up growth seems to be deleterious primarily if it is delayed or persists after early infancy (Adair 2003; Barker 2005; Ezzahir 2005; Sachdev 2005; Torre 2008; Wells, Hallal, 2005). In contrast, the growth effects of antenatal glucocorticoids appear to be transitory and this study has confirmed that repeat antenatal betamethasone treatment does not have any effect on somatic growth beyond the neonatal period. Although a study in very preterm infants found that even relatively mild acceleration of neonatal growth due to nutritional supplementation was associated with higher blood pressure and indirect markers of insulin resistance in adolescence, these effects were largely confined to high growth velocity in the first two weeks after birth but not later in the neonatal period (Singhal, Fewtrell, 2003; Singhal 2004). However, the effect of repeat antenatal glucocorticoid treatment occurs after this window (Battin 2011).

Third, a key feature of adverse catch-up growth appears to be a disproportionate gain in fat mass with delayed reconstitution of lean tissue mass, especially muscle (Dulloo 2006). Long-term deficits of muscle mass are strongly associated with poor fetal and infant growth (Eriksson 2002; Gale 2001; Kahn 2000; Kensara 2005; Loos 2001, 2002; Phillips 1995; Sachdev 2005; Sayer 2004; Yliharsila 2007), which may partly explain the
metabolic risk associated with these conditions, including excess adiposity. Muscle has a key role in thermogenesis and determination of metabolic rate, and is the main site for glucose clearance (Dulloo 2006). Therefore, reduced muscle mass may predispose to increased fat deposition. Although excess fat gain may not emerge until after infancy, most studies show that individuals at marked risk of future obesity (Cole 2004; De Kroon 2010; Gardner 2009; Taylor 2005; Williams 2009) or cardiovascular and metabolic disease (Barker 2010; Eriksson 2000; Eriksson 1999; Eriksson 2001; Eriksson 2003; Forsen 2000; Kajantie 2004) demonstrate a pattern of accelerated growth in weight for height by mid-childhood, sometimes referred to as early adiposity rebound. However, this was not evident in this study. Furthermore, direct measurement of soft tissue composition by DXA found that exposure to repeat doses of antenatal betamethasone was not associated with altered total or central fat mass, or lean mass at early school age. In particular, lean mass, which is highly correlated with skeletal muscle mass (Bridge 2009; Kim 2006; Shih 2000), did not differ between treatment groups. Thus, there is no evidence of altered body composition at early school age. Future differences between treatment groups in weight for height seem unlikely given that total body fat mass at five years of age has been shown to be predictive of later fat mass (Goulding 2003) and that body mass index shows moderate tracking stability from mid-childhood onwards (Freedman, Khan, 2005; Guo 2000; Howe 2010; Nader 2006; Power 1997; Vanhala 1998).

The Auckland Steroid Trial showed that adults exposed to a single course of antenatal betamethasone compared to those exposed to placebo had a greater proportion of stature in lower body segment, even though height was not significantly different (Dalziel, Fenwick, 2006). Some animal studies have suggested that this may be due to a differential effect of antenatal glucocorticoids on linear growth between the appendicular and axial skeleton. However, data are conflicting as both increases (Hauser 2008; Swolin-Eide 2002) and decreases (de Vries 2007) in long bone growth have been reported. In the present study, body segment proportions were identical between children exposed to repeat antenatal betamethasone and those exposed to a single course of antenatal glucocorticoids. This suggests that the difference in body segment proportions at final attained height is not due to altered long bone growth in childhood. A more likely explanation is a slight alteration in the timing of skeletal maturation at puberty, as appendicular growth is more rapid than axial growth before puberty and decelerates at puberty when axial growth accelerates. Indeed, there was a trend in one randomised trial for boys exposed to a single course of antenatal betamethasone to have slightly later pubarche compared to those exposed to
placebo (Smolders-de Haas 1990). There are also some data in animals to suggest that antenatal glucocorticoid exposure may affect testicular development (Pedrana 2008; Piffer 2009) and the timing of puberty (Smith 2000). Although the aim of the current study was to assess pre-pubertal outcomes, several children had entered early stages of puberty. However, the numbers of these children were too few to make any comparison between groups of the age of puberty onset.

Animal studies have raised concern that antenatal glucocorticoids, particularly repeat doses, may permanently reduce brain mass and volume (Liu 2001; Moss 2005). This was underscored by a human observational study that found that infants exposed to repeat doses had reduced cortical convolution (Modi 2001). The ACTORDS trial also showed that infants exposed to repeat doses of antenatal betamethasone had slightly reduced Z-scores for head circumference at birth (Crowther, Haslam, 2006), although this was not observed in this analysis of New Zealand subgroup. There were no differences between groups in head circumference by the time of primary hospital discharge, nor at two years’ corrected age (Crowther 2007), and this has been confirmed in three other trials (McKinlay 2012). These data are reassuring as head circumference is a reliable surrogate for whole-brain growth in young children and predicts later cognitive outcome (Bartholomeusz 2002; Cooke 1977; Epstein 1978; Gale 2006; Lange 2010). It is interesting to note that while children in both treatment groups demonstrated catch-up growth in height and weight, there was slowing of head growth after neonatal discharge such that both groups had a mean head circumference at two years’ corrected age of approximately half a standard deviation below the population mean. Although treatment groups did not differ in head circumference at 6 to 8 years’ corrected age, there was some suggestion that children exposed to repeat betamethasone had slower growth in head circumference after two years’ corrected age. However, the effect of treatment on the relative gain in head circumference Z-score was not significant after exclusion of children with known short stature or chronic medical illness, suggesting that the observed effect may be due to confounding. Ultimately, the significance of this finding will depend on the psychological and educational outcomes that were assessed as part of the early school-age follow-up.

Adequate accrual of bone mass in childhood is important not only for the prevention of paediatric fractures (Clark, Ness, 2006; Clark, Tobias, 2006; Flynn 2007) but also for the attainment of peak bone mass in early adulthood, which is a major determinant of the risk of osteoporosis in later life (Bonjour 2009). Multiple elements of bone influence its biomechanical strength, including size, morphology (shape and distribution of bone tissue),
mineral content, and the structural organization of the organic matrix (Seeman 2006). DXA assesses two of these components: bone size (two-dimensional) and mineral content (Fewtrell 2003). In a given individual, these traits tend to track from early life through to the completion of skeletal growth, at which time bone mass and strength reach a peak (Cooper, Harvey, 2009). This trajectory is determined in large part by genotype (Davies 2005; Harvey 2008; Ralston 2010), but there is increasing evidence that the perinatal environment, especially fetal nutrition, can have long-term effects on bone mass accrual (Cooper, Harvey, 2009). During fetal and early postnatal life there is rapid gain in bone mineral. In contrast, childhood gains in bone mass are primarily due to increases in bone size, and there is relatively little change in true volumetric mineral density until late puberty (Hangartner 1996; Lu 1996; Magarey 1999; Moyer-Mileur 2008; Neu 2001).

In mature bone, it is well known that glucocorticoids cause accelerated bone loss, especially of trabecular bone, principally due to osteoblast dysfunction, which reduces bone formation (van Staa 2006). Antenatal glucocorticoids can also affect fetal bone turnover and mineralisation (Fonseca 2009; Korakaki 2007; Mosier 1981; Saarela 2001), which could be due to a direct effect on bone, or it could occur indirectly as a result of reduced fetal plasma calcium concentration (Moss, Nitsos, 2003) or changes to the fetal somatotrophic axis (Cooper, Harvey, 2009). Although one study found that adult female rats exposed to antenatal glucocorticoids had reduced femoral cortical thickness (Swolin-Eide 2002), most evidence in animals and humans indicates that any effect of antenatal glucocorticoids on fetal bone metabolism is transient and does not lead to altered bone mass in the long-term (Dalziel, Fenwick, 2006; Korakaki 2007; Swolin-Eide 2002).

This study provides further evidence for the long-term safety of repeat doses of antenatal betamethasone compared to a single course of glucocorticoids with regard to bone mass accrual. Total skeletal size and mineral content were similar between treatment groups, for both raw data and Z-scores for sex and height. Although there was a trend to reduced areal bone mineral density in the repeat betamethasone group, it is unlikely that this reflects a true difference in bone mineralisation as there was no such trend in the adjusted analyses or after exclusion of the head region. Because the skull has a different developmental trajectory to the rest of the skeleton and its mass is less affected by environmental conditions, it has been suggested that sub-total assessment may be a more sensitive measure of change in bone mass in children (Taylor 1997).
Furthermore, although areal bone mineral density is a widely used index of bone mineralisation and has the advantage that it minimises measurement error, it is also susceptible to size-related artifacts, as bone mineral content is frequently not directly proportional to bone area (Prentice 1994). Correction of bone mineral content for the antero-posterior bone area tends to overestimate bone mineral when bones are larger than average and underestimate it when they are smaller than average (Carter 1992; Prentice 1994). This is a particular problem for total body DXA because it combines results for multiple bones of varying shape, depth and thickness, and estimation of bone volume based on projected area is possible for only a limited number of sites with predictable geometry (Leonard 2004). Consequently, areal density may not adequately adjust for the volume of bone over which mineral is distributed. In other words, bone mineral density measured by two-dimensional densitometry represents a mixture of true mineral density and skeletal size (Molgaard 1997). Therefore, in paediatric analysis it is recommended that bone mineral content be assessed directly with adjustment for body size (Prentice 1994). In a study comparing total body DXA and peripheral quantitative computed tomography of the tibial diaphysis, Leonard et al (2004) found that bone mineral content and bone area adjusted for height were strongly correlated with cortical compartmental density and bone strength, but bone mineral content normalised to bone area and areal density normalised to age were poorly correlated with these quantitative measures. Importantly, in our study there were no significant differences between treatment groups in bone mineral content and bone area standard deviation scores for height, suggesting equivalent true mineral density and bone strength. This is supported by results for fracture incidence, which also did not differ between groups. Although radiological reports were not obtained, large cohort studies have shown that parental recall of fracture is reliable (Clark, Ness, 2006). Thus, the apparent decrease in total body areal mineral density is inconsistent with the other more reliable DXA indices that show that bone mass at early school-age is not affected by exposure to repeat antenatal betamethasone. Given the high degree of tracking of bone mass from mid childhood through to adolescence (Foley 2009; Kalkwarf 2010; Loro 2000; Magarey 1999; Moyer-Mileur 2008), it is also highly likely that treatment groups will attain similar peak bone mass.

A limitation of total body DXA is that it largely reflects cortical bone, as this makes up approximately 80% of total skeletal mass (Leonard 2004). However, many fractures occur in cancellous bones or the trabecular regions of long bones, such as the distal radial metaphysis in children or the femoral neck and vertebrae in adults with osteoporosis.
Although the tracking of cancellous sites and total skeletal mass has been shown to be similar (Foley 2009), there are subtle differences in the development of trabecular and cortical bone, especially during puberty (Kalkwarf 2010; Wang 2008). In addition, diseases and drugs may differentially affect these two bone types. For example, growth hormone deficiency causes greater deficits in cortical bone whereas postnatal glucocorticoid induced bone loss predominantly affects trabecular bone (Fewtrell 2003). Therefore, detailed assessment of bone health in children requires measurements of the whole skeleton as well as regions such as the lumbar spine, distal radius and hip. For logistical reasons, it was not possible to perform separate regional DXA measurements, but data for the whole spine were extracted from the total body scan. Although this is less precise than dedicated lumbar anteroposterior measurements, due to larger pixel size and differences in bone edge detection (Boyanov 2008; Margulies 2005), there was no evidence of any difference between treatment groups in vertebral bone mass. This included analysis of raw data adjusted for height and estimated volumetric density. Thus, it can be concluded that children exposed to repeat antenatal betamethasone and those exposed to a single course of antenatal glucocorticoids have similar accrual of bone mass in both cortical and trabecular bone.

4.9 Summary
This is the first report from a human randomised trial on the effects of repeat doses of antenatal glucocorticoids on body size and composition at early school age. There was no evidence that exposure to repeat doses of antenatal betamethasone at <32 weeks’ gestation, 7 or more days after an initial course of antenatal glucocorticoids, alters body size, soft tissue composition, or bone mass at 6 to 8 years’ corrected age. These results, together with the finding of comparable childhood somatic growth, indicate that any long-term effect of repeat antenatal betamethasone treatment on body size and composition is unlikely.
5 Glucose and insulin metabolism

5.1 Summary of chapter contents
This chapter reports glucose and insulin metabolism of participants in the New Zealand ACTORDS early school-age physiological follow-up study who underwent a frequently sampled intravenous glucose tolerance test. Results for plasma cortisol and creatinine concentrations measured from blood taken as part of this test are reported separately.

5.2 Introduction
There is some evidence that repeat dose(s) of antenatal glucocorticoids improve glucose regulation in preterm infants compared to a single course (Koivisto 2007). However, animal studies have also shown that antenatal glucocorticoids can have adverse effects on glucose regulation in the long-term (de Vries 2007; Moss 2001; Nyirenda 1998; Sloboda 2005), including hepatic insulin resistance (de Vries 2007; Nyirenda 1998; Sloboda 2005). Furthermore, there is evidence of a dose-response effect, with greater changes in adult glucose metabolism following higher (de Vries 2007), more prolonged (Nyirenda 2001), or repeat doses (Sloboda 2005) of antenatal glucocorticoids. The clinical significance of these findings is not yet clear, as long-term human randomised data are limited to one trial and follow-up has extended only to early adulthood (Dalziel, Walker, 2005). That study showed increased insulin response to glucose challenge in subjects exposed to antenatal betamethasone but the changes were minimal and were not associated with altered glucose tolerance. It is currently not known whether exposure to repeat dose(s) of antenatal glucocorticoids has any additional effect on glucose metabolism in humans.

5.3 Chapter hypothesis
That administration of repeat doses of antenatal betamethasone to women at risk of preterm birth at <32 weeks’ gestation, 7 or more days after an initial course of antenatal glucocorticoids, has no effect on insulin sensitivity or other indicators of glucose metabolism in their children at 6 to 8 years’ corrected age.

5.4 Additional statistical methods used in the chapter analyses
The primary aim of the analyses in this chapter was to compare insulin sensitivity and other indicators of glucose and insulin metabolism between children exposed to repeat doses of antenatal betamethasone and those exposed to placebo (single course). An insulin-modified frequently sampled intravenous glucose tolerance test was used to determine
fasting plasma glucose and insulin concentrations, glucose tolerance (Kg), first phase insulin secretion (AIRg), and minimal model determined insulin-dependent (insulin sensitivity, Si) and insulin-independent (glucose effectiveness, Sg) glucose disposal. Fasting plasma glucose and insulin concentrations represent mean values of three baseline blood samples.

Primary analyses were unadjusted. Secondary analyses used mixed linear regression to adjust for the following factors, decided *a priori*: confounding by baseline variables of gestational age at trial entry, maternal preterm prelabour rupture of membranes as a reason for being at risk of preterm birth and European ethnicity (fixed effects); and the non-independence of data from twins (random effect). Further confounding by baseline variables with imbalance was explored using the change in estimates technique at the 10% level (Greenland 1989).

Subgroup analysis, using a test of interaction, was also performed for singletons and twins. Although this was a *post hoc* analysis, randomisation was stratified according to the number of fetuses in each pregnancy.

**5.5 Results**

**5.5.1 Recruitment and characteristics of participants**

After exclusion of triplets (nine repeat betamethasone group, six placebo group), eight children with severe neurosensory disability (three repeat betamethasone group, five placebo group) and one child with known diabetes (placebo group), 136 children in the repeat betamethasone group and 148 children in the placebo group were eligible for investigation of glucose and insulin metabolism. Of these, 84 (62%) in the repeat betamethasone group and 98 (66%) in the placebo group successfully completed the frequently sampled intravenous glucose tolerance test. In addition, testing was attempted in eleven children but was unsuccessful due to intolerance of the procedure (two each group), difficult intravenous access (three repeat betamethasone group, two placebo group) or contamination of blood samples with insulin (two placebo group). Recruitment rates were similar among ethnic groups (percentage of eligible children completing the test: 63% Maori, 75% Pacific Peoples, 76% Other, 61% European, P=0.28).

Of children who successfully completed the frequently sampled intravenous glucose tolerance test, there were no statistically significant differences between those exposed to repeat betamethasone or those exposed to placebo for baseline demographic and obstetric
characteristics (Table 5.1). Body size and body mass index at early school-age did not differ between treatment groups, both in absolute measures and standard scores for corrected age and sex (Table 5.2). Three children had entered puberty and were Tanner stage 2 (two repeat betamethasone group, one placebo group). The number of children of type 2 diabetic parents was not different between groups (repeat betamethasone group 5/84 (6%) versus placebo group 8/98 (8%); risk ratio 1.02, 95% CI 0.95 to 1.11, P=0.77).

5.5.2 Indicators of glucose and insulin metabolism
At early school age, insulin sensitivity index (Si) did not differ between children exposed to repeat antenatal betamethasone and those exposed to placebo (Table 5.3). There were also no differences between treatment groups for the other measures of glucose and insulin metabolism, including fasting plasma glucose and insulin concentrations, glucose effectiveness (Sg), first phase insulin secretion (AIRg), disposition index (Si x AIRg) and glucose tolerance (Kg).

5.5.3 Secondary analyses
There was imbalance between treatment groups in the proportion of European versus non-European children and these ethnic groups had significantly different insulin sensitivity (geometric mean Si x10^{-4} min^{-1}/[mIU/L] European children 9.6 versus non-European children 7.2; ratio of geometric means 1.32, 95% CI 1.11 to 1.58, P=0.002; adjusted for body mass index and sex P=0.02). However, secondary analyses that adjusted for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity and clustering of fetuses in multiple gestations did not alter the results for any of the indicators of glucose and insulin metabolism (Table 5.3).

There was also imbalance between treatment groups for the baseline variables of parity, maternal smoking, and twin pregnancy. Twins had increased insulin sensitivity compared to singletons (geometric mean Si x10^{-4} min^{-1}/[mIU/L] twins 9.8 versus singleton 7.8; ratio of geometric means 1.26, 95%CI 1.04 to 1.52, P=0.02; adjusted for body mass index, sex, and European ethnicity P=0.03). However, inclusion of these baseline variables as additional covariates in the secondary analysis of insulin sensitivity did not alter the estimate of treatment effect, using the change-in-estimates technique at the 10% level (Greenland 1989).

In sensitivity analysis, exclusion of pubertal children or children of type 2 diabetic parents did not alter the results.
In subgroup analysis, the effect of treatment on insulin sensitivity did not differ between singletons and twins (unadjusted treatment effect: singletons [n=124] ratio of geometric means 0.89, 95%CI 0.71 to 1.11 versus twins [n=58] ratio of geometric means 0.88, 95%CI 0.63 to 1.21; test for interaction P=0.96).
Table 5.1: Baseline characteristics of children exposed to repeat betamethasone or placebo who completed the frequently sampled intravenous glucose tolerance test, and of their mothers.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of women</td>
<td>73</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Maternal age—yr</td>
<td>29.9 (5.3)</td>
<td>30.1 (5.4)</td>
<td>0.18</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>0</td>
<td>30 (41)</td>
<td>26 (33)</td>
<td></td>
</tr>
<tr>
<td>1 to 3</td>
<td>35 (48)</td>
<td>45 (55)</td>
<td></td>
</tr>
<tr>
<td>≥ 4</td>
<td>8 (11)</td>
<td>10 (12)</td>
<td></td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>19 (28)</td>
<td>28 (35)</td>
<td>0.38</td>
</tr>
<tr>
<td>Gestational age at first glucocorticoids—wk†</td>
<td>26.6 (24.9, 28.6)</td>
<td>27.1 (24.7, 28.9)</td>
<td>0.79</td>
</tr>
<tr>
<td>Gestational age at trial entry—wk†</td>
<td>28.7 (26.4, 30.3)</td>
<td>28.7 (26.0, 30.2)</td>
<td>0.84</td>
</tr>
<tr>
<td>Twin pregnancy</td>
<td>11 (15)</td>
<td>19 (23)</td>
<td>0.22</td>
</tr>
<tr>
<td>Main reasons for risk of preterm birth§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm prelabour rupture of membranes</td>
<td>19 (26)</td>
<td>24 (30)</td>
<td>0.72</td>
</tr>
<tr>
<td>Preterm labour</td>
<td>12 (16)</td>
<td>13 (16)</td>
<td>1.00</td>
</tr>
<tr>
<td>Severe fetal growth restriction</td>
<td>11 (15)</td>
<td>10 (12)</td>
<td>0.65</td>
</tr>
<tr>
<td>Preeclampsia or eclampsia</td>
<td>15 (21)</td>
<td>17 (21)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cervical incompetence</td>
<td>5 (7)</td>
<td>7 (9)</td>
<td>0.77</td>
</tr>
<tr>
<td>Antepartum haemorrhage</td>
<td>19 (26)</td>
<td>19 (23)</td>
<td>0.85</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>6 (8)</td>
<td>2 (2)</td>
<td>0.15</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1)</td>
<td>5 (6)</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Infant characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of children</td>
<td>84</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Sex of child—female</td>
<td>35 (42)</td>
<td>43 (44)</td>
<td>0.88</td>
</tr>
<tr>
<td>Ethnicity of child‡</td>
<td></td>
<td></td>
<td>0.27</td>
</tr>
<tr>
<td>Maori</td>
<td>23 (27)</td>
<td>28 (29)</td>
<td></td>
</tr>
<tr>
<td>Pacific Peoples</td>
<td>14 (17)</td>
<td>13 (13)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>9 (11)</td>
<td>4 (4)</td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>38 (45)</td>
<td>53 (54)</td>
<td></td>
</tr>
</tbody>
</table>

Data are number (percent), mean (standard deviation) or †median (interquartile range). § Not mutually exclusive. ‡ Ethnicity prioritised in order of Maori, Pacific Peoples, Other, and European.
<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone (n=84)</th>
<th>Placebo (n=98)</th>
<th>Treatment effect: mean difference, ratio of geometric means, or risk ratio (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: mean difference or ratio of geometric means (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height—cm</td>
<td>125.3 (8.1)</td>
<td>125.6 (10.1)</td>
<td>-0.3 (-3.0, 2.4)</td>
<td>0.83</td>
<td>-0.7 (-3.5, 2.1)</td>
<td>0.60</td>
</tr>
<tr>
<td>Weight—kg†</td>
<td>25.1 (21.1, 29.4)</td>
<td>25.1 (21.4, 32.1)</td>
<td>0.98 (0.91, 1.06)</td>
<td>0.65</td>
<td>0.96 (0.88, 1.04)</td>
<td>0.31</td>
</tr>
<tr>
<td>Body mass index†</td>
<td>15.7 (14.5, 17.5)</td>
<td>16.1 (14.8, 18.4)</td>
<td>0.98 (0.94, 1.03)</td>
<td>0.44</td>
<td>0.97 (0.92, 1.02)</td>
<td>0.20</td>
</tr>
<tr>
<td>Mid-arm circumference—cm†</td>
<td>19.2 (17.6, 21.1)</td>
<td>19.4 (17.9, 21.6)</td>
<td>0.99 (0.95, 1.03)</td>
<td>0.64</td>
<td>0.98 (0.94, 1.02)</td>
<td>0.35</td>
</tr>
<tr>
<td>Upper to lower segment ratio</td>
<td>0.55 (0.02)</td>
<td>0.55 (0.01)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.94</td>
<td>0.00 (-0.01, 0.00)</td>
<td>0.90</td>
</tr>
<tr>
<td>Z-score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.31 (1.09)</td>
<td>0.34 (1.24)</td>
<td>-0.03 (-0.38, 0.31)</td>
<td>0.85</td>
<td>-0.09 (-0.45, 0.28)</td>
<td>0.63</td>
</tr>
<tr>
<td>Weight</td>
<td>0.33 (1.31)</td>
<td>0.38 (1.44)</td>
<td>-0.06 (-0.46, 0.35)</td>
<td>0.78</td>
<td>-0.19 (-0.61, 0.23)</td>
<td>0.37</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.14 (1.34)</td>
<td>0.33 (1.32)</td>
<td>-0.19 (-0.58, 0.20)</td>
<td>0.33</td>
<td>-0.26 (-0.68, 0.14)</td>
<td>0.20</td>
</tr>
<tr>
<td>Sitting height</td>
<td>0.78 (1.11)</td>
<td>0.85 (1.20)</td>
<td>-0.07 (-0.41, 0.28)</td>
<td>0.70</td>
<td>-0.21 (-0.58, 0.15)</td>
<td>0.25</td>
</tr>
<tr>
<td>Subischial leg length</td>
<td>-0.24 (1.02)</td>
<td>-0.22 (1.12)</td>
<td>-0.02 (-0.35, 0.30)</td>
<td>0.88</td>
<td>-0.05 (-0.39, 0.30)</td>
<td>0.78</td>
</tr>
<tr>
<td>Pubarche¶</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2.3 (0.2, 25.3)</td>
<td>0.60</td>
<td>Convergence failure</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean (standard deviation), number (percent) or †median (interquartile range). CI, confidence interval. Body mass index is the weight in kilograms divided by the square of the height in metres. Age and sex specific standard scores (Z-score) were derived from the 1990 British growth reference centiles (Cole 1998). For skewed data† the estimate of treatment effect is the ratio of geometric means. ‡Analyses adjusted for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity (fixed effect), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model. ¶All pubertal children were Tanner stage 2.
Table 5.3: Indicators of glucose and insulin metabolism of children exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone (n=84)</th>
<th>Placebo (n=98)</th>
<th>Treatment effect: mean difference, ratio of geometric means (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: mean difference or ratio of geometric means (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting plasma concentrations</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose—mmol/L</td>
<td>4.7 (0.4)</td>
<td>4.8 (0.4)</td>
<td>-0.1 (-0.2, 0.0)</td>
<td>0.25</td>
<td>-0.1 (-0.2, 0.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Insulin—mIU/L†</td>
<td>5.2 (3.6, 7.5)</td>
<td>4.8 (3.4, 6.3)</td>
<td>1.06 (0.89, 1.26)</td>
<td>0.50</td>
<td>1.02 (0.85, 1.21)</td>
<td>0.87</td>
</tr>
<tr>
<td>Glucose disappearance constant [Kg]—x10^-2 min^-1†</td>
<td>2.5 (1.8, 3.0)</td>
<td>2.5 (2.0, 3.4)</td>
<td>0.95 (0.83, 1.09)</td>
<td>0.47</td>
<td>0.93 (0.83, 1.05)</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Minimal model†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin sensitivity index—x10^-4 min^-1.mIU^-1.L</td>
<td>7.8 (4.8, 11.9)</td>
<td>9.7 (5.8, 13.9)</td>
<td>0.86 (0.72, 1.04)</td>
<td>0.11</td>
<td>0.90 (0.74, 1.08)</td>
<td>0.26</td>
</tr>
<tr>
<td>Glucose effectiveness—x10^-2 min^-1</td>
<td>2.7 (2.0, 3.3)</td>
<td>2.9 (1.9, 3.7)</td>
<td>0.96 (0.84, 1.08)</td>
<td>0.49</td>
<td>0.93 (0.82, 1.07)</td>
<td>0.30</td>
</tr>
<tr>
<td>Acute insulin release—mIU.L^-1.min</td>
<td>313.8 (187.3, 579.9)</td>
<td>268.8 (169.5, 485.2)</td>
<td>1.12 (0.90, 1.41)</td>
<td>0.30</td>
<td>1.04 (0.84, 1.30)</td>
<td>0.70</td>
</tr>
<tr>
<td>Disposition index</td>
<td>0.24 (0.15, 0.39)</td>
<td>0.27 (0.15, 0.49)</td>
<td>0.94 (0.75, 1.18)</td>
<td>0.58</td>
<td>0.91 (0.72, 1.14)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation) or †median (interquartile range). For skewed data† the estimate of treatment effect is the ratio of geometric means. *Fasting values are the average of three baseline samples. CI, confidence interval. ‡ Analyses adjusted for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity (fixed effect), and clustering of fetuses in multiple gestations (random effect) using a linear mixed regression model.
5.6 Discussion

This chapter reports glucose and insulin metabolism of 182 New Zealand children at early school age whose mothers participated in the ACTORDS randomised trial. The main outcome measure was insulin sensitivity, as determined by the minimal model. At 6 to 8 years’ corrected age, there was no significant difference in insulin sensitivity between children exposed to repeat doses of antenatal betamethasone at <32 weeks’ gestation and those not so exposed, 7 or more days after an initial course of antenatal glucocorticoids. There was also no difference between treatment groups in other minimal model parameters, glucose tolerance, or fasting glucose and insulin concentrations.

Insulin is a key anabolic hormone and primary regulator of blood glucose. After a meal, glucose and other intestinal secretagogues stimulate pancreatic beta cells to secrete insulin, which acts to restore plasma glucose by suppressing endogenous hepatic glucose production and by promoting glucose uptake, utilisation and storage in liver and peripheral tissues (DeFronzo 2004). Skeletal muscle is the principal site for insulin-mediated glucose disposal (Shulman 1990). In myocytes, insulin increases glucose uptake by promoting the translocation of the glucose transporter type 4 (GLUT4) to the cell membrane, and stimulates glucose metabolism by activating key enzymes involved in glucose phosphorylation (hexokinase), glycogenesis (glycogen synthetase), and glycolysis / glucose oxidation (phosphofructokinase, pyruvate dehydrogenase) (DeFronzo 2004). Although insulin does not stimulate glucose uptake in liver, it enhances glucose utilisation by activating glycogen synthetase and increasing the expression of glycolytic and fatty-acid synthetic enzymes, while inhibiting the expression of gluconeogenic enzymes (Pilkis 1992). Insulin promotes glucose uptake in adipose tissue, via GLUT4, where it is stored as lipid due to activation of lipid synthetic enzymes (Saltiel 2001). It is also a potent inhibitor of lipolysis, owing to decreased activity of hormone-sensitive lipase through reductions in cellular cAMP levels. Insulin acts on cells by binding to the insulin receptor, which when activated phosphorylates secondary messenger molecules, most importantly the insulin-receptor substrate proteins (Saltiel 2001). These, in turn, activate a diverse series of signalling pathways, of which phosphatidylinositol 3-kinase has a pivotal role (Saltiel 2001).

Reduced tissue sensitivity to insulin action is a cardinal feature of type 2 diabetes and plays a key role in its pathogenesis. Indeed, reduced insulin sensitivity or insulin resistance is often evident several decades before diagnosis (Martin 1992), but glucose intolerance is
initially prevented by compensatory hyperinsulinaemia, secondary to increases in beta cell functional capacity and mass. At this early stage, the reduction in insulin sensitivity, as measured by whole body assessments of insulin-mediated glucose metabolism such as the hyperinsulinaemic clamp or minimal model, primarily reflects reduced glucose uptake in muscle. Although the minimal model also measures hepatic insulin resistance, inability to suppress hepatic glucose production typically develops later in the course of type 2 diabetes, and is marked by the onset of fasting hyperglycaemia (DeFronzo 2004). While adipose has a relatively minor role in glucose clearance, insulin resistance in fat is a key determinant of whole-body insulin sensitivity, as increased lipolysis leads to raised plasma free fatty acids, which inhibits glucose uptake and metabolism in myocytes (Belfiore 1998; Kahn 2006; Petersen 2002). In the natural history of typical type 2 diabetes, there is a progressive decline in insulin sensitivity and glucose intolerance occurs when there is severe insulin resistance, especially in muscle (DeFronzo 2004). Eventually beta cell function also declines, possibly due to glucose and lipid toxicity, such that insulin secretion is inadequate for the degree of insulin resistance, heralding the onset of frank diabetes (DeFronzo 2004).

The molecular basis of insulin resistance is complex and likely involves defects at several levels, including the insulin receptor, post-receptor signalling pathways, glucose transport, and activation or expression of enzymes in metabolic pathways (Pessin 2000). However, impaired post-receptor signalling leading to reduced GLUT4 translocation and activation of hexokinase and glycogen synthetise appears to be a common and early defect in muscle (DeFronzo 2004). This results in decreased insulin-stimulated glycogenesis, which is the principal physiological defect in type 2 diabetes (Shulman 1990). It is likely that such individuals have an underlying decreased capacity for insulin signalling, which over time is exacerbated by acquired defects of insulin action due to hyperinsulinaemia and disturbed cellular metabolism (DeFronzo 2004). Indeed, it has been shown that normoglycaemic offspring of diabetic patients have reduced glycogenic synthetic function (Rothman 1995).

Insulin resistance is important in the pathogenesis of not only diabetes but also atherosclerosis and possibly hypertension, thus explaining the strong association between diabetes and vascular disease. Insulin-stimulated mitogenic pathways are usually not affected in insulin resistance and so physiologic hyperinsulinaemia leads to excessive cellular growth, collagen formation and cytokine activation in vascular tissues (Montagnani 2002). This predisposes to arterial wall thickening and atheroma formation.
In addition, hyperinsulinaemia activates the sympathetic nervous system, while insulin resistance in fat contributes to dyslipidaemia (Ginsberg 2000; Reaven 1996).

Insulin sensitivity is a difficult variable to assess because it is dynamic with physiologic fluctuations occurring throughout the life cycle, including puberty (Cutfield 1990; Moran 1999), and in response to environmental factors, such as exercise (Goodyear 1998; Ku 2000; Schmitz 2002) and carbohydrate intake (Chen 1988). Nevertheless, childhood measurements have predictive value as reduced insulin sensitivity has been demonstrated at an early age in groups at increased risk of diabetes. For example, this has been seen in certain ethnic groups, such as African-Americans (Casazza 2009; Ku 2000) and Pima Indians (Thearle 2009), and in some (Danadian 1999) but not all (Goran 2003) children of diabetic parents. Furthermore, there is evidence of tracking of insulin sensitivity and beta-cell function from an early age in subjects without apparent risk factors, who later develop glucose intolerance or diabetes (Nguyen 2010).

Importantly, life-long patterns of insulin sensitivity may be determined not only by genetic inheritance but also by environmental factors during early development. For example, there is now a large body of evidence in animals and humans showing that poor nutrition and growth in fetal and early postnatal life can permanently alter glucose metabolism (McMillen 2005; Newsome 2003). In term or near term populations, being born small for gestational age has been associated with reduced insulin sensitivity in childhood (Hofman 1997; Veening 2002) and early adulthood (Jaquet 2000), and an increased risk of type 2 diabetes in later life (Whincup 2008). This effect on insulin sensitivity may also underlie the known association between low birthweight and adult cardiovascular disease (Barker 2002). In addition, very preterm birth has been associated with reduced insulin sensitivity from childhood to adulthood (Hofman 2004; Hovi 2007; Rotteveel 2008), which may be indicative of a postnatal nutritional insult on fetal development (Hofman, Regan and Cutfield 2006).

Concern regarding the effect of antenatal glucocorticoid treatment on long-term glucose metabolism comes from two lines of evidence. First, it has been proposed that increased exposure to maternal glucocorticoids, due to either increased circulating maternal glucocorticoid concentrations or impaired placental 11β-HSD2 activity, may be one of the mechanisms linking low birthweight with later coronary artery disease and its risk factors, which in turn are related to insulin resistance (Seckl and Walker 2001). In addition, in rats maternal undernutrition was associated with glucocorticoid-induced impairment of fetal
pancreatic beta cell development (Blondeau 2001; Lesage 2001). However, current evidence is far from conclusive that maternal and more specifically fetal undernutrition inevitably leads to increased fetal glucocorticoid exposure (Bloomfield 2006).

Second, adult rat and juvenile monkey offspring of mothers treated with exogenous glucocorticoids had increased plasma glucose and insulin responses to glucose challenge, consistent with insulin resistance (de Vries 2007; Nyirenda 1998; O'Regan 2004). It is possible that this was of hepatic origin, as antenatal glucocorticoid exposure was associated with upregulation of the hepatic rate limiting gluconeogenic enzyme phospho-phenolpyruvate carboxykinase, which would act to increase hepatic glucose output. Antenatal glucocorticoid treatment has also been associated with impaired insulin signalling in muscle in rats (O'Brien 2008) and reduced beta cell number in monkeys (de Vries 2007). However, the results of these studies may not be applicable to the clinical context as the duration of antenatal glucocorticoid exposure was relatively long. Notably, a brief period of antenatal glucocorticoid exposure in rats had no effect on adult glucose regulation (Nyirenda 2001). Perhaps, the most relevant animal data for humans comes from an ovine study, in which ewes were administered saline placebo or either single or serial injections of betamethasone, with a dosage regimen similar to that used clinically (Moss 2001; Sloboda 2005). In early life, the betamethasone exposed offspring tended to have increased basal and stimulated insulin responses but blood glucose concentrations were generally similar among groups (Moss 2001). In later adulthood, no significant differences could be detected in basal and stimulated insulin and glucose responses between betamethasone exposed and control animals (Sloboda 2005). Despite the lack of evidence for altered long-term glucose metabolism, betamethasone exposed animals did have increased hepatic glucose-6-phosphatase activity, a key determinant of hepatic glucose output (Sloboda 2005). This may have explained the tendency to increased insulin secretion in early life.

Human randomised trial data on the long-term effects of glucocorticoids on glucose regulation are limited to the Auckland Steroid Trial. In early adulthood, subjects exposed to a single course of antenatal betamethasone had fasting glucose and insulin concentrations similar to those of subjects exposed to placebo, but appeared to have slightly increased first phase insulin secretion on oral glucose challenge (Dalziel, Walker, 2005). However, 2-hour plasma glucose concentrations were slightly lower in the betamethasone group, though plasma insulin concentrations were not different. This has been interpreted as possible evidence of insulin resistance, but assessment of insulin
sensitivity during oral glucose tolerance testing is complex and can be confounded by physiological factors such as the rate of glucose absorption and beta cell sensitivity to secretagogues (Hucking 2008). The findings of the Auckland Steroid Trial follow-up study are also consistent with a slight increase in the rate of glucose absorption or beta cell sensitivity in the betamethasone group, and may not be indicative of altered insulin sensitivity. In support of this interpretation, an observational study of subjects born very preterm found that those exposed to a single course of betamethasone actually had increased insulin sensitivity, as determined by the homeostatic model (Finken 2008).

Ours is the first study to examine the long-term effects of repeat doses of antenatal glucocorticoids on glucose and insulin metabolism. Importantly, it has shown that repeat betamethasone treatment compared with a single course of antenatal glucocorticoids is not associated with insulin resistance in mid-childhood. Although the adjusted confidence interval for the ratio of geometric means includes the possibility that repeat betamethasone treatment could reduce insulin sensitivity by up to 26%, a clinically relevant type 2 error seems unlikely for two reasons. First, if repeat doses of antenatal betamethasone did have a substantial effect on long-term insulin action it is likely that this would be evident by mid-childhood, given that the minimal model is a highly accurate method (Cutfield 2005). Furthermore, reduced insulin sensitivity has been clearly demonstrated in other groups of children with genetic or perinatal risk factors for impaired glucose metabolism, and the changes have generally been large, exceeding the confidence intervals for treatment effect seen in this study. For example, compared to control children, minimal model insulin sensitivity was reduced by 60% in children with intrauterine growth retardation (Hofman 1997), 35% to 40% in children born very preterm (Hofman 2004), 45% in twin children born moderately preterm (Jefferies 2004), and 32% to 43% in African American children (Casazza 2009; Ku 2000).

Second, even if a small isolated reduction in insulin sensitivity was present but not detected in this study, it is unlikely that this would present a major risk for future diabetes. Although insulin resistance is central to the pathogenesis of diabetes, longitudinal studies have shown that conversion to diabetes generally requires the presence of several predisposing factors, including other defects of glucose disposal, such as reduced glucose effectiveness; disturbed lipid metabolism or obesity; hepatic insulin resistance; and reduced beta cell reserve (Faerch 2009; Goldfine 2003; Lorenzo 2010; Martin 1992; Weyer 1999). While the animal data have suggested that antenatal glucocorticoids may have long-term effects on endogenous glucose production and beta cell capacity, which could
increase the risk of glucose intolerance in later life, there was no evidence of such effects in this study. In particular, because the minimal model incorporates the effect of insulin on hepatic glucose production, a decrease in $S_i$ would be expected in the presence of significant hepatic insulin resistance. Furthermore, fasting plasma glucose and insulin concentrations, which relate closely to endogenous glucose production and hepatic insulin sensitivity, were not different between groups. The disposition index was also not different between treatment groups. This is sensitive to changes in beta cell function because of the hyperbolic relationship that exists between $S_i$ and $AIR_g$ in the minimal model, though reduced beta cell function may not be fully apparent in the absence of insulin resistance. Although preterm birth is another risk factor for disturbed glucose metabolism in this cohort, it has also only been associated with an isolated reduction in insulin sensitivity and not altered beta cell capacity or glucose effectiveness (Hofman 2004; Kajantie 2011).

Although not the primary aim of this study, it is interesting to note that certain subgroups in this cohort had altered insulin sensitivity. For example, non-European children, of whom the majority were of Maori or Pacific heritage, had a 25% lower insulin sensitivity than European children, which was not accounted for by differences in body mass index. In New Zealand, Maori and Pacific people have a lifetime risk for type 2 diabetes that is more than twice that of Europeans (Ministry of Health 2002), and the incidence of diabetes in these groups remains high even after adjusting for confounding factors such as higher rates of obesity and lower socioeconomic status (Joshy 2006; Scragg 1991). For Maori, very high rates of insulin resistance are present by early adulthood (Tipene-Leach 2004), and individuals who develop type 2 diabetes often do so at an earlier age than expected (McGrath 1999). Our study indicates that the reduction in insulin sensitivity is present from childhood, suggesting an underlying defect of insulin action in this population.

Our cohort also included a large number of twins, which is typical of the preterm population (Bornstein 2009; Lee, Cleary-Goldman, 2006). Twins were found to have a modest increase in sensitivity compared with singletons. In contrast, Jefferies et al (2004) found that insulin sensitivity was reduced by 45% in twins compared with normal birthweight term singleton controls. The reason for this divergence in results is unclear, but it may relate to the different makeup of these cohorts, in particular the fact that most of the singleton children in ACTORDS were also born preterm.
5.7 Summary
This is the first report from a human randomised trial on the effects of repeat doses of antenatal glucocorticoids on glucose and insulin metabolism beyond the neonatal period. There was no evidence that exposure to repeat doses of antenatal betamethasone at <32 weeks’ gestation, 7 or more days after an initial course of antenatal glucocorticoids, alters insulin sensitivity at 6 to 8 years’ corrected age, or alters any other indicators of glucose and insulin metabolism.
6 Blood pressure and renal function

6.1 Summary of chapter contents
This chapter reports ambulatory blood pressure, heart rate and renal function of participants in the New Zealand ACTORDS early school-age physiological follow-up study.

6.2 Introduction
In animals and humans, antenatal glucocorticoids promote maturation of the fetal kidneys and cardiovascular system, which results in increased neonatal blood pressure, glomerular filtration, urine flow, and enhanced electrolyte and acid-base balance following preterm birth (al-Dahan 1987; Baum 1991; Berry 1997; Dimitriou 2005; Ervin 1996; Hill 1988; Kari 1994; Koenen 2002; Smith 2004; Stein 1993; Stonestreet 1983). These effects are generally transient, as the kidneys and cardiovascular system normally undergo considerable maturation in the early postnatal period (Smith, Ervin, 2003; van den Anker 1994). Nevertheless, it is likely that maturation of these organ systems contributes to the overall clinical benefit of antenatal glucocorticoids, as initial haemodynamic instability is common in very preterm infants and may contribute to the risk of intraventricular haemorrhage and neurological injury (Martens 2003).

Administration of repeat doses of antenatal glucocorticoids does not appear to have any additional effect on neonatal blood pressure compared to a single dose or course (Mildenhall 2009; Smith, Ervin, 2003). However, in humans, repeat dose(s) have been associated with a reduced need for inotropic support, indicating improved cardiovascular stability (Crowther, Haslam, 2006; Moise 1995; Peltoniemi 2007). Repeat doses also reduce the risk of combined serious neonatal outcome, including intraventricular haemorrhage and periventricular leukomalacia (McKinlay 2012), which may be partly due to improved systemic haemodynamics.

Despite these short-term benefits, there is concern that antenatal glucocorticoids may adversely affect renal and cardiovascular function in the long-term. Animal studies have shown that antenatal glucocorticoids can reduce nephron number and cause elevated adult blood pressure and impaired renal function (Benediktsson 1993; Celsi 1998; Dagan 2010; Dodic 1998; Figueroa 2005; Levitt 1996; Massmann 2006; Ortiz 2001). In humans, these adverse effects seem to be less apparent, although data from randomised trials are limited.

The effect of antenatal glucocorticoids on renal function has not been assessed in
randomised controlled trials, and data regarding the long-term effects of repeat doses on blood pressure are limited to two trials with follow-up not extending beyond three years of age (Crowther 2007; Wapner 2007).

6.3 Chapter hypotheses
That administration of repeat doses of antenatal betamethasone to women at risk of preterm birth at <32 weeks’ gestation, 7 or more days after an initial course of antenatal glucocorticoids, has no effect in their children at 6 to 8 years’ corrected age on:

- 24-hour ambulatory blood pressure
- Indicators of blood pressure and heart rate diurnal rhythm
- Estimated glomerular filtration rate

6.4 Additional statistical methods used in the chapter analyses
The primary aim of the analyses in this chapter was to compare 24-hour ambulatory blood pressure, indicators of blood pressure and heart rate diurnal rhythm, and renal function between children exposed to repeat doses of antenatal betamethasone and those exposed to placebo (single course). Mean values for ambulatory blood pressure and heart rate were used for analysis.

Primary analyses were unadjusted. Secondary analyses used mixed linear regression to adjust for the following factors, decided a priori: confounding by baseline variables of gestational age at trial entry, maternal preterm prelabour rupture of membranes as a reason for being at risk of preterm birth and European ethnicity (fixed effects); and the non-independence of data from twins (random effect). Further confounding by baseline variables with imbalance was explored using the change in estimates technique at the 10% level (Greenland 1989).

Subgroup analysis, using a test of interaction, was also performed for children from single and multiple pregnancy. Although this was a post hoc analysis, randomisation was stratified according to the number of fetuses in each pregnancy.
6.5 Results: ambulatory blood pressure and heart rate

6.5.1 Recruitment and characteristics of participants
After exclusion of eight children with severe neurosensory disability (three repeat betamethasone group, five placebo group), 300 children were eligible for investigation (145 repeat betamethasone group, 155 placebo group), of whom 242 underwent ambulatory blood pressure monitoring (116 repeat betamethasone group, 126 placebo group). However, results for ten children in each group were excluded from analysis because fewer than seven daytime recordings or fewer than seven night-time recordings were obtained. Thus, of eligible children, 106 (73%) children in the repeat betamethasone group and 116 (75%) in the placebo group successfully completed the assessment.

There were no statistically significant differences between participants in the ambulatory blood pressure study exposed to repeat betamethasone and those exposed to placebo for baseline demographic and obstetric characteristics (Table 6.1). Four children (4%) in the repeat betamethasone group and one (1%) in the placebo group had entered puberty and were Tanner stage 2. One child in the placebo group had a history of recurrent post-streptococcal glomerulonephritis but did not have active renal disease at the time of assessment.

6.5.2 Quality of ambulatory blood pressure monitoring
The majority of children (64%) underwent monitoring on a non-school day, and most wore the monitor for approximately 24 hours (Table 6.2). Monitoring was performed on the non-dominant arm, which was the left side for the majority of children (82%). The mean (SD) percentage of attempted recordings that were successfully obtained was 64% (15), resulting in a mean (SD) number of recordings of 16.6 (6.4) in the daytime and 13.0 (3.1) at night (total recordings 29.6 [8.3]). A total of 16 (7%) children reported night-time disturbance but their data were not excluded from the analyses. It was common for children (65%) to have at least one period of more than 2 hours when no recordings were obtained. However, the presence of more than two such episodes was uncommon (7% of children). Children in the repeat betamethasone group had a slightly longer median duration of monitoring but there were no statistically significant differences between treatment groups for any of the indicators of monitoring quality, such as the total number of recordings and the percentage of attempted recordings that were successfully obtained (Table 6.2).
Table 6.1 Baseline characteristics of children exposed to repeat betamethasone or placebo who completed ambulatory blood pressure monitoring and renal function testing, and of their mothers.

<table>
<thead>
<tr>
<th></th>
<th>Ambulatory blood pressure monitoring</th>
<th>Renal function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Repeat betamethasone</td>
<td>Placebo</td>
</tr>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of women</td>
<td>90</td>
<td>96</td>
</tr>
<tr>
<td>Maternal age—yr</td>
<td>30.1 (5.8)</td>
<td>31.0 (5.5)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>38 (42)</td>
<td>28 (29)</td>
</tr>
<tr>
<td>1 to 3</td>
<td>44 (49)</td>
<td>55 (57)</td>
</tr>
<tr>
<td>≥ 4</td>
<td>8 (9)</td>
<td>13 (14)</td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>25 (29)</td>
<td>30 (32)</td>
</tr>
<tr>
<td>Gestational age at first glucocorticoids—wk†</td>
<td>26.8 (24.9, 29.0)</td>
<td>27.1 (24.7, 28.9)</td>
</tr>
<tr>
<td>Gestational age at trial entry—wk†</td>
<td>28.5 (26.2, 30.5)</td>
<td>28.6 (26.0, 30.2)</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>17 (19)</td>
<td>21 (22)</td>
</tr>
<tr>
<td>Main reasons for risk of preterm birth§</td>
<td>25 (28)</td>
<td>27 (28)</td>
</tr>
<tr>
<td>Preterm prelabour rupture of membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm labour</td>
<td>17 (19)</td>
<td>14 (15)</td>
</tr>
<tr>
<td>Severe fetal growth restriction</td>
<td>13 (14)</td>
<td>14 (15)</td>
</tr>
<tr>
<td>Preeclampsia or eclampsia</td>
<td>19 (21)</td>
<td>22 (23)</td>
</tr>
<tr>
<td></td>
<td>Ambulatory blood pressure monitoring</td>
<td>Renal function</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td>Repeat betamethasone</td>
<td>Placebo</td>
</tr>
<tr>
<td>Cervical incompetence</td>
<td>7 (8)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Antepartum haemorrhage</td>
<td>21 (23)</td>
<td>22 (23)</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>9 (10)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Infant characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of children</td>
<td>106</td>
<td>116</td>
</tr>
<tr>
<td>Sex of child—female</td>
<td>50 (47)</td>
<td>52 (45)</td>
</tr>
<tr>
<td>Ethnicity of child‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maori</td>
<td>34 (32)</td>
<td>33 (28)</td>
</tr>
<tr>
<td>Pacific Peoples</td>
<td>13 (12)</td>
<td>12 (10)</td>
</tr>
<tr>
<td>Other</td>
<td>9 (8)</td>
<td>6 (5)</td>
</tr>
<tr>
<td>European</td>
<td>50 (47)</td>
<td>65 (56)</td>
</tr>
</tbody>
</table>

Data are number (percent), mean (standard deviation) or †median (interquartile range). P values are for the comparison between treatment groups. § Not mutually exclusive. ‡ Ethnicity prioritised in order of Maori, Pacific Peoples, Other, and European.
Table 6.2 Quality of ambulatory blood pressure monitoring of children exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th></th>
<th>Total (n=222)</th>
<th>Repeat betamethasone (n=106)</th>
<th>Placebo (n=116)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of recordings‡</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime</td>
<td>16.6 (6.4)</td>
<td>17.0 (0.6)</td>
<td>16.3 (0.6)</td>
<td>0.41</td>
</tr>
<tr>
<td>Night-time (sleep)</td>
<td>13.0 (3.1)</td>
<td>12.7 (0.3)</td>
<td>13.3 (0.3)</td>
<td>0.16</td>
</tr>
<tr>
<td>Percentage successful recordings</td>
<td>63.5 (14.5)</td>
<td>63.4 (15.6)</td>
<td>63.7 (13.6)</td>
<td>0.88</td>
</tr>
<tr>
<td>Duration—h†</td>
<td>24.0 (23.0, 25.0)</td>
<td>24.0 (23.5, 25.0)</td>
<td>23.9 (22.5, 24.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Sleep disturbance reported</td>
<td>16 (7)</td>
<td>8 (8)</td>
<td>8 (7)</td>
<td>1.00</td>
</tr>
<tr>
<td>Performed on a school day</td>
<td>81 (36)</td>
<td>34 (32)</td>
<td>47 (41)</td>
<td>0.21</td>
</tr>
<tr>
<td>No successful recordings for &gt;2 h</td>
<td>144 (65)</td>
<td>67 (63)</td>
<td>77 (66)</td>
<td>0.67</td>
</tr>
<tr>
<td>More than 2 episodes with no recordings &gt; 2 h</td>
<td>16 (7)</td>
<td>8 (8)</td>
<td>8 (7)</td>
<td>0.79</td>
</tr>
<tr>
<td>Performed on left (non-dominant) arm</td>
<td>181 (82)</td>
<td>88 (83)</td>
<td>93 (80)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Data are number (percent), mean (standard deviation) or ‡median (interquartile range). P values are for comparisons (two-tailed) between the two treatment groups. ‡ Daytime and night-time periods were defined by the child’s own diurnal pattern. Monitors were programmed to take 42 recordings per 24-hour period.
6.5.3 Ambulatory blood pressure
At early school age, there was no difference in systolic or diastolic 24-hour ambulatory blood pressure between children exposed to repeat antenatal betamethasone and those exposed to placebo (Table 6.3). There were also no differences between groups in systolic and diastolic blood pressure or Z-scores for the daytime or night-time.

Systolic and diastolic diurnal variation did not differ between groups, assessed as the percentage drop in daytime blood pressure compared with the night-time, or the proportion of children with a diurnal drop in blood pressure of <10% (Table 6.3).

The overall risk of having blood pressure in the hypertensive range was 11%, defined as a systolic or diastolic ambulatory blood pressure above the 95th percentile for sex and height in the daytime or night-time (Table 6.3). This risk was not different between treatment groups. There was also no difference between groups in systolic or diastolic blood pressure load during the daytime or night-time, at both the 90th and 95th percentile limits.

6.5.4 Heart rate
Heart rate did not differ between groups for the 24-hour, daytime or night-time periods. There was also no difference between groups in heart rate Z-scores during the daytime or night-time (Table 6.3).

6.5.5 Secondary analyses
Adjustment for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity, and clustering of fetuses in multiple gestations did not alter the results for 24-hour ambulatory blood pressure or any of the other indicators of diurnal blood pressure and heart rate control (Table 6.3). There was imbalance between treatment groups for the additional baseline variables of parity and smoking. However, inclusion of parity and smoking as additional covariates in the secondary analysis of 24-hour systolic and diastolic ambulatory blood pressure did not alter the estimate of treatment effect, using the change-in-estimates technique at the 10% level (Greenland 1989). Multiple pregnancy as a reason for being at risk of preterm birth also showed imbalance, but actual rates of multiple pregnancy were not unbalanced.

In sensitivity analysis, exclusion of pubertal children did not alter the results.

In subgroup analysis, the effect of treatment on 24-hour systolic and diastolic ambulatory blood pressure was similar between children from single and multiple pregnancy
(unadjusted mean difference mmHg [95% CI]: 24-hour systolic pressure singletons [n=147] 0 [-3 to 2], twins and triplets [n=75] 0 [-4 to 3], test for interaction P=0.81; 24-hour diastolic singletons [n=147] 0 [-2 to 1], twins and triplets [n=75] 0 [-2 to 2], test for interaction P=0.72).
Table 6.3 Ambulatory blood pressure, diurnal variation, and heart rate of children exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone</th>
<th>Placebo</th>
<th>Treatment effect: mean difference or risk ratio (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: mean difference or risk ratio (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=106)</td>
<td>(n=116)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour blood pressure—mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>102 (7)</td>
<td>102 (8)</td>
<td>0 (-2, 2)</td>
<td>0.79</td>
<td>0 (-2, 1)</td>
<td>0.63</td>
</tr>
<tr>
<td>Diastolic</td>
<td>62 (5)</td>
<td>62 (5)</td>
<td>0 (-1, 1)</td>
<td>0.98</td>
<td>0 (-1, 1)</td>
<td>0.81</td>
</tr>
<tr>
<td>Daytime systolic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure—mmHg</td>
<td>107 (7)</td>
<td>108 (9)</td>
<td>-1 (-3, 1)</td>
<td>0.32</td>
<td>-1 (-4, 1)</td>
<td>0.28</td>
</tr>
<tr>
<td>Z-score</td>
<td>-0.48 (1.01)</td>
<td>-0.34 (1.25)</td>
<td>-0.14 (-0.44, 0.16)</td>
<td>0.37</td>
<td>-0.16 (-0.47, 0.16)</td>
<td>0.33</td>
</tr>
<tr>
<td>Percent recordings &gt;90th percentile†</td>
<td>5 (0, 17)</td>
<td>6 (0, 22)</td>
<td>-0.14 (-0.44, 0.16)</td>
<td>0.37</td>
<td>-0.16 (-0.47, 0.16)</td>
<td>0.33</td>
</tr>
<tr>
<td>Percent recordings &gt;95th percentile†</td>
<td>0 (0, 11)</td>
<td>0 (0, 13)</td>
<td>-0.14 (-0.44, 0.16)</td>
<td>0.37</td>
<td>-0.16 (-0.47, 0.16)</td>
<td>0.33</td>
</tr>
<tr>
<td>Daytime diastolic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure—mmHg</td>
<td>67 (5)</td>
<td>68 (6)</td>
<td>-1 (-2, 1)</td>
<td>0.44</td>
<td>-1 (-2, 1)</td>
<td>0.38</td>
</tr>
<tr>
<td>Z-score</td>
<td>-0.70 (0.94)</td>
<td>-0.60 (1.04)</td>
<td>-0.10 (-0.36, 0.16)</td>
<td>0.46</td>
<td>-0.12 (-0.39, 0.15)</td>
<td>0.39</td>
</tr>
<tr>
<td>Percent recordings &gt;90th percentile†</td>
<td>9 (0, 18)</td>
<td>8 (0, 18)</td>
<td>-0.10 (-0.36, 0.16)</td>
<td>0.46</td>
<td>-0.12 (-0.39, 0.15)</td>
<td>0.39</td>
</tr>
<tr>
<td>Percent recordings &gt;95th percentile†</td>
<td>6 (0, 15)</td>
<td>7 (0, 14)</td>
<td>-0.10 (-0.36, 0.16)</td>
<td>0.46</td>
<td>-0.12 (-0.39, 0.15)</td>
<td>0.39</td>
</tr>
<tr>
<td>Night-time systolic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure—mmHg</td>
<td>95 (7)</td>
<td>96 (7)</td>
<td>0 (-2, 2)</td>
<td>0.80</td>
<td>0 (-2, 2)</td>
<td>0.73</td>
</tr>
<tr>
<td>Z-score</td>
<td>-0.11 (0.93)</td>
<td>-0.09 (1.09)</td>
<td>-0.02 (-0.29, 0.25)</td>
<td>0.89</td>
<td>-0.03 (-0.32, 0.25)</td>
<td>0.83</td>
</tr>
<tr>
<td>Percent recordings &gt;90th percentile†</td>
<td>0 (0, 11)</td>
<td>0 (0, 22)</td>
<td>-0.02 (-0.29, 0.25)</td>
<td>0.89</td>
<td>-0.03 (-0.32, 0.25)</td>
<td>0.83</td>
</tr>
<tr>
<td>Percent recordings &gt;95th percentile†</td>
<td>0 (0, 0)</td>
<td>0 (0, 11)</td>
<td>-0.02 (-0.29, 0.25)</td>
<td>0.89</td>
<td>-0.03 (-0.32, 0.25)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Repeat betamethasone (n=106)</td>
<td>Placebo (n=116)</td>
<td>Treatment effect: mean difference or risk ratio (95% CI)</td>
<td>P</td>
<td>Adjusted treatment effect: mean difference or risk ratio (95% CI)‡</td>
<td>Adjusted P‡</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-------------------------------</td>
<td>-----------------</td>
<td>----------------------------------------------------------</td>
<td>----</td>
<td>------------------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>Night-time diastolic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure—mmHg</td>
<td>55 (5)</td>
<td>56 (5)</td>
<td>0 (-1, 1)</td>
<td>0.80</td>
<td>0 (-2, 1)</td>
<td>0.53</td>
</tr>
<tr>
<td>Z-score</td>
<td>0.11 (0.94)</td>
<td>0.16 (0.97)</td>
<td>-0.05 (-0.30, 0.21)</td>
<td>0.72</td>
<td>-0.09 (-0.35, 0.18)</td>
<td>0.51</td>
</tr>
<tr>
<td>Percent recordings &gt;90th percentile†</td>
<td>11 (0, 22)</td>
<td>11 (0.22)</td>
<td></td>
<td>0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent recordings &gt;95th percentile†</td>
<td>0 (0, 13)</td>
<td>0 (0, 14)</td>
<td></td>
<td>0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Systolic diurnal variation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage dip</td>
<td>11 (5)</td>
<td>11 (5)</td>
<td>-1 (-2, 1)</td>
<td>0.37</td>
<td>-1 (-2, 1)</td>
<td>0.40</td>
</tr>
<tr>
<td>No dip (&lt; 10%)</td>
<td>49 (46)</td>
<td>48 (42)</td>
<td>1.11 (0.82, 1.49)</td>
<td>0.59</td>
<td>1.04 (0.90, 1.21)</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Diastolic diurnal variation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage dip</td>
<td>18 (6)</td>
<td>18 (7)</td>
<td>0 (-2, 1)</td>
<td>0.71</td>
<td>0 (-2, 2)</td>
<td>0.87</td>
</tr>
<tr>
<td>No dip (&lt; 10%)</td>
<td>11 (10)</td>
<td>17 (15)</td>
<td>0.70 (0.34, 1.43)</td>
<td>0.42</td>
<td>0.84 (0.58, 1.20)</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Blood pressure in hypertensive range</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour</td>
<td>12 (11)</td>
<td>12 (10)</td>
<td>1.08 (0.51, 2.31)</td>
<td>0.83</td>
<td>Convergence failure</td>
<td></td>
</tr>
<tr>
<td>Daytime</td>
<td>3 (3)</td>
<td>6 (5)</td>
<td>0.55 (0.14, 2.13)</td>
<td>0.50</td>
<td>Convergence failure</td>
<td></td>
</tr>
<tr>
<td>Sleep</td>
<td>10 (9)</td>
<td>12 (10)</td>
<td>0.91 (0.41, 2.02)</td>
<td>1.00</td>
<td>Convergence failure</td>
<td></td>
</tr>
</tbody>
</table>
### Heart rate

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone (n=106)</th>
<th>Placebo (n=116)</th>
<th>Treatment effect: mean difference or risk ratio (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: mean difference or risk ratio (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour Heart rate</td>
<td>88 (8)</td>
<td>87 (9)</td>
<td>1 (-1, 3)</td>
<td>0.48</td>
<td>1 (-1, 3)</td>
<td>0.42</td>
</tr>
<tr>
<td>Daytime Heart rate</td>
<td>94 (8)</td>
<td>95 (9)</td>
<td>0 (-2, 2)</td>
<td>0.93</td>
<td>0 (-2, 2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Daytime Z-score</td>
<td>-0.39 (0.84)</td>
<td>-0.38 (0.95)</td>
<td>-0.01 (-0.25, 0.23)</td>
<td>0.94</td>
<td>-0.01 (-0.26, 0.23)</td>
<td>0.91</td>
</tr>
<tr>
<td>Night-time Heart rate</td>
<td>79 (9)</td>
<td>78 (10)</td>
<td>1 (-2, 3)</td>
<td>0.56</td>
<td>1 (-2, 4)</td>
<td>0.46</td>
</tr>
<tr>
<td>Night-time Z-score</td>
<td>0.41 (1.06)</td>
<td>0.25 (1.03)</td>
<td>0.15 (-0.12, 0.43)</td>
<td>0.27</td>
<td>0.18 (-0.12, 0.47)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Data are number (percent), mean (standard deviation) or †median (interquartile range). CI, confidence interval. Hypertension is defined as a systolic or diastolic mean blood pressure > 95th percentile. † For skewed data groups are compared by Wilcoxon’s rank sum test. ‡ Analyses for normally distributed continuous data adjusted for gestational age and preterm prelabour rupture of membranes at trial entry, European ethnicity (fixed effects), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model. Analyses for categorical data adjusted for gestational age and, preterm prelabour rupture of membranes at trial entry, and European ethnicity using log binomial regression.

6.6
6.7 Results: renal function

6.7.1 Recruitment and characteristics of participants
Children who underwent the frequently sampled intravenous glucose tolerance test had blood taken at baseline for measurement of plasma creatinine concentration. Recruitment to this test is described in chapter five. Plasma samples were obtained for 89 out of 136 (65%) eligible participants in the repeat betamethasone group and 100 out of 148 (68%) in the placebo group. There were no statistically significant differences between participants exposed to repeat betamethasone and those exposed to placebo for baseline demographic and obstetric characteristics (Table 6.1).

Two children (2%) in the repeat betamethasone group and one (1%) in the placebo group had entered puberty and were Tanner stage 2. One child in the placebo group had a history of recurrent post-streptococcal glomerulonephritis but was not excluded from analysis.

One child in the New Zealand follow-up cohort (repeat betamethasone group) had known chronic renal disease due to congenital multicystic kidneys and underwent a renal transplant several years before the early school-age follow-up. She participated in the neurodevelopmental follow-up but declined to undergo the frequently sampled intravenous tolerance test and so did not contribute data to the renal function results.

6.7.2 Estimated glomerular filtration rate
At early school-age, there was no difference in estimated glomerular filtration rate or plasma creatinine concentration between children exposed to repeat antenatal betamethasone and those exposed to placebo (Table 6.4). There was also no difference between groups in the proportion of children with a mildly reduced glomerular filtration rate (60 to 89 mL/min/1.73m²). No child had renal impairment (glomerular filtration rate <60 mL/min/1.73m²).

6.7.3 Secondary analyses
Adjustment for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity, and clustering of fetuses in multiple gestations did not alter the results for estimated glomerular filtration rate or plasma creatinine concentration (Table 6.4).

There was imbalance between treatment groups for the additional baseline variables of parity, smoking, and multiple pregnancy. However, inclusion of these variables as additional covariates in the secondary analysis of estimated creatinine clearance did not
alter the estimate of treatment effect, using the change-in-estimates technique at the 10% level (Greenland 1989).

In sensitivity analysis, exclusion of pubertal children did not alter the results.

In subgroup analysis, there was some evidence that the effect of treatment on estimated glomerular filtration rate was different between singletons and twins (unadjusted mean difference mL/min/1.73m2 [95% CI] singletons [n=132] –0.9 [–7.7 to 5.9], twins [n=57] 10.8 [1.1 to 20.6]). However, the test for interaction between the effect of twin status and treatment group on estimated glomerular filtration rate was not statistically significant (P=0.06).
Table 6.4 Renal function of children exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone (n=89)</th>
<th>Placebo (n=100)</th>
<th>Treatment effect: mean difference or risk ratio (95% CI)</th>
<th>P</th>
<th>Treatment effect: adjusted mean difference (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma creatinine concentration—µmol/L</td>
<td>37.2 (4.9)</td>
<td>38.0 (5.3)</td>
<td>-0.7 (-2.2, 0.8)</td>
<td>0.33</td>
<td>-0.6 (-2.2, 1.0)</td>
<td>0.49</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate—mL/min/1.73m2</td>
<td>106.8 (14.4)</td>
<td>104.9 (14.6)</td>
<td>1.9 (-2.3, 6.1)</td>
<td>0.37</td>
<td>1.0 (-3.5, 5.5)</td>
<td>0.67</td>
</tr>
<tr>
<td>Level of renal function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild impairment (60 to 89 mL/min/1.73m2)</td>
<td>11 (12)</td>
<td>15 (15)</td>
<td>0.82 (0.40, 1.70)</td>
<td>0.68</td>
<td>Convergence failure</td>
<td></td>
</tr>
<tr>
<td>Normal (&gt;90 mL/min/1.73m2)</td>
<td>78 (88)</td>
<td>85 (85)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean (standard deviation) or number (%). ‡ Analyses for continuous data adjusted for gestational age and preterm prelabour rupture of membranes at trial entry, European ethnicity (fixed effects), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model. Analyses for categorical data are adjusted for gestational age and, preterm prelabour rupture of membranes at trial entry, and European ethnicity using log binomial regression.
6.8 Discussion

The research in this chapter examines ambulatory blood pressure in 242 and renal function in 189 New Zealand children at early school-age whose mothers participated in the ACTORDS randomised trial. The main outcome measures were 24-hour systolic and diastolic blood pressure, and estimated glomerular filtration rate. There were no significant differences in these variables at 6 to 8 years’ corrected age between children exposed to repeat doses of antenatal betamethasone at <32 weeks’ gestation and those not so exposed, 7 or more days after an initial course of antenatal glucocorticoids.

Blood pressure is a major risk factor for coronary artery disease, stroke and end-stage renal disease (Stamler 1993). The relationship between systolic and diastolic blood pressure and cardiovascular disease is continuous and graded, and extends into the high-normal range (Vasan 2001). Although cardiovascular complications are uncommon before early adulthood in males and middle age in females (Stamler 1993), assessment of childhood blood pressure is important for two reasons. First, there is modest tracking of blood pressure from early life, such that elevated childhood blood pressure is likely to predict adult hypertension, especially in populations with a high incidence of essential hypertension (Chen 2008). Second, there is evidence that atherosclerotic lesions develop in childhood and advance steadily through adolescence and early adulthood (Berenson 1998; Strong 1969; Strong 1999). The progression of this pre-clinical arterial disease is influenced by the presence of cardiovascular risk factors in childhood, including elevated blood pressure (Berenson 1998; Davis 2001; Dawson 2009; Juonala 2005; Li 2004; Mahoney 1996; McMahan 2006; Raitakari 2003).

Assessment of blood pressure is complicated by considerable intra-subject variability and the fact that clinic measurements can be falsely high (white coat effect) (Sorof 2000) or low (masked hypertension) (Lurbe 2005; Pickering 2007). Twenty-four hour ambulatory monitoring has higher sensitivity and specificity for hypertensive states compared with clinic blood pressure, especially the nocturnal component and variables relating to circadian rhythm (Fagard 2009; Fan 2010; Hansen 2011). In adults, this results in better prediction of cardiovascular disease and mortality (Conen 2008; Verdecchia 2000). Although longitudinal data are not yet available to correlate childhood ambulatory blood pressure levels with hard clinical end-points, ambulatory blood pressure does predict functional changes in target organs in children and adolescents, such as left ventricular hypertrophy (Belsha 1998; Kapuku 1999; McNiece 2007; Sorof 2002) and increased
arterial wall thickness (Lande 2006; Stabouli 2005). Ambulatory monitoring also appears to have better tracking stability than clinic blood pressure (Li 2009; O'Sullivan 2000).

In this study, confidence intervals for the estimate of treatment effect on 24-hour ambulatory blood pressure were narrow and excluded small differences between treatment groups (≥2 to 3 mmHg for systolic and ≥2 mmHg for diastolic pressure). The minimum change in childhood blood pressure that confers a significant increase in risk of cardiovascular disease in adulthood is unclear, but appears to be greater than these limits. In the Bogalusa Heart Study, children who subsequently developed hypertension as adults had higher systolic and diastolic blood pressure by approximately 10 mmHg (Bao 1995). In the Muscatine study, children with blood pressure above the 90th percentile compared to those with blood pressure at the 50th percentile, which at 6 to 8 years of age represents a difference of 3 to 5 mmHg (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents 2004), had two and a half times greater risk of high adult blood pressure (Lauer 1989). Preterm birth, which is known to be a risk factor for high blood pressure in adulthood (Cooper, Atherton, 2009; Crump 2011; Dalziel 2007; Johansson 2005; Leon 2000), has been associated with a 2 to 4 mmHg increase in childhood systolic or diastolic blood pressure in some studies (Bonamy 2007; Norman, Johansson, 2010), and a 4 mmHg increase in night-time ambulatory systolic pressure in one study (Bayrakci 2007). By adolescence, differences of 5 to 10 mmHg have been detected (Bonamy 2005; Doyle 2003). Thus, the fact that ambulatory blood pressure values at mid-childhood were virtually identical between treatment groups, including 24-hour, daytime and night-time periods, suggests that exposure to repeat doses of antenatal betamethasone is unlikely to affect blood pressure in the long-term.

Several other lines of evidence support this conclusion. First, groups did not differ in any of the other ambulatory monitoring parameters including blood pressure load (90th and 95th percentile), heart rate, percentage change in blood pressure from the daytime to sleep, and the proportion of children with a non-dipping diurnal rhythm (<10% change). This implies similar cardiovascular development and regulation. Second, in animal studies permanent effects on blood pressure occurred primarily with prolonged (de Vries 2007) or early (Dodic, Wintour, 1999) drug exposure, but were not seen with clinically relevant doses, either single or repeat, in late gestation (Moss 2005). Results from rodent studies need to be interpreted cautiously because the period of drug exposure is often quite long relative to the short gestation length. Lastly, two human randomised trials have shown that a single course of antenatal glucocorticoids either does not affect adult blood pressure (Dalziel,
Walker, 2005) or may be associated with a slight reduction in adult systolic pressure (Dessens 2000).

The percentage of successful recordings achieved by children in this cohort was less than has been reported in other paediatric studies using the Spacelabs 90207 monitor (Krull 1993; Lurbe 1994; Lurbe 1999; Portman 1991), especially given that ten children in each group were excluded from these data because of insufficient recordings. Although most (96%) of the included children achieved ≥20 recordings, which is sufficient for good reproducibility (Coats 1990), accurate prediction of individual outcomes may require more frequent recordings (Kikuya 2011). However, monitoring quality was unlikely to confound the results of this study, as there was no difference between groups in the total number of recordings or the percentage of successful recordings. Several factors may explain the lower percentage of successful recordings in this cohort, including young age and relatively low systolic blood pressure values (Lurbe 1999), and high levels of physical activity (Portman 1991). The total number of recordings could have been improved by programming the monitors to record more frequently, although this may have reduced compliance with the test.

Glomerular filtration rate was used to determine the effect of repeat antenatal betamethasone treatment on kidney development because it reflects functioning renal mass and is the most useful single measure of renal function (Hogg 2003; Schwartz 2007). Other functions of the kidney, such as production of erythropoietin, activation of vitamin D, and ion and solute transport, decline as glomerular filtration rate declines. Glomerular filtration rate represents the volume of plasma ultrafiltrate presented to the nephrons per unit time in the process of urine formation. It is measured indirectly through the concept of clearance, which is defined as the equivalent volume of plasma from which a substance would have to be totally removed to account for its rate of excretion in urine per unit of time (Schwartz 2007).

As for blood pressure, confidence intervals for the estimate of treatment effect on calculated glomerular filtration rate were narrow, ranging from a 2% decrease to a 6% increase in renal function. Changes of this magnitude are unlikely to be clinically relevant, at least in the short term, as the kidneys have considerable functional reserve and systemic complications of renal disease usually do not occur until glomerular filtration rate is reduced by more than 50% (<60 mL/min/1.73m2) (Hogg 2003). Importantly, the confidence interval for the difference in means was not much greater than the precision of
the plasma creatinine assay itself, and both groups had normal mean glomerular filtration rate for age (Schwartz 2009). Therefore, a clinically significant effect of repeat antenatal betamethasone treatment on glomerular filtration rate in childhood can be confidently excluded.

Concern about the effects of antenatal glucocorticoids on long-term renal function arises mainly from the observation in animals that glucocorticoids can impair nephrogenesis, resulting in reduced total nephron mass (Celsi 1998; Figueroa 2005; Ortiz 2001; Ortiz 2003; Singh 2007; Wintour 2003). This may predispose to early loss of renal function and hypertension later in life (Abitbol 2009; Brenner 1988; Brenner 1994; Moritz, Singh, 2009). However, the timing of antenatal glucocorticoid exposure appears to be important, with effects being most marked during early kidney development and before peak nephrogenesis has occurred (Moritz 2003). Therefore, inter-species differences in the timing of nephrogenesis need to be considered when interpreting animal data. For example, in rats the kidney is still mostly mesenchyme until day 15 (~0.7 of gestation). Peak nephrogenesis occurs around term and continues into the first postnatal week (Bertram 2000). Thus, much of the data from rats may not be applicable to the clinical situation in humans. In contrast, the majority of nephrons are formed in the human fetus from the 20th week of gestation onwards and nephrogenesis is complete by weeks 34 to 36 (Hinchliffe 1991). Studies in non-human primates suggest that nephron number is not affected by exposure to antenatal glucocorticoids during this period (Bramlage 2009; Gubhaju 2009). Nevertheless, in one ovine study there was a significant reduction in nephron number after exposure to betamethasone at mid-gestation, during peak nephrogenesis (Figueroa 2005).

In humans, long-term renal outcome data following exposure to antenatal glucocorticoids are limited to a single observational study, which found that estimated glomerular filtration rate was reduced in very preterm young adults exposed to a single course of antenatal betamethasone compared to those unexposed (Finken 2008). Both groups had normal renal function and the mean difference in glomerular filtration rate was only 5 mL/min/1.73m2. Importantly, this cohort was born at a time when antenatal glucocorticoid treatment of women was not routine and only 20% of the cohort were exposed to antenatal betamethasone. Thus, the results of this study could be confounded by selection bias.

The effects of antenatal glucocorticoids on renal development need to be interpreted in the context of preterm birth, which may be an important risk factor for nephron deficit. Although there is ongoing kidney development after preterm birth, it appears that the
duration of active glomerulogenesis is shorter and fewer glomeruli are formed (Faa 2010; Gubhaju 2009; Rodriguez 2004). This results in reduced kidney volume in infancy (Schmidt 2005), especially when combined with intrauterine growth restriction (Drougia 2009). A study in preterm baboons also found that the glomeruli that were formed postnatally were frequently abnormal (Gubhaju 2009). However, animals exposed to antenatal glucocorticoids had an increased percentage of mature glomeruli compared with those not exposed to glucocorticoids, suggesting that prenatal acceleration of glomerular development may help to preserve normal renal tissue. In addition, the improved postnatal course of infants treated with antenatal glucocorticoids is likely to reduce ischaemic injury and nephrotoxic exposures. For example, in the ACTORDS trial, infants exposed to repeat doses of antenatal betamethasone were less likely to experience complications such as persistent patent ductus arteriosus and sepsis (Crowther, Haslam, 2006), which would reduce exposure to non-steroidal anti-inflammatory drugs and antibiotics (especially aminoglycosides), respectively.

The clinical manifestations of total nephron deficit may be delayed for several decades, and occur when there is critical decline in glomerular number and renal filtration surface due to progressive hyperfiltration-induced glomerulosclerosis or other secondary renal insults (Brenner 1994; Nenov 2000). Therefore, although glomerular filtration rate did not differ between children exposed to repeat antenatal betamethasone and those exposed to a single course of glucocorticoids, later effects of treatment cannot be entirely excluded. However, the experimental data in baboons, the close similarity of blood pressure between treatment groups, and the fact that there was some evidence of better glomerular filtration rate in the subgroup of twins are all reassuring.

A limitation of this study was that glomerular filtration rate was estimated from plasma creatinine concentration rather than measured clearance. In a population of children with mild to severe renal impairment, Bland-Altman analysis of the prediction equation showed that there was a small degree of underestimation compared with values determined by the plasma disappearance of iohexal (-1.8 mL/min/1.73m2) (Schwartz 2009). However, accuracy was poor with only 79% and 37% of estimated values within 30% and 10% of the measured glomerular filtration rate, respectively. In another validation study that used iothalamate clearance, there was a greater degree of bias (-9.1 mL/min/1.73m2) in children with normal glomerular filtration rate (>90 mL/min/1.73m2), but accuracy was similarly poor with only 85% and 35% of estimated values within 30% and 10% of the measured glomerular filtration rate, respectively (Staples 2010). Thus, prediction equations are
inadequate for precise determination of glomerular filtration rate in individual subjects. However, bias and accuracy would be expected to be similar among randomised intervention groups, and hence the comparison of estimated glomerular filtration rate is still valid.

Sensitivity for the assessment of total nephron mass could have been improved by evaluation of stimulated glomerular filtration rate (Bosch 1984; Chan 2010; Hellerstein 2004). The kidney filtration rate can increase in response to a variety of conditions, including oral protein loading. The difference between stimulated and basal glomerular filtration rate is the renal functional reserve, which is more indicative of the functional and anatomical status of the renal parenchyma than the resting rate alone. A reduction in renal reserve means that the available nephrons are working at closer to maximal capacity due to reduced relative nephron mass. Ultrasound measurement of renal volume has also been used as a surrogate marker of total glomerular number but results can be confounded by increased glomerular volume due to hyperfiltration (Nyengaard 1992). Unfortunately, it was not possible to perform additional renal investigations as part of the follow-up schedule.

6.9 Summary
This is the first report from a human randomised trial on the effects of repeat doses of antenatal glucocorticoids on ambulatory blood pressure and renal function at early school age. Exposure to repeat doses of antenatal betamethasone at <32 weeks’ gestation, 7 or more days after an initial course of antenatal glucocorticoids, did not affect 24-hour blood pressure, indicators of blood pressure and heart rate diurnal rhythm, and estimated glomerular filtration rate at 6 to 8 years’ corrected age. 24-hour
7 Hypothalamic-pituitary-adrenal axis

7.1 Summary of chapter contents
This chapter reports basal diurnal salivary cortisol concentrations of participants in the New Zealand ACTORDS early school-age physiological follow-up study, and stimulated plasma cortisol concentrations of children who underwent the insulin-modified frequently sampled intravenous glucose tolerance test (modified insulin stress test).

7.2 Introduction
The hypothalamic-pituitary-adrenal (HPA) axis has a major role in homeostasis under normal conditions and in adaptation to physical and psychological stress. Cortisol, which is the main effector hormone of HPA axis in humans, influences a wide range of physiological systems and functions, including vascular responsiveness, fluid and electrolytes, the central nervous system, immunity, inflammation, glucose and fat metabolism, and reproduction (Munck 1984; Sapolsky 2000). Abnormalities of HPA axis function, including changes to the basal circadian cortisol rhythm and reactivity to environmental stimuli, are associated with a number of chronic medical and psychiatric disorders (Anagnostis 2009; Fries 2005; Goodyer 2001; Nijm 2009). Moreover, permanent resetting of HPA axis activity is a central candidate mechanism in the known epidemiological link between adverse early life conditions, such as fetal growth restriction, and increased risk of adult cardiovascular disease (Levitt 2000; Phillips 1998; Phillips 2000; Reynolds 2001). Animal experiments have shown that fetal over-exposure to glucocorticoids, endogenous or exogenous, can permanently alter central corticosteroid receptor systems, thereby altering glucocorticoid feedback and hence set points in the HPA axis (Kapoor 2006; Welberg 2001). Therefore, evaluation of the long-term effects of antenatal glucocorticoid treatment on HPA axis function is important.

Despite these concerns, data from human randomised trials on this issue are limited. In one small study, a single course of antenatal betamethasone caused a transient decrease in fetal plasma cortisol concentrations, but there was rapid recovery of neonatal HPA axis function (Teramo 1980). In the Auckland steroid trial, a single course of betamethasone did not increase the risk of neonatal adrenal insufficiency (Liggins 1972) and had no effect on basal plasma cortisol concentrations in adulthood (Dalziel, Walker, 2005). However, results from two of the repeat dose trials, including ACTORDS, suggested that repeated exposure to antenatal betamethasone may be associated with HPA axis suppression in the
first postnatal weeks, although the data were somewhat inconsistent (Ashwood 2006; Battin 2007; McEvoy 2002). There are currently no data from human randomised trials on the longer-term effects of repeat antenatal glucocorticoids on HPA axis function.

7.3 Chapter hypothesis
That administration of repeat doses of antenatal betamethasone to women at risk of preterm birth at <32 weeks’ gestation, 7 or more days after an initial course of antenatal glucocorticoids, has no effect on basal and stimulated cortisol concentrations in their children at 6 to 8 years’ corrected age.

7.4 Additional statistical methods used in the chapter analyses
The primary aim of the analyses in this chapter was to compare morning and evening basal salivary cortisol concentrations, including diurnal variation, between children exposed to repeat doses of antenatal betamethasone and those exposed to placebo (single course). In addition, stimulated plasma cortisol concentrations in response to mild hypoglycaemia during an insulin-modified intravenous glucose tolerance test (modified insulin stress test) were compared between these two groups.

For salivary cortisol, values for the three collection days were averaged and the mean morning and evening cortisol concentrations for each child were used in analysis (Goodyer 2003; Patel 2004), with logarithmic transformation to reduce positive skewness. Evening values that were below the lower limit of quantification of the assay (0.14 nmol/L) were assigned an arbitrary cortisol concentration of 0.1 nmol/L. Evening samples with an extremely high value were excluded from analysis, defined as a cortisol concentration greater than the 97th percentile for morning cortisol in the whole cohort and at least ten-fold higher than the remaining evening samples for a given child. These samples were excluded because they were likely to represent stressed rather than typical basal conditions or inadequate compliance with the collection protocol.

An index of diurnal variation for each child was derived as the slope of the linear least-square regression line of the logarithm of salivary cortisol concentrations against time from awakening, using all data from the three collection days (Kraemer 2006; Smyth 1997). Logarithms, rather than raw values, were used because the daytime basal salivary cortisol rhythm approximates an exponential decay curve (Schmidt-Reinwald 1999; Tzortzi 2009). Time from awakening rather than time of collection was used to account for the effect of both the awakening cortisol response (Clow 2004) and the time interval between samples.
If collection times were not available, median values of the whole cohort were used. Lower (more negative) slope coefficients indicate a more rapid decline in cortisol concentrations.

For analysis of the modified insulin stress test, plasma cortisol concentrations at time 60 min after the insulin intravenous bolus were compared between treatment groups using analysis of co-variance with regression on the plasma cortisol concentration immediately before insulin administration (Vickers 2001).

Primary analyses were unadjusted. Secondary analyses used mixed linear regression to adjust for the following factors, decided \textit{a priori}: confounding by baseline variables of gestational age at trial entry, maternal preterm prelabour rupture of membranes as a reason for being at risk of preterm birth and European ethnicity (fixed effects); and the non-independence of data from twins (random effect). Further confounding by baseline variables with imbalance was explored using the change in estimates technique at the 10\% level (Greenland 1989). Secondary analysis of stimulated plasma cortisol concentrations was also adjusted for the nadir plasma glucose concentration, as this is the primary determinant of the cortisol rise in the glucose counter-regulatory response (Streeten 1984). Further analysis was performed for subgroups defined by glucose nadir concentrations of 3.8 and 2.5 mmol/L, which represent glycaemic thresholds for initiation of the cortisol counter-regulatory response and maximal adrenocortical stimulation, respectively (Erturk 1998; Streeten 1984).

Subgroup analysis was also performed for children from single and multiple pregnancy. Although this was a \textit{post hoc} analysis, randomisation was stratified according to the number of fetuses in each pregnancy.

\textbf{7.5 Results: Salivary cortisol}

\textbf{7.5.1 Recruitment and characteristics of participants}

After exclusion of eight children with severe neurosensory disability (three repeat betamethasone group, five placebo group) and one child on long-term glucocorticoid treatment (repeat betamethasone group), 299 children were eligible for investigation (144 repeat betamethasone group, 155 placebo group). Of these, 35 declined salivary cortisol testing (19 repeat betamethasone group, 16 placebo group), 12 were not able to provide adequate volumes of saliva (six each group), and 40 did not return any samples (17 repeat betamethasone group, 23 placebo group). Thus, saliva was collected from 102 (71\%) eligible children in the repeat betamethasone group and 110 (71\%) in the placebo group.
No specimens showed visual evidence of blood contamination. Four children in the repeat betamethasone groups and six in the placebo group returned only five out of six saliva samples. In addition, five children had one evening sample excluded from analysis because of an extremely high outlying value (one child repeat betamethasone group, cortisol concentration 39.3 nmol/L; four children placebo group, cortisol concentrations 29.6, 34.0, 35.6, and >82.8 nmol/L) (97th percentile for cortisol concentration of all morning saliva samples 15.8 nmol/L). Six children (five repeat betamethasone group, one placebo group) had an evening sample that was below the lower limit of quantification of the assay, and were assigned a cortisol concentration of 0.1 nmol/L.

There were no statistically significant differences between participants in the salivary cortisol study exposed to repeat betamethasone and those exposed to placebo for baseline demographic and obstetric characteristics (Table 7.1). Overall, of children who returned saliva samples, 95% and 78% provided complete date and time data, respectively, and postal marks were clearly identified for 91% of children (Table 7.2). There were no significant differences between treatment groups for the time of sample collection, delay between waking and collection of the morning sample (morning awakening delay), season of sampling, number of days that samples spent in the post, or the duration that samples spent in frozen storage before being assayed (Table 7.2). However, more children in the placebo group compared to the repeat betamethasone group collected their saliva samples on school days than on the weekend or during holiday periods (Table 7.2). Four children in the repeat betamethasone group and one child in the placebo group had entered puberty and were Tanner stage 2.
Table 7.1 Baseline characteristics of children exposed to repeat betamethasone or placebo who underwent salivary and plasma cortisol testing, and of their mothers.

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Salivary cortisol</th>
<th>Plasma cortisol</th>
<th>P*</th>
<th>Salivary cortisol</th>
<th>Plasma cortisol</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women</td>
<td>86</td>
<td>88</td>
<td></td>
<td>75</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Maternal age—yr</td>
<td>31.0 (5.4)</td>
<td>31.5 (5.1)</td>
<td>0.53</td>
<td>30.0 (5.3)</td>
<td>31.0 (5.4)</td>
<td>0.22</td>
</tr>
<tr>
<td>Parity</td>
<td>0</td>
<td>35 (41)</td>
<td></td>
<td>0</td>
<td>31 (41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 to 3</td>
<td>42 (49)</td>
<td></td>
<td>1 to 3</td>
<td>36 (48)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 4</td>
<td>9 (10)</td>
<td></td>
<td>≥ 4</td>
<td>8 (11)</td>
<td></td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>24 (29)</td>
<td>22 (26)</td>
<td>0.61</td>
<td>20 (28)</td>
<td>29 (35)</td>
<td>0.39</td>
</tr>
<tr>
<td>Gestational age at first glucocorticoids—wk†</td>
<td>26.5 (24.5, 28.7)</td>
<td>26.9 (24.7, 28.7)</td>
<td>0.83</td>
<td>26.9 (24.9, 28.6)</td>
<td>27.1 (24.7, 29.0)</td>
<td>0.73</td>
</tr>
<tr>
<td>Gestational age at trial entry—wk†</td>
<td>28.4 (26.1, 30.3)</td>
<td>28.6 (26.0, 30.0)</td>
<td>0.78</td>
<td>28.4 (26.4, 30.3)</td>
<td>28.7 (26.0, 30.1)</td>
<td>0.83</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>17 (20)</td>
<td>22 (25)</td>
<td>0.47</td>
<td>11 (15)</td>
<td>19 (23)</td>
<td>0.23</td>
</tr>
<tr>
<td>Main reasons for risk of preterm birth§</td>
<td>25 (29)</td>
<td>27 (31)</td>
<td>0.87</td>
<td>20 (27)</td>
<td>27 (32)</td>
<td>0.49</td>
</tr>
<tr>
<td>Preterm prelabour rupture of membranes</td>
<td>15 (17)</td>
<td>11 (13)</td>
<td>0.40</td>
<td>12 (16)</td>
<td>13 (15)</td>
<td>1.00</td>
</tr>
<tr>
<td>Preterm labour</td>
<td>12 (14)</td>
<td>13 (15)</td>
<td>1.00</td>
<td>11 (15)</td>
<td>10 (12)</td>
<td>0.64</td>
</tr>
<tr>
<td>Severe fetal growth restriction</td>
<td>18 (21)</td>
<td>20 (23)</td>
<td>0.86</td>
<td>15 (20)</td>
<td>17 (20)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Salivary cortisol</td>
<td></td>
<td>Plasma cortisol</td>
<td></td>
<td>P*</td>
<td></td>
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<td>-----------------------------------</td>
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</tr>
<tr>
<td></td>
<td>Repeat betamethasone</td>
<td>Placebo</td>
<td>P*</td>
<td>Repeat betamethasone</td>
<td>Placebo</td>
<td>P*</td>
</tr>
<tr>
<td>Cervical incompetence</td>
<td>7 (8)</td>
<td>7 (8)</td>
<td>1.00</td>
<td>5 (7)</td>
<td>7 (8)</td>
<td>0.77</td>
</tr>
<tr>
<td>Antepartum haemorrhage</td>
<td>22 (26)</td>
<td>20 (23)</td>
<td>0.72</td>
<td>20 (27)</td>
<td>19 (23)</td>
<td>0.58</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>10 (12)</td>
<td>5 (6)</td>
<td>0.19</td>
<td>6 (8)</td>
<td>2 (2)</td>
<td>0.15</td>
</tr>
<tr>
<td>Other</td>
<td>2 (2)</td>
<td>5 (6)</td>
<td>0.44</td>
<td>1 (1)</td>
<td>5 (6)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

**Infant characteristics**

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of children</td>
<td>102</td>
<td>110</td>
<td>86</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Sex of child—female</td>
<td>57 (56)</td>
<td>62 (56)</td>
<td>1.00</td>
<td>50 (58)</td>
<td>57 (57)</td>
</tr>
<tr>
<td>Ethnicity of child‡</td>
<td></td>
<td></td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maori</td>
<td>26 (25)</td>
<td>29 (26)</td>
<td>24 (28)</td>
<td>28 (28)</td>
<td></td>
</tr>
<tr>
<td>Pacific Peoples</td>
<td>14 (13)</td>
<td>13 (12)</td>
<td>15 (17)</td>
<td>14 (14)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>10 (10)</td>
<td>6 (5)</td>
<td>9 (10)</td>
<td>4 (4)</td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>52 (51)</td>
<td>62 (56)</td>
<td>38 (44)</td>
<td>54 (54)</td>
<td></td>
</tr>
</tbody>
</table>

Data are number (percent), mean (standard deviation) or †median (interquartile range). § Not mutually exclusive. ‡ Ethnicity prioritised in order of Maori, Pacific Peoples, Other, and European. * P values are for the comparison between treatment groups (two-tailed).
7.5.2 Salivary cortisol concentrations and diurnal variation

At early school age, there were no significant differences in morning and evening mean salivary cortisol concentrations between children exposed to repeat antenatal betamethasone and those exposed to placebo (Table 7.3). There was substantial within-subject variation in cortisol concentration across collection days, especially in the evening, although this was similar between treatment groups (Table 7.3). Both treatment groups demonstrated a strong diurnal rhythm with high morning and low evening salivary cortisol concentrations. There was, on average, approximately a six-fold difference in cortisol concentration between these two time points. However, children in the repeat betamethasone group had a slightly steeper diurnal rhythm, as determined from the slope of the individual regression lines (Table 7.3). This appeared to be due to more children in the placebo group having a flat diurnal pattern on one or more collection days, defined as a less than two-fold decrease in diurnal cortisol concentration or an increasing diurnal pattern (Table 7.3). Very few children had a flat diurnal rhythm on all collection days.

To determine if flat diurnal patterns were due to a decrease in morning or an increase in evening cortisol concentration, paired diurnal samples for the whole cohort with flat or negative diurnal patterns were compared. Although there was a significant difference in cortisol concentrations between these two patterns for both time points, the change in evening cortisol levels was greater (flat diurnal rhythm versus non-flat diurnal rhythm: morning salivary cortisol concentration geometric mean 3.5 v. 4.1 nmol/L, P=0.01, evening salivary cortisol geometric mean 1.3 v. 0.5 nmol/L, P<0.0001). Consequently, mean evening salivary cortisol concentrations differed between children with an inconsistent daily diurnal rhythm compared to those with a consistently negative daily rhythm (geometric means 1.7 v. 0.58 nmol/L, P<0.0001), but mean morning salivary cortisol concentrations did not (geometric means 4.1 v. 4.3 nmol/L, P=0.49). Of children who had a consistently negative daily cortisol rhythm, there was no difference in mean evening salivary cortisol concentration between treatment groups (geometric means repeat betamethasone versus placebo group 0.55 v. 0.62 nmol/L, P=0.23).

7.5.3 Secondary analyses

Adjustment for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity and clustering of fetuses in multiple gestations did not alter the results for any of the analyses of salivary cortisol at early school age (Table 7.3). There was additional imbalance between treatment groups for the baseline variables of parity and multiple pregnancy. However, inclusion of these baseline variables as additional covariates
In the secondary analyses of mean salivary cortisol concentration and diurnal rhythm did not alter the estimate of treatment effect, using the change-in-estimates technique at the 10% level (Greenland 1989). Furthermore, adjustment of both analyses for the type of collection day (school vs non-school day), or adjustment of diurnal rhythm for the morning mean salivary cortisol concentration did not alter results.

In sensitivity analysis, exclusion of pubertal children did not alter the results.

In subgroup analysis, the effect of treatment on morning and evening mean salivary cortisol concentrations was similar between children from single and multiple pregnancy (unadjusted ratio of geometric means [95% CI]: AM singletons [n=135] 0.99 [0.66 to 1.14], twins and triplets [n=77] 1.17 [0.87 to 1.58], test for interaction P=0.25; PM singletons [n=135] 0.83 [0.54 to 1.28], twins and triplets [n=77] 0.87 [1.22 to 1.46], test for interaction P=0.99). There was also no interaction between the effect of treatment on diurnal rhythm and single or multiple pregnancy status (P=0.26).
Table 7.2 Saliva sampling characteristics of children exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone</th>
<th>n</th>
<th>Placebo</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Collection days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>School only</td>
<td>36 (38)</td>
<td>96</td>
<td>57 (54)</td>
<td>105</td>
<td>0.02</td>
</tr>
<tr>
<td>Non-school only</td>
<td>21 (22)</td>
<td>96</td>
<td>11 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>39 (41)</td>
<td>96</td>
<td>37 (35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean collection time</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM time—h</td>
<td>7.5 (0.6)</td>
<td>96</td>
<td>7.5 (0.5)</td>
<td>99</td>
<td>0.51</td>
</tr>
<tr>
<td>PM time—h†</td>
<td>17.3 (17.0, 17.8)</td>
<td>92</td>
<td>17.2 (17.0, 17.7)</td>
<td>97</td>
<td>0.44</td>
</tr>
<tr>
<td>AM awakening delay—min†</td>
<td>15 (8, 29)</td>
<td>92</td>
<td>19 (10, 30)</td>
<td>96</td>
<td>0.30</td>
</tr>
<tr>
<td>AM awakening delay &gt;30 min</td>
<td>17 (19)</td>
<td>91</td>
<td>24 (25)</td>
<td>95</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring / summer</td>
<td>35 (34)</td>
<td>102</td>
<td>35 (32)</td>
<td>110</td>
<td>0.77</td>
</tr>
<tr>
<td>Autumn / winter</td>
<td>67 (66)</td>
<td></td>
<td>75 (68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Days in post‡</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 3</td>
<td>82 (86)</td>
<td>95</td>
<td>78 (80)</td>
<td>98</td>
<td>0.22</td>
</tr>
<tr>
<td>4 to 7</td>
<td>12 (13)</td>
<td></td>
<td>20 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 7</td>
<td>1 (1)</td>
<td></td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weeks in frozen storage†</strong></td>
<td>60 (40, 91)</td>
<td>102</td>
<td>73 (50, 90)</td>
<td>110</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Data are number (percent), mean (standard deviation) or †median (interquartile range). Collection time data represent mean values for each child. P values are for the comparison between treatment groups (two-tailed).
Table 7.3 Basal salivary cortisol concentration of children exposed to repeat betamethasone or placebo.

<table>
<thead>
<tr>
<th>Mean salivary cortisol concentration—nmol/L†</th>
<th>Repeat n</th>
<th>Placebo n</th>
<th>Treatment effect: ratio of geometric means, mean difference or risk ratio (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: ratio of geometric means, mean difference or risk ratio (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>4.3 (3.2, 5.8)</td>
<td>4.3 (3.2, 5.7)</td>
<td>1.05 (0.91, 1.20)</td>
<td>0.52</td>
<td>1.04 (0.89, 1.20)</td>
<td>0.63</td>
</tr>
<tr>
<td>PM</td>
<td>0.7 (0.4, 1.1)</td>
<td>0.8 (0.5, 1.3)</td>
<td>0.86 (0.68, 1.08)</td>
<td>0.19</td>
<td>0.85 (0.67, 1.07)</td>
<td>0.17</td>
</tr>
<tr>
<td>Diurnal rhythm</td>
<td>102</td>
<td>110</td>
<td>Slope (β) of regression line—ln(nmol/L)/h§</td>
<td>-0.186 (0.076)</td>
<td>-0.163 (0.068)</td>
<td>-0.024 (-0.043, -0.004)</td>
</tr>
<tr>
<td>Variation across days¶</td>
<td>98</td>
<td>100</td>
<td>AM coefficient of variation—%†</td>
<td>31 (22, 50)</td>
<td>39 (21, 57)</td>
<td>0.89 (0.72, 1.09)</td>
</tr>
<tr>
<td></td>
<td>PM coefficient of variation—%†</td>
<td>50 (34, 68)</td>
<td>43 (31, 68)</td>
<td>1.11 (0.93, 1.33)</td>
<td>0.23</td>
<td>1.11 (-0.07, 0.28)</td>
</tr>
<tr>
<td>Flat diurnal rhythm*</td>
<td>22 (22)</td>
<td>38 (38)</td>
<td>0.59 (0.38, 0.92)</td>
<td>0.02</td>
<td>0.90 (0.82, 0.98)</td>
<td>0.03</td>
</tr>
<tr>
<td>One or more days</td>
<td>1 (1)</td>
<td>3 (3)</td>
<td>0.34 (0.04, 3.21)</td>
<td>0.62</td>
<td>Non-convergence</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean (standard deviation), number (percent) or †median (interquartile range). CI, confidence interval. Saliva collected on waking and at 5pm on 3 days, and mean values at each time point were used for analysis. For skewed data† the estimate of treatment effect is the ratio of geometric means. ¶ Includes only children with paired diurnal samples for all 3 days. § Regression of the logarithm of salivary cortisol concentration on time from awakening for all collection days for each child. ‡ Analyses for continuous data adjusted for gestational age and preterm prelabour rupture of membranes at trial entry, European ethnicity (fixed effects), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model. Analyses for categorical data are adjusted for gestational age and preterm prelabour rupture of membranes at trial entry, and European ethnicity using log binomial regression. * Flat diurnal rhythm defined as less than two-fold decrease or an increase in salivary cortisol concentration from AM to PM.
7.6 Results: Modified insulin stress test

7.6.1 Recruitment and characteristics of participants
All children who completed the frequently sampled intravenous glucose tolerance test (84 repeat betamethasone group, 98 placebo group) had plasma cortisol concentrations measured immediately before and 60 minutes after the insulin bolus. The baseline demographic and obstetric characteristics of children recruited to this test are described in chapter 5. There were no significant differences between participants exposed to repeat betamethasone and those exposed to placebo. Two children in the repeat betamethasone group and one in the placebo group had entered puberty and were Tanner stage 2.

7.6.2 Modified insulin stress test
Plasma glucose concentrations were significantly elevated above fasting values at the time of insulin administration due to the prior intravenous dextrose load (P<0.0001 for within-subject change, both groups (Table 7.4). Despite the relatively small intravenous insulin bolus (0.015 IU/kg), plasma glucose concentrations fell below fasting values in all children, except for one child in the repeat betamethasone group. However, endogenous plasma insulin concentrations were also raised above fasting values at the time that exogenous insulin was given (P<0.0001 for ratio of geometric means, both groups [Table 7.4]), which may have contributed to the ongoing decline in plasma glucose concentration. A total of 20 (24%) children in the repeat betamethasone group and 19 (19%) in the placebo group developed significant hypoglycaemia (<2.5 mmol/L), and approximately half of the children in each group had mild hypoglycaemia (2.5 to 3.8 mmol/L) (Table 7.5). Mean plasma glucose concentrations at the nadir were similar between treatment groups (Table 7.4). This occurred at a median of 30 minutes after insulin administration, so plasma cortisol concentrations were measured approximately 30 minutes after the plasma glucose nadir.

Children exposed to repeat antenatal betamethasone and those exposed to placebo had similar plasma cortisol concentrations before administration of insulin (Table 7.5). This represented a resting sample taken between 9:00 and 9:30 am, 40 minutes after intravenous cannulation and 20 minutes after a dextrose load. The number of children who had an increase in plasma cortisol concentration post-insulin was similar in each group (repeat betamethasone group 58 (69%) v. placebo group 73 (74%), P=0.51). However, children in the repeat betamethasone group tended to have a smaller increase in plasma cortisol
concentration at 60 minutes (ratio of geometric means 0.87, 95% CI 0.76 to 1.00, P=0.05) (Table 7.5).

7.6.3 Secondary analyses
The effect of treatment on stimulated plasma cortisol concentrations was statistically significant after adjustment for gestational age at trial entry, preterm prelabour rupture of membranes, European ethnicity, and clustering of fetuses in multiple gestations (Table 7.5). This was even more apparent with further adjustment for the level of the glucose nadir. There was also imbalance between treatment groups for the baseline variables of parity, smoking, and twin pregnancy. However, inclusion of these baseline variables as additional covariates in the secondary analysis of stimulated plasma cortisol concentration did not alter the estimate of treatment effect, using the change-in-estimates technique at the 10% level (Greenland 1989).

In sensitivity analysis, exclusion of pubertal children did not alter the results.

In subgroup analysis, the effect of treatment on stimulated plasma cortisol concentrations did not differ between singletons and twins (unadjusted ratio of geometric means [95% CI]: singletons [n=125] 0.81 [0.65 to 1.01], twins [n=57] 1.03 [0.74 to 1.43], test for interaction P=0.12). Further subgroup analysis was conducted according to the level of glucose nadir (Table 7.5). There was no significant interaction between treatment effect and glucose nadir group (P=0.67), suggesting that the effect was not restricted to any one subgroup. Accordingly, the point estimate for the ratio of geometric means for stimulated plasma cortisol concentrations appeared to be similar at all glycaemic thresholds (Table 7.5). Of the children who developed significant hypoglycaemia (<2.5 mmol/L), only four (20%) in the repeat betamethasone group and nine (47%) in the placebo group had a stimulated plasma cortisol concentration >500 nmol/L, a commonly used clinical cutoff for normal pituitary and adrenal reserve (Grinspoon 1994). Of the children with a plasma glucose nadir of >3.8 mmol/L, nine (43%) in the repeat betamethasone group and 16 (67%) in the placebo group had a plasma cortisol concentration at 60 min that was greater than baseline (P=0.14). However, in this subgroup, only children exposed to placebo had a significant change in plasma cortisol concentration between the two time points (ratio of geometric means repeat betamethasone group 0.91, P=0.43; placebo group 1.31, P=0.02). Several children with plasma glucose nadir concentrations of >3.8 mmol/L had plasma cortisol concentrations at 60 minutes of >500 nmol/L (one repeat betamethasone group, six placebo group).
Table 7.4 Glucose and insulin concentrations of children exposed to repeat antenatal betamethasone or placebo during a modified insulin stress test.

<table>
<thead>
<tr>
<th></th>
<th>Repeat</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>betamethasone</td>
<td>placebo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=84)</td>
<td>(n=98)</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose concentration—mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before insulin IV bolus (20 min after 0.3 g/kg dextrose IV bolus)</td>
<td>7.9 (1.2)</td>
<td>7.9 (1.3)</td>
<td>0.97</td>
</tr>
<tr>
<td>Glucose nadir after insulin IV bolus</td>
<td>3.1 (0.8)</td>
<td>3.2 (0.8)</td>
<td>0.67</td>
</tr>
<tr>
<td>Time to glucose nadir after insulin IV bolus—min†§</td>
<td>30 (20, 40)</td>
<td>30 (30, 40)</td>
<td>0.98</td>
</tr>
<tr>
<td>Plasma insulin concentration—mIU/L†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endogenous before IV insulin bolus (20 min after 0.3 g/kg dextrose IV bolus)</td>
<td>12.4 (9.0, 21.2)</td>
<td>11.7 (8.9, 17.4)</td>
<td>0.32</td>
</tr>
<tr>
<td>Peak after IV insulin bolus</td>
<td>191.3 (113.5, 390.9)</td>
<td>169.3 (100.9, 341.5)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation), number (percent) or †median (interquartile range). Insulin bolus was given as part of a frequently sampled intravenous glucose tolerance test. IV, intravenous. § Glucose measured frequently for the first 15 min then at 10 min intervals until 70 min after insulin IV bolus. See chapter five for fasting plasma glucose and insulin concentrations.
Table 7.5 Plasma cortisol concentrations of children exposed to repeat antenatal betamethasone or placebo during a modified insulin stress test.

<table>
<thead>
<tr>
<th></th>
<th>Repeat betamethasone</th>
<th>Placebo</th>
<th>Treatment effect: ratio of geometric means (95% CI)</th>
<th>P</th>
<th>Adjusted treatment effect: ratio of geometric means (95% CI)‡</th>
<th>Adjusted P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cortisol concentration before insulin IV bolus—nmol/L</td>
<td>213.7 (180.0, 266.8) 84</td>
<td>211.0 (168.2, 262.7) 98</td>
<td>1.05 (0.94, 1.17)</td>
<td>0.43</td>
<td>1.06 (0.95, 1.20)</td>
<td>0.28</td>
</tr>
<tr>
<td>Plasma cortisol concentration 60 min after insulin IV bolus—nmol/L§</td>
<td>268.9 (204.8, 390.9) 84</td>
<td>299.2 (228.2, 530.9) 98</td>
<td>0.87 (0.76, 1.00)</td>
<td>0.05</td>
<td>0.86 (0.75, 0.99)</td>
<td>0.04</td>
</tr>
<tr>
<td>Glucose nadir subgroup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3.8 mmol/L</td>
<td>212.4 (193.1, 289.6) 21</td>
<td>244.1 (204.1, 493.7) 24</td>
<td>0.80 (0.60, 1.05)</td>
<td>0.11</td>
<td>0.79 (0.59, 1.07)</td>
<td>0.12</td>
</tr>
<tr>
<td>2.5 to 3.8 mmol/L</td>
<td>292.3 (217.9, 399.9) 43</td>
<td>303.4 (237.2, 499.2) 55</td>
<td>0.90 (0.76, 1.08)</td>
<td>0.26</td>
<td>0.90 (0.75, 1.07)</td>
<td>0.22</td>
</tr>
<tr>
<td>&lt;2.5 mmol/L</td>
<td>326.8 (218.6, 462.7) 20</td>
<td>499.2 (267.5, 628.8) 19</td>
<td>0.79 (0.57, 1.1)</td>
<td>0.16</td>
<td>0.79 (0.54, 1.13)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Data are median (interquartile range) or number (percent). CI, confidence interval. IV, intravenous. § Analysis of covariance with regression on the plasma cortisol concentration immediately before the insulin IV bolus (logarithmically transformed). ‡ Analyses adjusted for gestational age and preterm prelabour rupture of membranes at trial entry, European ethnicity (fixed effects), and clustering of fetuses in multiple gestations (random effect) using a linear mixed model.
7.7 Discussion

This chapter reports basal diurnal salivary cortisol concentrations and stimulated plasma cortisol concentrations of 212 and 182 New Zealand early school age children, respectively, whose mothers participated in the ACTORDS randomised trial. There were no significant differences in morning and evening basal salivary cortisol concentrations at 6 to 8 years’ corrected age between children exposed to repeat doses of antenatal betamethasone at <32 weeks’ gestation and those not so exposed, 7 or more days after an initial course of antenatal glucocorticoids. Furthermore, children in the repeat betamethasone group appeared to have a more consistent diurnal rhythm. However, their plasma cortisol response to mild hypoglycaemia during an insulin-modified intravenous glucose tolerance test was attenuated compared to children exposed to placebo.

The clinical disorders of Cushing’s syndrome and Addison’s disease illustrate the wide-ranging health effects of marked over- or underproduction of cortisol. Key symptoms of Cushing’s syndrome include abdominal obesity, impaired glucose tolerance, elevated blood pressure, depression, impaired cognitive function and osteoporosis (Carroll 2010; Pereira 2010), whereas glucocorticoid insufficiency is associated with fatigue, weakness, weight loss, gastrointestinal disturbance, and arthralgia (Nieman 2006). However, even within the normal cortisol range, chronic hyper- or hypoactivity of the HPA axis may contribute to disease pathogenesis or progression by altering allostatic load (Chrousos 1992; McEwen 1998). Functional resetting of the axis can result from altered hypothalamic drive or internal dysregulation due to changes in the responsiveness of the pituitary and adrenal glands, or the sensitivity of the axis to glucocorticoid feedback. These changes can affect total daily basal cortisol production, the circadian pattern of cortisol release, and the magnitude and duration of superimposed stress responses. Changes may occur in one aspect of HPA axis function without necessarily affecting other components (Streeten 1984). In addition, cortisol synthesis can be stimulated directly by inflammatory cytokines, the sympathetic nervous system and medullary catecholamines, independently of adrenocorticotrophic hormone (Bornstein 1999).

Functional hyperactivity of the HPA axis is associated with a range of adverse health effects that resemble Cushing’s syndrome. Hypercortisolaemia is common in depression, including children and adolescents, and is correlated with illness severity and chronicity (Lopez-Duran 2009; Stetler 2011). Moreover, elevated morning salivary cortisol concentrations may precede the onset of depression in at risk individuals (Goodyer 2000;
Goodyer 2003; Halligan 2007). In depression, the HPA axis is characterised by loss of diurnal variation, primarily due to elevated afternoon and evening cortisol concentrations, increased limbic-hypothalamic activity with enhanced production of corticotrophic-releasing hormone and vasopressin, and reduced glucocorticoid negative feedback (Stetler 2011; Swaab 2005). Depressive symptoms relate not only to elevated glucocorticoid concentrations but also to the neurotransmitter effects of raised corticotrophic-releasing hormone levels within the brain (Lloyd 2011; Swaab 2005).

In addition to effects on mood, a typical finding in central obesity is functional hyperactivity of the HPA axis, especially increased stress reactivity (Marin 1992; Moyer 1994; Pasquali 1993; Pasquali 1996) and decreased glucocorticoid negative feedback (Ljung 1996; Rosmond 1998). Some studies have reported elevated basal cortisol secretion (Marin 1992; Vicennati 2009; Wallerius 2003), while others have demonstrated lower morning cortisol concentrations but flat diurnal rhythms (Ljung 1996; Power 2006). Although obesity is associated with systemic inflammation (Hotamisligil 2006), which can activate the HPA axis at multiple levels (John 2003), several lines of evidence suggest that hypercortisolism has a causal relationship with obesity. For example, visceral obesity is characteristic of Cushing’s syndrome; glucocorticoids promote lipoprotein lipase activity and adipocyte growth (Ottosson 1994; Rebuffe-Scrive 1992); and there are several genetic polymorphisms that present with both functional alterations of HPA axis function and central obesity (Barat 2005; Gelernter-Yaniv 2003; Manenschijn 2009). Furthermore, although the acute effects of glucocorticoids on metabolism are primarily catabolic (Kyrou 2009), chronic hypercortisolism promotes positive energy balance. This is due to increased intake of energy-dense foods, via effects on central appetite regulation and leptin signalling (Adam 2007; la Fleur 2006; Leal-Cerro 2001; Nieuwenhuizen 2008), and to lowering of basal metabolic rate as a result of reduced skeletal mass and decreased activation of thyroxine (Nieuwenhuizen 2008). Some of the effects of glucocorticoids on eating patterns and lipid accumulation are also mediated by hyperinsulinaemia, which occurs secondary to insulin resistance (Fried 1993; la Fleur 2004). The net effect of positive energy balance is fat accumulation, and glucocorticoids preferentially stimulate growth of abdominal fat (Rebuffe-Scrive 1992). This may be due to the higher density of glucocorticoid receptors in visceral fat depots (Rebuffe-Scrive 1990) and differential expression of 11β-hydroxysteroid-dehydrogenase-1, which enhances tissue cortisol concentrations (Seckl and Walker 2001).
Functional hypercortisolism also contributes to other components of the metabolic syndrome, including dyslipidaemia, hypertension and impaired glucose tolerance, which may occur secondary to visceral obesity or other direct effects on metabolism and vascular function (Anagnostis 2009; Reynolds 2010; Rosmond 2005). The associations between hypercortisolism, central obesity and other aspects of metabolic syndrome may explain why central obesity carries a much higher risk of cardiovascular disease than a more peripheral fat distribution (Björntorp 1997; Wajchenberg 2000).

In contrast to the hypercortisolism seen in depression and metabolic disease, a number of chronic stress-related conditions are associated with decreased HPA axis activity, including low morning cortisol concentrations and a blunted cortisol response to acute stress. These include post-traumatic stress disorder, fibromyalgia and chronic fatigue syndrome (Heim 2000; Parker 2001; Yehuda 2006). The hypocortisolism appears to be due to decreased pituitary responsiveness to corticotrophin-releasing hormone and increased sensitivity to glucocorticoid feedback, which may represent an adaptation to prior chronic stress and prolonged HPA axis activation (Fries 2005; Heim 2000; McEwen 1998). Low morning cortisol concentrations have also been observed in adolescents and children with functional somatic complaints (Janssens 2011; Tornhage 2006), infants with colic (White 2000) and children experiencing maltreatment, social deprivation or trauma (Gunnar 2001). However, responses to chronic stress may be more variable in children than adults, as paediatric post-traumatic stress disorder has also been associated with normal morning but high evening cortisol concentrations (Carrion 2002).

Flattening of the diurnal cortisol rhythm per se may be associated with reduced resilience. For example, loss of diurnal variation in cancer patients was associated with shortened survival, regardless of whether cortisol concentrations were high or low (Sephton 2000). Similarly, in large epidemiological studies, cardiovascular disease risk and mortality is predicted by flattening of the cortisol rhythm but not by morning or average cortisol concentrations (Kumari 2011; Matthews 2006). This may relate to decreased nocturnal blood pressure dipping (Holt-Lunstad 2007), which is a known risk factor for cardiovascular disease (Fagard 2009; Hansen 2011).

Some systemic inflammatory disorders, such as rheumatoid arthritis and atopic diseases, are associated with normal basal cortisol levels but diminished HPA axis stress responses, suggesting impaired suppression of stress-induced inflammation. In atherosclerosis, while hypercortisolism may favour plaque formation (Dekker 2008; Reynolds 2010), blunted
cortisol responses to stress may actually increase the risk of myocardial infarction as plaque instability is related to inflammation (Nijm 2009).

Therefore, even in the absence of an endocrine defect, moderate hyper or hypoactivity of the HPA axis increases the risk of affective disorders and a number of chronic diseases. Although HPA axis dysfunction can result from frequent or persistent axis stimulation during times of stress, exposure to excess glucocorticoids during fetal life may also play a part. Elevated fetal glucocorticoid levels can permanently alter HPA axis regulation, which may render an individual more susceptible to clinically relevant HPA axis dysfunction, especially when under stress. The fetal HPA axis is responsive to stress, but significant increases in fetal glucocorticoid levels are most likely to originate from the maternal circulation, either endogenous or exogenous (Barbazanges 1996). Normally the fetus is protected from maternal glucocorticoids by placental 11β-HSD2 (Lindsay, Lindsay, Edwards, 1996), which converts physiological glucocorticoids (cortisol or corticosterone) to inactive products. However, this barrier is incomplete with 10% to 20% of maternal glucocorticoids reaching the fetus intact (Benediktsson 1993; Benediktsson 1997). Furthermore, placental 11β-HSD2 activity can be reduced by multiple factors, including maternal stress (Glover 2009; Mairesse 2007), protein restriction (Bertram 2001; Langley-Evans 1996), catecholamines (Sarkar 2001), hypoxia (Alfaidy 2002), inflammatory cytokines (Chisaka 2005) and pre-eclampsia (Kajantie, Dunkel, 2003; McCalla 1998; Schoof 2001). In contrast, synthetic glucocorticoids pass readily to the fetus because they have much lower affinity for 11β-HSD2 (Murphy 2007).

Experimentally, fetal glucocorticoid overexposure has been induced by maternal stress such as repeated restraint, 11β-HSD2 antagonists and synthetic glucocorticoids. Although these exposures are somewhat different, the predominant pattern that has emerged from various animal studies is that fetal overexposure to glucocorticoids causes transient suppression of the fetal HPA axis but subsequently tends to increase HPA axis activity. For example, juvenile and adult offspring of rat dams exposed to repeated restraint or other physical stress in the last week of pregnancy consistently demonstrated increased corticosterone stress responses (Barbazanges 1996; Fride 1986; Henry 1994; Maccari 1995; Peters 1982; Takahashi 1988; Vallee 1999). Conversely, inactivation of 11β-HSD2 throughout pregnancy increased basal corticosterone concentrations in adult rat offspring but not HPA axis stress responses (Welberg 2000). Similarly, exposure to synthetic antenatal glucocorticoids increased basal HPA activity in juvenile guinea pigs (Dean 2001) and adult rats (Levitt 1996; O'Regan 2004; Shoener 2006), and the effects were greatest in
males. In non-human primates (de Vries 2007; Uno 1994) and sheep (Sloboda 2002), basal and stimulated cortisol concentrations were increased by antenatal glucocorticoid exposure. Mild chronic glucocorticoid excess can be further exacerbated by increased tissue glucocorticoid activity secondary to upregulation of 11β-hydroxysteroid-dehydrogenase-1 (Cleasby 2003; Nyirenda 2009), which regenerates active glucocorticoids from 11-keto metabolites (Seckl 2004).

However, the long-term picture may be different. Longitudinal studies in rats (Vallee 1999) and sheep (Sloboda 2002; Sloboda 2007), and cross-sectional studies in guinea pigs (Banjanin 2004; Dean 2001; Liu 2001) have shown that glucocorticoid-induced HPA axis hyperactivity diminishes over time. Furthermore, sheep who had been exposed to antenatal betamethasone were found to have adrenocortical hyporesponsiveness in late adulthood, which was more apparent in animals exposed to repeat than single doses (Sloboda 2007).

The long-term effects of fetal glucocorticoid exposure on HPA axis activation may be due to changes in the expression of corticosteroid receptors in regions of the brain that regulate hypothalamic corticosteroid-releasing hormone secretion, especially the hippocampus (De Kloet 1998). For example, in adult rats, exposure to either antenatal glucocorticoids (Levitt 1996; Shoener 2006) or maternal stress (Barbazanges 1996; Henry 1994; Maccari 1995) decreased the density of corticosteroid receptors in the hippocampus, thereby attenuating glucocorticoid negative feedback. Antenatal inhibition of 11β-HSD2 did not affect hippocampal receptors but was associated with increased expression of glucocorticoid receptors in the amygdala and downregulation in the hypothalamus, changes that also enhance HPA axis activity (Welberg 2000). Downregulation of corticosteroid receptor expression may be mediated by increased methylation of the promoter regions of these genes (Meaney 2007). However, HPA axis feedback control is complex and not all observations fit expected patterns. For instance, basal hypercortisolism in male guinea pigs was associated with increased hippocampal glucocorticoid receptor expression (Dean 2001). The switch in HPA axis activity that appears to occur in later life may be due to subsequent upregulation of corticosteroid receptors in the hippocampus (Banjanin 2004; Liu 2001; Vallee 1999) or declining adrenocortical responsiveness to adrenocorticotropic hormone (Sloboda 2007).

In line with these animal data, observational studies in humans have shown that maternal stress or anxiety in pregnancy increases basal and stress salivary cortisol concentrations in childhood (Gutteling 2004, 2005; O'Connor 2005). However, by early adulthood this effect
was not evident, and adrenocortical responsiveness to adrenocorticotropic hormone appeared to be reduced (Entringer 2009). Small size at birth, another potential marker of fetal glucocorticoid excess, has also been associated with long-term changes in HPA axis function, especially increased stimulated cortisol concentrations (Jones 2006; Levitt 2000; Phillips 2000; Reynolds 2001; Wust 2005), although hypo-reactivity may develop in the long-term (Kajantie 2007).

In a randomised trial, exposure to a single course of antenatal betamethasone did not alter morning plasma cortisol concentrations in early adulthood (Dalziel, Walker, 2005). The discrepancy between this trial and the results of antenatal glucocorticoid experiments in animals may relate to the relatively low dose and brief period of glucocorticoid exposure in the clinical setting or to differences in brain maturation. Accordingly, antenatal glucocorticoid exposure did not lead to changes in brain corticosteroid receptor expression in the human fetus (Noorlander 2006), unlike in animals (Dean 1999; McCabe 2001; Owen 2003; Sloboda 2008).

The salivary cortisol results in this study provide further evidence that human fetal glucocorticoid exposure, at clinically relevant doses, does not adversely affect basal cortisol secretion in the long-term. In particular, there was no evidence that mean salivary cortisol concentrations differed between children exposed to single or repeat doses of glucocorticoids at the two time points tested, awakening and early evening. Given the animal and human observational data described above, an increase in basal cortisol concentrations in children exposed to repeat antenatal betamethasone may have been expected. However, confidence intervals for the effect of treatment on mean cortisol concentrations showed that an increase in geometric means as small as 10% to 20% or greater could be excluded. There are no longitudinal data in humans to determine tracking of cortisol from childhood to adulthood, but the similar basal cortisol concentrations at mid-childhood suggest that future differences between groups are unlikely. Although animal studies have raised the possibility of delayed hypocortisolism, this appears to follow a period of HPA axis hyperactivity, and there is no evidence of it in human studies.

Both treatment groups demonstrated a strong diurnal rhythm, comparable to that reported for healthy school-age children (Groschl 2003; Tornhage 2006; Tzortzi 2009). However, despite the similar morning and evening cortisol concentrations, children exposed to repeat antenatal betamethasone had slightly greater diurnal variation, as indicated by a steeper log-cortisol slope. This represents an index of cortisol decline that accounts for the timing
of samples and incorporates data from multiple collection days. Although one study found that steeper diurnal cortisol rhythms were associated with post-traumatic stress disorder due to accentuation of the circadian nadir (Yehuda 1996), most of the evidence presented in this chapter shows that a strong diurnal rhythm is associated with better psychological and physical health, especially when combined with a normal morning cortisol peak. Analysis of individual daily patterns suggested that the difference in slope was due to more children in the placebo group having a flat diurnal rhythm on one or more collection days. In other words, children in the repeat betamethasone group had a more consistent diurnal rhythm. The lower limit of the confidence interval for the effect of treatment on the evening mean salivary cortisol concentration was wider than for the morning, with up to a 30% decrease in the geometric means, which may reflect this difference in daily pattern. The clinical significance of variation in the cortisol diurnal rhythm is not known, and up to 30% of normal adults have inconsistent diurnal cycles (Smyth 1997). Nevertheless, it is likely that the long-term effect of greater inconsistency in the cortisol rhythm is an increase in the evening allostatic load, thereby increasing an individual’s risk of chronic disease (Kumari 2011; Matthews 2006). Although the small difference in diurnal slope may simply represent a type I error, it is certainly not an unfavourable finding for children in the repeat betamethasone group.

There are several limitations to the assessment of the basal cortisol rhythm in this study. First, as only two saliva samples were collected per day, the diurnal slope could be unduly influenced by measurements that deviate from basal conditions. For example, evening samples are particularly susceptible to the effects of emotional upset or exercise (Kertes 2004). The inclusion of additional time points, such as before lunch and mid-afternoon, would have facilitated better characterisation of the diurnal decline and permitted calculation of the area under the curve, which provides an estimate of average daily cortisol exposure (Adam 2009). However, given the considerable day-to-day intra-subject variation in cortisol concentration, priority was given to collecting saliva over a greater number of days, so that mean values at the time points sampled would be more reflective of an individual’s typical state. It has been recommended that the basal salivary cortisol rhythm should be assessed at four or more time points over at least four days (Goodyer 2001). However, the complexity of the sampling protocol must be balanced against the desirability of achieving high return rates (Adam 2009) and the likelihood that the sampling protocol will be adhered to (Broderick 2004; Kudielka 2003). Furthermore, because children generally need assistance with saliva collection, inclusion of samples
during the middle of the day necessitates that testing be carried out on weekends, which limits the number of available collection days.

Second, no assessment was made of the morning surge in cortisol secretion that occurs immediately on waking, also known as the awakening cortisol response. This has been proposed as a discrete marker of HPA axis activity (Clow 2004; Pruessner 1997). Alterations in the awakening cortisol response are increasingly being recognised in a variety of physical and mental disorders (Kudielka 2010). Although influenced by circadian rhythmicity, it is a distinct neuroendocrine response to waking (Clow 2010; Wilhelm 2007), and is affected by psychological factors, such as perceived stress and anticipated workload (Kunz-Ebrecht 2004; Schlotz 2004; Wust 2000). It thus reflects both HPA axis basal activity and reactivity (Schmidt-Reinwald 1999). Several studies have shown that children have an awakening cortisol response, but it appears to be less marked and may be less consistent than in adults (O'Connor 2005; Pruessner 1997; Rosmalen 2005). Because assessment of the awakening cortical response requires collection of several morning samples and strict adherence to collection times (Kudielka 2003), it was felt that this was unlikely to be successfully implemented in this age group. Indeed, the majority of children in the study collected their morning saliva sample from 10 to 30 min after waking, even though they were instructed to collect the sample immediately on waking. Consequently, the morning salivary cortisol concentrations observed in this study may reflect near peak concentrations, as the awakening cortisol response is maximal at around 30 min after waking (Pruessner 1997).

Third, paired adrenocorticotropic hormone concentrations were not be obtained because of the difficulty in achieving unstressed blood sampling in children and because saliva samples were collected at home. Thus, it was not possible to determine the level of pituitary drive to the adrenal glands. However, given that salivary cortisol concentrations were very similar between treatment groups, especially in the morning, it seems reasonable to assume that adrenocortical sensitivity and pituitary secretion of adrenocorticotropic hormone were also similar between groups. Nevertheless, peripheral cortisol concentrations may not reflect altered cortisol production if compensatory changes occur in cortisol clearance (Walker 2002).

Due to the extensive range of assessments included in the school-age follow-up, it was not possible to perform dedicated dynamic testing of the HPA axis. However, the insulin-modified frequently sampled intravenous glucose tolerance test, which was undertaken to
measure insulin sensitivity, provided an opportunity to also investigate HPA axis responsiveness to mild hypoglycaemia. Hypoglycaemia is a powerful stimulus to cortisol production via activation of the hypothalamus and pituitary gland, and insulin stress tests are used clinically to assess the integrity of the entire HPA axis (Grinspoon 1994). It reliably identifies primary and secondary hypoadrenalinism and predicts adrenocortical responses to surgical stress (Plumpton 1969), although partial deficiencies of corticotrophic releasing hormone may go undetected (Tsatsoulis 1988). In formal insulin stress testing, marked symptomatic hypoglycaemia (<2.2 to 2.5 mmol/L) is induced using intravenous insulin boluses of between 0.1 to 0.2 IU/kg (Erturk 1998; Finucane 2008; Grinspoon 1994). Plasma glucose nadir and peak adrenocorticotropic hormone concentrations typically occur after 30 min and peak plasma cortisol concentrations are detected from 60 to 90 min (Erturk 1998; Finucane 2008; Streiten 1984).

However, activation of cortisol secretion occurs not only at low plasma glucose concentrations but also as part of the normal glucose counter-regulatory mechanism that facilitates the transition from exogenous to endogenous glucose metabolism in the postprandial period. This occurs in combination with glucagon, adrenaline, and growth hormone (Cryer 1993; De Feo 1989; Tse 1983). Because of the importance of a continuous blood glucose supply to the brain, secretion of these hormones is initiated when the plasma glucose concentration falls into the low physiological range. The glycaemic threshold for glucose counter-regulation is around 3.8 mmol/L (Cryer 1993; De Feo 1989; Genter 1994; Schwartz 1987), which is approximately the point at which brain glucose uptake starts to fall (Boyle 1994). A cortisol response was thus expected after the insulin administration, even though the dose was small, as plasma glucose concentrations typically fall into the low physiological range during the modified intravenous glucose tolerance test. Indeed, nearly three-quarters of children had an increase in plasma cortisol concentration after administration of insulin.

It was somewhat unexpected that stimulated cortisol responses were diminished in the repeat betamethasone group, given that in young animals antenatal glucocorticoid exposure, including maternal stress, is associated with either increased or unchanged HPA axis reactivity. This finding also seems inconsistent with the salivary cortisol results, as changes in stress responses are generally accompanied by similar perturbations in the cortisol circadian rhythm. The similarity of plasma cortisol concentrations between treatment groups prior to insulin administration supports the absence of any effect of treatment on basal cortisol secretion. A hypocortisolaemic stress response with normal
cortisol diurnal rhythm may not necessarily be maladaptive but it could predispose to exaggerated inflammatory immune responses and stress related physical disorders (Fries 2005). Due to limited plasma sample volumes, adrenocorticotropic hormone concentrations were not measured. Therefore, it remains to be determined whether the altered stimulated cortisol response is due to differences in hypothalamic-pituitary drive or adrenal sensitivity.

These plasma cortisol data need to be interpreted cautiously, because they were not collected as part of a formal insulin stress test. Furthermore, the insulin stress test is more qualitative than quantitative. Clinically, a normal HPA axis response is defined by a peak plasma cortisol concentration >500 nmol/L at any time point, rather than by an incremental change above baseline (Erturk 1998). The fact that this threshold was reached by fewer than half of the children with significant hypoglycaemia (<2.5 mmol/L) suggests that peak plasma cortisol concentrations may have occurred after the 60 min sample. Adult data indicate that the cortisol peak can occur at any time from 60 to 120 min after insulin administration (Finucane 2008). Unfortunately, because the modified insulin stress test was conducted as part of the intravenous glucose tolerance test, later plasma samples were not available. Although treatment groups did not differ in plasma glucose concentration at the nadir or in the time to the nadir, slight differences in these parameters could have affected the cortisol rise. It is also interesting that a number of children without hypoglycaemia (>3.8 mmol/L) had substantial increases in plasma cortisol concentration, especially in the placebo group. At this level of plasma glucose concentration, cortisol stimulation would not be expected, although individual thresholds may vary. Therefore, other unidentified factors may have confounded results.

Although the insulin stress test is a useful and physiologically relevant test of pituitary and adrenocortical reserve, it does not assess all aspects of HPA axis function. Given that hypocortisolism may be due to enhanced glucocorticoid feedback, it would have been interesting to perform a low dose dexamethasone suppression test to assess differences in negative feedback (Reynolds 1998). It would have also been valuable to measure cortisol responses to psychosocial stress, which activates the HPA axis via different central pathways, including the frontal cortex and limbic system. A common method is to measure the salivary cortisol response to the Trier Social Stress Test, but this test is complex and time-consuming to administer (Jones 2006; Kudielka 2010).
7.8 Summary
This is the first report from a human randomised trial on the effects of repeat doses of antenatal glucocorticoids on HPA axis function beyond the neonatal period. There was no evidence that exposure to repeat doses of antenatal betamethasone at <32 weeks’ gestation, 7 or more days after an initial course of antenatal glucocorticoids, has any adverse effects on basal cortisol concentrations or the diurnal rhythm. Further investigation of the effect of repeat doses of antenatal betamethasone on HPA axis stress reactivity is needed.
8 Cardiovascular risk factors and preterm birth

8.1 Summary of chapter contents
This chapter examines the effect of preterm birth and fetal growth on cardiovascular risk factors in participants of the New Zealand ACTORDS early school-age physiological follow-up study.

8.2 Introduction
It is now well established that the pathogenesis of coronary artery disease and its biological risk factors has its origins in fetal and early postnatal life (Barker 2004). During this critical period of development, the structural organisation and functional units within organs are largely determined and the homeostatic systems that regulate organ function are established. This period of early growth can be viewed as the formation of an individual’s metabolic capacity, upon which is imposed the load of later growth, mature body size, disease and environmental conditions (Wells 2011). Therefore, disturbances during early development can have a major influence on life long physiological function and reserve. A key determinant of fetal growth is nutritional substrate supply, which is the product of a complex supply chain involving the mother, placenta, and uterine and umbilical blood flow (Bloomfield 1998). This supply chain can be interrupted in many ways, resulting in sub-optimal fetal nutrition and leads to slowing of growth, altered fetal metabolism, and redirection of systemic circulation to critical organs. If chronic, these changes can permanently alter the function and capacity of vascular and metabolic tissues. Not surprisingly, epidemiological studies have shown that a major pathway to adult chronic disease involves poor fetal growth, slow infant growth, and accelerated growth in later childhood (Osmond 2000), indicating a mismatch between metabolic capacity and load. Similarly, low birthweight, a proxy for impaired fetal growth, has been extensively associated with increased risk of hypertension, diabetes, coronary artery disease, stroke and metabolic syndrome (Barker 2002; Huxley 2000; Whincup 2008).

With the advent of modern neonatal intensive care, increasing numbers of very preterm infants are being born and surviving at a time when fetal organ systems have yet to undergo a major part of their growth and maturation (Philip 2005). Not only do the antecedents of preterm birth, such as infection and maternal illness, have the potential to disturb normal development, but preterm infants also face a vastly different postnatal environment, where medical illness and impaired nutrition are common. Thus, there is
concern that preterm birth may represent an alternative pathway of impaired early development leading to chronic disease. Indeed, there is evidence from cohorts born in the first half of the 20th century, before the modern era of neonatology, that preterm birth is associated with an increased risk of stroke and diabetes (Kaijser 2009; Koupil, Leon, 2005; Lawlor 2005; Lawlor 2006), but not coronary artery disease (Kaijser 2008; Koupil, Leon, 2005). The first large cohorts of very preterm infants are only now entering adulthood, and the presence of cardiovascular risk factors in these groups is being investigated. However, it is unclear if these results will apply equally to contemporary cohorts due to rapid changes in neonatal practice and declining morbidity of preterm infants in recent decades (Darlow 2003). It is also unknown to what extent antenatal growth influences later outcomes independently of preterm birth. Therefore, the data collected as part of the New Zealand ACTORDS physiological follow-up study were examined to determine the independent effects of gestation length and fetal growth on cardiovascular risk factors in a contemporary cohort of preterm children.

8.3 Chapter hypothesis
That shorter gestation length and impaired fetal growth are not associated with changes in physiological variables relating to cardiovascular function and health at early school age, including:

- Ambulatory blood pressure and heart rate
- Insulin and glucose metabolism, as assessed by minimal model analysis of an insulin-modified frequently sampled intravenous glucose tolerance test
- Soft tissue composition, as assessed by total body dual energy X-ray absorptiometry (DXA)
- Basal and diurnal variation in salivary cortisol concentration
- Renal function, as determined by estimated glomerular filtration rate

8.4 Additional statistical methods used in the chapter analyses
The aim of the analyses in this chapter was to determine the independent influence of gestation length and fetal growth. Birthweight standard deviation scores for gestational age (Z-score) were used as a marker of fetal growth. These were calculated from the 1990 British growth reference, which is derived from cross-sectional population birth data (Cole
Gestation length and birthweight data were collected prospectively as part of the ACTORDS trial, with gestational age at birth calculated from the best available obstetric estimate of the due date (Crowther, Haslam, 2006).

The relationship between outcome variables and gestation length or birthweight Z-score was assessed by linear regression, initially in univariate analysis and followed by multivariate analysis adjusting for age at follow-up, sex, multiple status (twin or triplet), number of antenatal glucocorticoid courses, and European ethnicity decided a priori. In addition, multivariate analyses for the effect of gestation length were adjusted for birthweight Z-score. Positively skewed data were logarithmically transformed for analysis. To account for the normal physiological effect of growth on blood pressure and heart rate, outcomes were analysed as standard deviation scores specific for height and sex (Wuhl 2002). Data are presented as regression coefficient ($\beta$) with standard error (SE). In view of the large number of statistical tests performed and in order to reduce the risk of a type I error, P values <0.01 were deemed to be statistically significant. The goodness of fit of significant univariate associations was determined from the $R^2$ value, which estimates the proportion of the variation in the dependent variable around the mean that is attributed to the independent variable in the regression model rather than to random error.

For outcomes other than body composition, a second multivariate analysis was performed with additional adjustment for body mass index. The aim of this analysis was to investigate whether any of the effects of gestation length or birthweight Z-score were mediated through changes in current body fat, which has an important influence on cardiovascular risk factor variables (Botton 2007; Cutfield 1990; Marin 1992; Paradis 2004; Reich 2003). Although body mass index is an imperfect index of adiposity (Freedman, Wang, 2005; Wells 2000), it was the best measure available for all children in the physiological study.

Additional multivariate analysis of fat and lean mass was performed with adjustment for height to determine the effect of gestation length or birthweight Z-score on body composition independent of body size (Wells 2002; Wells and Cole 2011). The effect of the predictor variables on relative fatness was determined by adjusting the regression of fat mass for lean mass. Similarly, central fatness was assessed as android (abdominal) fat mass adjusted for height, lean mass or gynoid (thigh) fat mass (Wells and Victora 2005). Limb lean mass was included as a more direct measure of skeletal muscle mass (Kim 2006).

Primary analyses were performed on all children in the physiological study with relevant outcome data. Sensitivity analyses were performed that excluded children with chronic
medical illness, who had entered puberty, or who were born at term (≥37 weeks’ gestation).

Cardiovascular risk factor data were also compared among children born very preterm (<32 weeks’ gestation), moderate and late preterm (32 to 36 weeks’ gestation), and at term (≥37 weeks’ gestation), using linear regression to determine linear trends. Although this analysis is less powerful than assessment of gestation length as a continuous variable, it allows for easier characterisation of the children in clinically relevant categories. For this analysis, lean and fat mass were adjusted for body size using indices for height raised to the power of two and six, respectively (Wells and Victora 2005). This adjustment ensures that soft tissue mass is independent of height and allows for effects on linear growth and body composition to be separated.

8.5 Results

8.5.1 Characteristics of participants

The baseline and neonatal characteristics of the 264 children who participated in the New Zealand ACTORDS early school-age physiological study have been described (chapter 3). Gestation length ranged from 24 to 41 weeks with 90% of children born preterm (<37 weeks’ gestation) and more than 50% born very preterm (Figure 8.1). A total of 125 (47%) children had very low birth weight (<1500g). Birthweight Z-scores ranged from –3.1 to +3.2, and the cohort mean was significantly different to the zero mean of the reference population (mean difference –0.33, 95% CI –0.46 to –0.20, P<0.001). There was a trend towards a positive association between birthweight Z-scores and gestation length in the whole cohort (β 0.045 per week increase in gestation, SE 0.019, P=0.02, R² 0.02), but this was less significant in children born preterm (β 0.044, SE 0.025, P=0.09, R² 0.01 [n=238]) (Figure 8.2). Birthweight Z-scores did not differ significantly across gestation groups (mean [SD] very preterm –0.45 [0.09], moderate and late preterm –0.21 [1.09], term 0.00 [1.13], P=0.08 for linear trend). However, the lower gestation groups tended to include a larger proportion of babies born small for gestational age, defined either as below the 10th percentile (very preterm 23%, moderate and late preterm 16%, term 4%, P=0.05) or as birthweight Z-score less than –2 (very preterm 10%, moderate and late preterm 1%, term 0%, P=0.01).

Shorter gestation length and lower birthweight Z-score were both significantly associated with reduced Z-scores for height, weight and body mass index at early school age (Table
These associations remained after exclusion of children born at term. However, in preterm children weight Z-score was not related to gestation length after adjustment for height Z-score.

Seven children in the physiological study had chronic medical illness but completed some of the tests. One child each had lymphoblastic lymphoma (DXA and salivary cortisol), chronic cystic renal disease requiring renal transplant (DXA), cystic fibrosis with secondary diabetes (ambulatory blood pressure and salivary cortisol), idiopathic bronchiectasis (ambulatory blood pressure, salivary cortisol), celiac disease (DXA and salivary cortisol, two children) and steroid-resistant nephrotic syndrome (DXA).

Five children had entered puberty (Tanner stage 2). These children completed all of the physiological tests except for two who did not undergo DXA and two who did not undergo blood sampling.

Figure 8.1 Gestation length of children in the physiological study cohort (n=264).

![Figure 8.1 Gestation length of children in the physiological study cohort (n=264).](image)

Figure 8.2 Size at birth of children in the physiological study cohort (n=264).

![Figure 8.2 Size at birth of children in the physiological study cohort (n=264).](image)
Table 8.1 Effect of gestational length and fetal growth (birthweight Z-score) on body size at early-school age in participants of the physiological study.

<table>
<thead>
<tr>
<th>(n=264 [preterm 238])</th>
<th>Effect of 1 week increase in gestation length</th>
<th>Effect of 1 SD increase in birthweight Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate</td>
<td>Multivariate†</td>
</tr>
<tr>
<td></td>
<td>β (SE)</td>
<td>P</td>
</tr>
<tr>
<td>Height</td>
<td>0.094 (0.021)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Preterm</td>
<td>0.108 (0.027)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight</td>
<td>0.122 (0.023)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Preterm</td>
<td>0.118 (0.030)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.096 (0.021)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Preterm</td>
<td>0.076 (0.028)</td>
<td>0.0007</td>
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<tr>
<td>Weight adjusted for height‡</td>
<td>0.037 (0.014)</td>
<td>0.008</td>
</tr>
<tr>
<td>Preterm</td>
<td>0.174 (0.045)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

β, linear regression coefficient; SE, standard error; SD, standard deviation. *Derived from the British growth reference; Z-scores are specific for sex and corrected age. Preterm <37 weeks’ gestation. †Adjusted for twin or triplet status, number of antenatal glucocorticoid courses, European ethnicity, and sex. In addition, regression on gestation length adjusted for birthweight Z-score. ‡Height Z-score included as covariate in the regression of weight Z-score on gestation length or birthweight Z-score.
8.5.2 Effect of gestation length

Shorter gestation length at birth tended to be associated with reduced fat mass at early school age, adjusted for height or lean mass, but was not significantly related to android (abdominal) fat mass adjusted for height, lean mass or gynoid fat mass (Table 8.2). There was also no significant relationship with lean mass, either total body or limb, after adjustment for height (Table 8.2). There was a significant relationship between gestation length and estimated glomerular filtration rate in univariate but not multivariate analysis (Table 8.2). Shorter gestation length tended to be associated with higher daytime systolic ambulatory blood pressure, but only after adjustment for body mass index (Table 8.2). There were no statistically significant associations between gestation length and other outcomes, including ambulatory blood pressure and heart rate, indicators of glucose and insulin metabolism, and diurnal salivary cortisol concentration and rhythm.

In sensitivity analysis, exclusion of children with chronic medical illness or who had entered puberty did not alter results. However, exclusion of children born at term abolished the trend for the association between gestation length and fat mass, adjusted for height (log fat mass $\beta$ 0.018, SE 0.014, P=0.21, adjusted model [n=172]) or lean mass (log lean mass $\beta$ 0.025, SE 0.014, P=0.08, adjusted model [n=172]). The association between gestation length and estimated glomerular filtration rate was also not significant after exclusion of children born at term (univariate analysis $\beta$ 0.373, SE 0.486, P=0.05; multivariate analysis $\beta$ 0.620, SE 0.419, P=0.14 [n=171]). Results for other outcomes were not substantially altered by exclusion of children born at term.

Similar results were obtained when the effect of preterm birth was analysed as a categorical factor but P values were generally larger (Table 8.3).

8.5.3 Effect of fetal growth

At early school age, lower birthweight Z-score was associated with reduced lean mass, both total body and limbs, even after adjusting for height (Table 8.2). There was no association between birthweight Z-score and total fat mass or android fat mass after adjustment for height or lean mass. However, there was a trend to increased android fat mass with lower birthweight Z-score when adjusted for gynoid fat mass. In multivariate analysis, including adjustment for height, lean tissue mass increased by 372 g (95% CI 159 to 586) per standard deviation increase in birthweight for gestational age (for preterm [n=172] 398 g, 95% CI 190 to 607). This amounted to a 980 g (95% CI 420 to 1560) difference in adjusted lean tissue mass, on average, between children in the top and bottom
birthweight Z-score tertiles (median birthweight Z-score for top and bottom tertiles 0.67 and –1.58, respectively; lean mass geometric mean 19.46 vs 18.48 kg, P<0.001 [n=121]).

Lower birthweight Z-score tended to be associated with lower daytime ambulatory blood pressure, but this relationship was not significant after adjustment for current body mass index (Table 8.2). There were trends to increased acute insulin release (AIRg) and glucose effectiveness (Sg), and thus increased disposal index (DI) and glucose disappearance (Kg), in children with lower birthweight Z-score after adjustment for body mass index (Table 8.2). There were no statistically significant associations between birthweight Z-score and outcomes for ambulatory heart rate, diurnal salivary cortisol concentrations and rhythm, or renal function.

Inclusion of gestation length in the multivariate analyses did not alter results for any outcomes.

In sensitivity analysis, exclusion of children with chronic medical illness or who had entered puberty did not alter results. Exclusion of children born at term also did not substantially alter results.
Table 8.2 Effect of gestational length and fetal growth (birthweight Z-score) on cardiovascular risk factor outcomes at early-school age in participants of the physiological study.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n</th>
<th>Effect of 1 week increase in gestation length</th>
<th>Effect of 1 SD increase in birthweight Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β (SE)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambulatory blood pressure Z-score</td>
<td>222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime systolic</td>
<td></td>
<td>-0.012 (0.022)</td>
<td>0.57</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>-0.0485 (0.022)</td>
<td>0.03</td>
</tr>
<tr>
<td>Daytime diastolic</td>
<td></td>
<td>-0.005 (0.019)</td>
<td>0.81</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>-0.033 (0.018)</td>
<td>0.07</td>
</tr>
<tr>
<td>Night-time systolic</td>
<td></td>
<td>-0.003 (0.019)</td>
<td>0.88</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>-0.022 (0.021)</td>
<td>0.29</td>
</tr>
<tr>
<td>Night-time diastolic</td>
<td></td>
<td>-0.010 (0.019)</td>
<td>0.59</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>-0.018 (0.020)</td>
<td>0.38</td>
</tr>
<tr>
<td>Ambulatory heart rate Z-score</td>
<td>222</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime</td>
<td></td>
<td>0.002 (0.017)</td>
<td>0.91</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>-0.020 (0.018)</td>
<td>0.27</td>
</tr>
<tr>
<td>Night-time</td>
<td></td>
<td>-0.488 (0.026)</td>
<td>0.20</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>0.022 (0.022)</td>
<td>0.30</td>
</tr>
<tr>
<td>Glucose and insulin metabolism</td>
<td>182</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose concentration—mmol/L</td>
<td></td>
<td>0.006 (0.008)</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.044 (0.023)</td>
<td>0.06</td>
</tr>
<tr>
<td>Outcome</td>
<td>n</td>
<td>Effect of 1 week increase in gestation length</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>-----</td>
<td>---------------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β (SE)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β (SE)</td>
<td>P</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>0.002 (0.008)</td>
<td>0.76</td>
</tr>
<tr>
<td>Log (Fasting insulin concentration—mIU/L)</td>
<td></td>
<td>0.018 (0.013)</td>
<td>0.17</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>-0.005 (0.012)</td>
<td>0.66</td>
</tr>
<tr>
<td>Log (Insulin sensitivity—x10^{-4} min^{-1}.mIU^{-1}.L)</td>
<td></td>
<td>-0.010 (0.014)</td>
<td>0.48</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>0.000 (0.011)</td>
<td>0.99</td>
</tr>
<tr>
<td>Log (Acute insulin release—mIU.L^{-1}.min)</td>
<td></td>
<td>0.005 (0.017)</td>
<td>0.78</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>-0.013 (0.015)</td>
<td>0.37</td>
</tr>
<tr>
<td>Log (Glucose disposal index)</td>
<td></td>
<td>-0.012 (0.017)</td>
<td>0.48</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>-0.02 (0.016)</td>
<td>0.21</td>
</tr>
<tr>
<td>Log (Glucose effectiveness—x10^{-2} min^{-1})</td>
<td></td>
<td>0.003 (0.010)</td>
<td>0.75</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>0.002 (0.010)</td>
<td>0.87</td>
</tr>
<tr>
<td>Log (Glucose disappearance constant [Kg]—x10^{-2} min^{-1})</td>
<td></td>
<td>-0.008 (0.011)</td>
<td>0.46</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>-0.012 (0.010)</td>
<td>0.26</td>
</tr>
<tr>
<td>DXA total body composition</td>
<td>185</td>
<td>Lean mass—kg</td>
<td>0.230 (0.133)</td>
</tr>
<tr>
<td>Outcome</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----</td>
<td>-----------------</td>
<td>----------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Effect of 1 week increase in gestation length</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>β (SE)</td>
<td>P</td>
</tr>
<tr>
<td>adjusted for height</td>
<td></td>
<td>0.026 (0.006)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Limb lean mass—kg</td>
<td></td>
<td>0.067 (0.015)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>adjusted for height</td>
<td></td>
<td>0.056 (0.015)</td>
<td>0.04</td>
</tr>
<tr>
<td>adjusted for lean mass</td>
<td></td>
<td>0.026 (0.014)</td>
<td>0.07</td>
</tr>
<tr>
<td>adjusted for log (gynoid mass)</td>
<td></td>
<td>0.002 (0.006)</td>
<td>0.78</td>
</tr>
<tr>
<td>Basal salivary cortisol</td>
<td>212</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.013 (0.010)</td>
<td>0.21</td>
</tr>
<tr>
<td>adjusted for BMI</td>
<td></td>
<td>0.000 (0.013)</td>
<td>0.49</td>
</tr>
<tr>
<td>Diurnal slope</td>
<td></td>
<td>0.000 (0.001)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

### Notes

1. Effect of 1 week increase in gestation length
2. Effect of 1 SD increase in birthweight Z-score
3. Adjusted for height
4. Adjusted for BMI
5. Adjusted for lean mass
6. Adjusted for log (gynoid mass)
<table>
<thead>
<tr>
<th>Outcome</th>
<th>n</th>
<th>Effect of 1 week increase in gestation length</th>
<th>Effect of 1 SD increase in birthweight Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β (SE) P</td>
<td>β (SE) P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate—mL/min/1.73m² adjusted for BMI</td>
<td>189</td>
<td>0.833 (0.310) 0.008^h</td>
<td>0.640 (0.321) 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.612 (0.328) 0.07</td>
<td>1.185 (0.919) 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.406 (0.990) 0.68</td>
<td>0.696 (0.950) 0.46</td>
</tr>
</tbody>
</table>

β, regression coefficient; SE, standard error; SD, standard deviation; DXA, dual energy absorptiometry; BMI, body mass index. For logarithmically transformed data the percentage change in the dependent variable per unit change in the independent variable is given by the regression coefficient x 100. Ambulatory blood pressure Z-scores are height and sex specific. †Adjusted for corrected age at assessment, sex, twin or triplet status, number of antenatal glucocorticoid courses, and European ethnicity. In addition, regression on gestation length is adjusted for birthweight Z-score. R² values: a=0.03, b=0.09, c=0.08, d=0.07, e=0.16, f=0.08, g=0.04, h=0.04, i=0.10, j=0.14.
Table 8.3 Effect of gestational age group on body size and cardiovascular risk factor characteristics of physiological study participants at early school age.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Very preterm (&lt;32 wk)</th>
<th>Moderate and late preterm (32 to 36 wk)</th>
<th>Term (≥37 wk)</th>
<th>n</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body size</td>
<td></td>
<td></td>
<td></td>
<td>152</td>
<td></td>
</tr>
<tr>
<td>Height Z score</td>
<td>-0.11 (1.23)</td>
<td>0.62 (1.00)</td>
<td>0.74 (1.03)</td>
<td>86</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight Z-score</td>
<td>-0.14 (1.37)</td>
<td>0.61 (1.18)</td>
<td>1.08 (1.03)</td>
<td>26</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body mass index Z-score</td>
<td>-0.07 (1.16)</td>
<td>0.36 (1.29)</td>
<td>0.98 (1.11)</td>
<td></td>
<td>0.0003</td>
</tr>
<tr>
<td>Ambulatory blood pressure Z-score</td>
<td></td>
<td></td>
<td></td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Daytime systolic</td>
<td>-0.42 (1.16)</td>
<td>-0.42 (1.09)</td>
<td>-0.32 (1.28)</td>
<td>74</td>
<td>0.93</td>
</tr>
<tr>
<td>Daytime diastolic</td>
<td>-0.68 (1.02)</td>
<td>-0.56 (0.88)</td>
<td>-0.69 (1.16)</td>
<td></td>
<td>0.70</td>
</tr>
<tr>
<td>Night-time systolic</td>
<td>-0.14 (0.88)</td>
<td>-0.09 (1.04)</td>
<td>0.07 (1.50)</td>
<td></td>
<td>0.65</td>
</tr>
<tr>
<td>Night-time diastolic</td>
<td>0.13 (0.84)</td>
<td>0.16 (0.99)</td>
<td>0.10 (1.37)</td>
<td></td>
<td>0.96</td>
</tr>
<tr>
<td>Ambulatory heart rate Z-score</td>
<td></td>
<td></td>
<td></td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Daytime</td>
<td>-0.43 (0.93)</td>
<td>-0.36 (0.83)</td>
<td>-0.24 (0.94)</td>
<td>74</td>
<td>0.62</td>
</tr>
<tr>
<td>Night-time</td>
<td>0.21 (1.01)</td>
<td>0.47 (1.07)</td>
<td>0.52 (1.13)</td>
<td></td>
<td>0.16</td>
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<tr>
<td>Glucose and insulin metabolism</td>
<td></td>
<td></td>
<td></td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose concentration—mmol/L</td>
<td>4.7 (0.3)</td>
<td>4.8 (0.3)</td>
<td>4.7 (0.5)</td>
<td>64</td>
<td>0.31</td>
</tr>
<tr>
<td>Fasting insulin concentration—mIU/L†</td>
<td>4.8</td>
<td>5.2</td>
<td>6.5</td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>Insulin sensitivity—x10⁻⁴ min⁻¹.mIU⁻¹.L †</td>
<td>8.8</td>
<td>7.8</td>
<td>8.0</td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>Acute insulin release mIU.L⁻¹.min †</td>
<td>305.5</td>
<td>306.9</td>
<td>407.2</td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td>Disposition index†</td>
<td>0.26</td>
<td>0.20</td>
<td>0.28</td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>Glucose effectiveness—x10⁻² min⁻¹†</td>
<td>2.6</td>
<td>2.7</td>
<td>2.5</td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>Outcome</td>
<td>Very preterm (&lt;32 wk)</td>
<td>Moderate and late preterm (32 to 36 wk)</td>
<td>Term (≥37 wk)</td>
<td>p*</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------------------------</td>
<td>-----------------------------------------</td>
<td>---------------</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>Glucose disappearance constant (Kg)—x10^{-2} min^{-1}†</td>
<td>2.6</td>
<td>2.4</td>
<td>2.4</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td><strong>DXA total body composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass—kg†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.5</td>
<td>5.0</td>
<td>6.3</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Android (AFM)</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.001</td>
<td></td>
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<tr>
<td>Gynoid (GFM)</td>
<td>0.9</td>
<td>1.2</td>
<td>1.4</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Lean mass—kg†</td>
<td>18.2</td>
<td>20.5</td>
<td>21.4</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Fat mass index (FM)—kg/m^6†</td>
<td>1.1E^{12}</td>
<td>1.1E^{11}</td>
<td>1.4E^{11}</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Lean mass index (LM)—kg/m^2</td>
<td>12.4</td>
<td>12.5</td>
<td>13.0</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>FM/[LM]^2 x10^{-3}†</td>
<td>0.10</td>
<td>0.12</td>
<td>0.14</td>
<td>0.11</td>
<td></td>
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<tr>
<td>AFM/[LM]^2 x10^{-3}†</td>
<td>0.69</td>
<td>0.80</td>
<td>0.96</td>
<td>0.15</td>
<td></td>
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<tr>
<td>AFM/GFM†</td>
<td>0.26</td>
<td>0.28</td>
<td>0.31</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Basal salivary cortisol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean morning concentration—nmol/L†</td>
<td>4.2</td>
<td>4.5</td>
<td>4.3</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Mean evening concentration—nmol/L†</td>
<td>0.8</td>
<td>0.9</td>
<td>0.7</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Diurnal slope†</td>
<td>-0.18 (0.07)</td>
<td>-0.17 (0.07)</td>
<td>-0.19 (0.07)</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Estimated glomerular filtration rate—mL/min/1.73m2</td>
<td>104.0 (13.6)</td>
<td>106.8 (14.3)</td>
<td>113.1 (17.9)</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean (standard deviation) or geometric mean†. Positively skewed data† were transformed to the natural logarithm for analysis. *P value for linear trend.
8.6 Discussion

This chapter reports the effect of gestation length and fetal growth, explored as birthweight Z-score, on cardiovascular risk factors in 264 children at early school age, who participated in the New Zealand ACTORDS physiological follow-up study. The study cohort included a wide range of gestational ages, although the majority of children were born between 28 to 34 weeks’ gestation and only 10% were born at term. Thus, the results of this study primarily reflect outcomes following moderate and very preterm birth. Term children were included in the analyses in order to examine continuous associations across the broadest range of gestation lengths and birthweight Z-scores, but their inclusion did not alter results for most outcomes. While there was considerable variation in birthweight at all gestational ages, fetal growth restriction tended to be more common at lower gestations. On average, infants in this cohort were a third of a standard deviation lighter at birth than the reference population, although most of the children born at term had normal birthweights.

The main positive findings in this study related to body composition. Shorter gestation length and lower birthweight Z-score were associated with smaller body size at early school age (height, length and body mass index Z-scores), and hence were related to lower total body and regional fat mass and lean mass. However, when adjusted for body size, as determined by height, the associations with body composition were substantially diminished or abolished except for a highly significant association between birthweight Z-score and lean mass, which remained after exclusion of children born at term. Therefore, it appeared that children who were born earlier remained smaller in mid-childhood but had proportionately reduced fat and lean mass, whereas children who had poorer fetal growth had persisting deficits in lean mass, even allowing for smaller body size. This may explain why, in the preterm subgroup, poorer fetal growth was associated with reduced weight for age and height but gestation length was not associated with reduced weight for height. Despite their reduced lean mass, children with poorer fetal growth did not have altered fat mass relative to height or lean mass. This apparent inconsistency may be due to the fact that birthweight Z-score explained only a modest amount of the variability in lean mass, even though the relationship was highly statistically significant. In contrast, shorter gestation length showed no relationship with later lean mass for height, but there was some suggestion in the whole cohort that shorter gestation length was associated with decreased relative fat mass.
Interpretation of paediatric body composition data is complex, as allometric relationships between tissue mass and parameters of body size are affected by growth. In adults, body and organ weights generally scale with the square of height (Heymsfield 2007), but in children, total mass and the components of body composition scale to height in different ways (Fewtrell 2004; Metcalf 2011). Correction of soft tissue mass for height can be achieved using log-log regression to determine the power by which to raise height to calculate tissue indices (typically a power of two for lean mass and six for fat mass) or by adjusting the relationship between variables for height using linear regression (Wells 2002; Wells and Victora 2005). However, complete normalisation of paediatric body composition data for height is controversial, as increased adiposity is associated with increased linear growth, and children who mature faster may have greater risk of later obesity and cardiovascular disease (Lever 1992; Metcalf 2011; Parsons 2001; Weder 1994). Nevertheless, if indices of weight and fat remain correlated to height (as is the case with fat mass indexed to the square of height), it is difficult to determine whether associations with other disease variables are mediated by actual changes in body composition or by effects of linear growth (Wells and Cole 2011). Thus, in this study relationships between tissue mass and gestation length and birthweight Z-score were fully adjusted for height.

In addition to the assessment of absolute fat and lean tissue mass for body size, establishing the relative proportions of these tissues provides further information regarding metabolic risk. Lean mass, especially skeletal muscle, can be thought of as reflecting metabolic capacity whereas fat mass can be viewed as a marker of metabolic load (Wells and Victora 2005; Wells 2011). An individual’s risk of metabolic and cardiovascular disease is increased when there is a greater metabolic load for a given metabolic capacity. Excess weight usually results from a disproportionate increase in fat mass, but in subjects with greater lean mass, relative fatness is overestimated if assessed by a simple one-to-one ratio to lean mass (Wells and Victora 2005). Rather, fat mass should be indexed to the square of lean mass, or adjusted for lean mass by linear regression (Wells and Victora 2005). Relative fatness is often expressed as a percentage of total mass, but this should be avoided, because inclusion of fat mass as both numerator and denominator underestimates fatness as weight increases (Cole 2008).

This study found of a positive relationship between lean mass for height and birthweight Z-score. This is consistent with a growing body of evidence in term or near term populations that birthweight is correlated with subsequent lean mass, which mainly consists of skeletal
Adequate growth of muscle during fetal life is important as the potential for myogenesis declines in early infancy and subsequent muscle growth is largely achieved by an increase in the length and size of existing muscle fibres (Gollnick 1981). Studies in sheep and pigs have shown that fetal undernutrition reduces muscle fibre number and the density of myonuclei, both of which limit the capacity for later hypertrophic growth (Greenwood 2000; Rehfeldt 2006). Similar histological changes have been observed in human infants born small for gestational age (Widdowson 1972), and growth restricted infants have reduced total body lean mass as well as fat mass (Ibanez, Sebastiani, 2008; Koo 2004; Verkauskiene 2007). Reduced muscle mass may be explained by the fact that fetuses adapt to reduced nutrient supply by substituting amino acids for glucose as a main oxidative fuel, thus limiting the availability of amino acids for protein synthesis (Bloomfield 1998; Hay 1994). This downregulates the mTOR pathway in skeletal muscle, which is a key regulator...
of myogenesis (Zhu 2004). Reduced placental supply of glucose also leads to lower fetal IGF-1 levels, which has a role in promoting musculoskeletal growth (Javaid 2004; Jensen 2000). Furthermore, the combination of intrauterine growth restriction and poor postnatal growth appears to have a compounding effect on muscle accretion (Greenwood 2000; Yliharsila 2008).

Although excess fat, especially abdominal fat, is a key risk factor for cardiovascular and metabolic disease (Yusuf 2005), there is some evidence that low muscle mass may also contribute independently to the risk of adult chronic disease. Skeletal muscle is an important determinant of energy expenditure, and reduced muscularity may predispose to adipose deposition, especially when combined with an energy-dense diet (Dulloo 2006). It is also the main site for glucose disposal, and while some studies have shown no association between muscle mass and glucose tolerance (Kuk 2008; Phillips 1995; Taylor 1995), others have demonstrated a positive relationship between muscle mass and insulin sensitivity (Atlantis 2009; Srikanthan 2010; Srikanthan 2011; Van Der Heijden 2010). In addition to effects on muscle mass, fetal growth restriction may induce functional changes in muscle fibres that contribute to insulin resistance, including reduced capacity to take up and utilise glucose and altered fatty acid oxidation (Ozanne 2003; Selak 2003; Vaag 2006; Zhu 2006). Theoretically, individuals with low muscle mass will have higher relative fat mass at any given weight and hence a greater metabolic demand on a lower functional capacity (Murphy 2006; Wells 2011). However, one study suggested that low birthweight subjects may have a compensatory increase in muscle metabolic activity, possibly as a result of increased sympathetic activity (Eriksson 2002), which is also a common feature of metabolic syndrome (Seematter 2005). This could explain why lower lean mass has been associated with impaired regulation of blood pressure during stress (Wilson 2004), even though both fat and lean mass are correlated with resting blood pressure (Brion 2007). Thus, there are several mechanisms by which a permanent reduction in lean mass for body size could confer an increased risk of metabolic disease, and this may partly explain the known association between small size at birth and adult cardiovascular disease, especially when fetal growth restriction is followed by poor infant growth (Barker 2009).

Variation in lean mass may also explain the apparent paradox that high birthweight is associated with increased adult body mass index (Parsons 1999; Parsons 2001; Sorensen 1997) but in several studies has been associated with a lower risk of cardiovascular disease compared with low birthweight (Barker 2002; Eriksson 2000; Frankel 1996; Leon 2000; Newsome 2003; Rich-Edwards 1997). This is because individuals who have a higher body
mass index due to increased muscle mass are likely to have a favourable cardiovascular risk factor profile (Murphy 2006). However, the long-term benefits of high birthweight may depend on whether such infants are longer or fatter at birth (Rogers 2006), and high birthweight may not be associated with decreased all-cause mortality due to the opposing effects of increased cancer mortality (Baker 2008).

In contrast to the relatively predictable relationship between birth size and lifetime trajectory of lean mass, associations with later fat mass or adiposity have been markedly less consistent. A large number of studies in term populations have variously shown negative, positive or non-significant relationships between birthweight, or other indicators of birth size, and measures of total body fat (Wells 2007). Differences in measurement technique, age at follow-up and postnatal growth may be responsible for some of this variation. However, studies in preterm subjects have been more consistent and have not shown any relationship between birthweight for gestational age and relative fatness (for weight or height), either in childhood (Fewtrell 2004; Peralta-Carcelen 2000) or young adulthood (Euser 2005; Rotteveel 2008). Similarly, in several very low birthweight cohorts, which are biased towards subjects with fetal growth restriction, percentage fat did not differ from that of normal birthweight term controls, but lean mass for height was reduced (Hovi 2007; Wang 2007; Weiler 2002). Thus, based on these data and the results of this study, it would appear that in preterm subjects fetal growth has little effect on later relative fat mass. Nevertheless, there was some suggestion in this study that poorer fetal growth was associated with increased central (abdominal) fat compared with peripheral (thigh) fat, a finding that has previously been reported in one study of children born preterm (Gianni 2008) and in several studies of children born at term (Barker 1997; Garnett 2001; Ibanez, Lopez-Bermejo, 2008; Malina 1996). Central fat deposition, especially intra-abdominal, is associated with increased risk of metabolic disease independently of overall fatness (Cnop 2002; Wajchenberg 2000). However, this risk probably relates to the absolute rather than relative amount of abdominal fat (Wells and Victora 2005), and no association was found between birthweight Z-score and abdominal fat when adjusted for height or lean mass.

It is surprising that gestation length, whether analysed as a continuous or categorical variable, had no impact on later lean mass for height given that peak myogenesis continues into the third trimester (Widdowson 1972), and that very preterm infants frequently have protein energy deficits (Hay 2008) and postnatal growth failure (Cooke 2004). Furthermore, very preterm birth and fetal growth restriction are both associated with
suppression of the somatotrophic axis, including decreased circulating concentrations of key growth factors such as IGF-1 and IGFBP-3 (Giudice 1995; Kajantie 2003; Verhaeghe 2003; Verkauskiene 2007). However, the absence of any association between gestation length and lean mass is consistent with several studies that have shown no difference in lean mass between children born preterm and those born at term, when adjusted for current weight or height (Fewtrell 2004; Gianni 2008; Peralta-Carcelen 2000; Willemsen 2008). Furthermore, in one of these studies there was no linear association between lean mass for height and gestation length within the preterm group (gestational age range 25 to 36 weeks) (Fewtrell 2004). This apparent differential effect of prenatal versus postnatal growth failure on lean tissue accretion has not been explained, but may relate to the fact that chronically growth restricted fetuses continue to have reduced capacity to synthesise protein once nutrition has been restored, and hence experience a combined prenatal and postnatal nutritional insult (Hay 2008). There may also be subtle differences in perturbations of the somatotrophic axis caused by pre-natal growth restriction and preterm birth (Cutfield 2004; Verhaeghe 2003).

In contrast, children born preterm have generally been found to have reduced fat mass for weight or height compared to term children (Fewtrell 2004; Gianni 2008; Willemsen 2008), although one study suggested that this was more of a categorical effect rather than a graded association with gestation length (Fewtrell 2004). The results for fat composition in this study are consistent with this view in that there were trends to a positive association between gestation length and relative fat mass in the whole cohort but not after exclusion of children born at term. Despite these data, there are concerns that very preterm birth may be associated with increased risk of obesity in later life, as very or extremely low birthweight subjects in several longitudinal cohort studies have demonstrated relatively greater catch-up growth in weight than height during puberty, especially females (Doyle 2004; Hack 2003; Saigal 2006). A limitation of these studies is that cohorts were weight-limited, and hence it is difficult to determine if effects are due to very preterm birth or fetal growth restriction. Reassuringly, no studies of very preterm or very low birthweight subjects have reported any increase in body mass index in early adulthood compared to term controls (Bonamy 2005; Doyle 2004; Evensen 2009; Hack 2003; Hovi 2007; Kistner 2000; Rogers 2005; Rotteveel 2008; Saigal 2006; Weiler 2002), or any difference in total percent body fat (Hovi 2007; Rotteveel 2008; Weiler 2002).

Data regarding the effect of preterm birth on fat distribution are mixed, with some studies showing an increase in central or visceral fat compared to control subjects (Bonamy 2005;
Evensen 2009; Gianni 2008; Uthaya 2005) and others showing no difference (Hovi 2007; Willemsen 2008). Again, many of these cohorts were weight-limited, making assessment of the independent effects of preterm birth more difficult. Our study showed no evidence of an inverse relationship between gestation length and abdominal fat mass at mid-childhood.

There was some evidence that children born at earlier gestations had reduced glomerular filtration rate, as estimated from plasma creatinine and height, although results were not significant in adjusted analyses or after exclusion of children born at term. This is in keeping with previous concerns that preterm birth reduces total nephron mass (Faa 2010; Rodriguez 2004; Rodriguez 2005). In humans, nephrogenesis normally occurs from 20 to 36 weeks’ gestation after which no new glomeruli are formed (Hinchliffe 1991). After preterm birth, nephrogenesis continues for a short period but is likely to be incomplete (Faa 2010; Rodriguez 2004), and the glomeruli that are formed may be morphologically abnormal (Gubhaju 2009). Nephrogenesis is further impaired by neonatal renal failure and poor postnatal nutrition (Abitbol 2003; Bacchetta 2009). Individuals with low nephron mass compensate for reduced filtration surface area by increasing glomerular volume and pressure, but this can lead to progressive hyperfiltration-induced glomerulosclerosis and premature age-related loss of renal function (Brenner 1988; Brenner 1994; Hodgin 2009; Hoy 2003). They also have less kidney reserve to compensate for other renal insults or disease (Nenov 2000), and are more sensitive to salt load, which may contribute to the development of hypertension (Sanders 2005; Simonetti 2008).

Despite these concerns, data on long-term renal outcomes after preterm birth are limited. One study found that children with extremely low birthweight had a small reduction in estimated glomerular filtration rate and altered tubular function compared to term children (Rodriguez-Soriano 2005), but several studies did not show any difference between very preterm and term subjects in basal or stimulated glomerular filtration, either in childhood (Chan 2010; Rakow 2008) or early adulthood (Keijzer-Veen 2007; Kistner 2005). One study found that preterm infants had reduced renal volume for age, an indirect measure of renal mass, but there was no difference between preterm and term subjects at 18 months of age after correction for weight (Schmidt 2005). Similarly, two childhood studies found no difference in renal volume between very preterm and term children (Rakow 2008; Rodriguez-Soriano 2005). However, a study of young adults suggested that those born with very low birthweight had reduced renal volume compared to term controls (Keijzer-Veen, Devos, 2010). All of these studies were relatively small and so had limited power and are
at risk of selection bias. A larger study of 422 very preterm young adults found no linear association between estimated glomerular filtration rate or microalbuminuria and gestation length, but control subjects were not included (Keijzer-Veen, Schrevel, 2005). As the effects of low nephron mass may not appear until after the third decade (Anderson 1986; Muntner 2009), longer-term follow-up of large cohorts, with control subjects, is needed to determine the impact of preterm birth on nephrogenesis and renal reserve.

There is also strong evidence that fetal growth restriction results in impaired nephrogenesis and reduced total nephron mass. Low birth weight at term or small size for gestational age has been associated with low glomerular number at autopsy in infants (Hinchliffe 1992) and adults (Hughson 2003; Manalich 2000), reduced renal volume on ultrasound in early life (Konje 1997; Schmidt 2005; Silver 2003; Simonetti 2008; Spencer 2001), lower renal function and filtration reserve (Chan 2010; Hallan 2008), and an increased risk of early end-stage renal disease (Vikse 2008; White 2009). Such associations are strongest with severe fetal growth restriction but extend throughout the birthweight range, possibly in a U-shaped manner. Congenital oligonephropathy is strongly implicated in the pathogenesis of essential hypertension (Hughson 2006; Keller 2003; Kett 2004), and hence is likely to be one of the mechanisms linking small size at birth with increased risk of adult cardiovascular disease (Barker 2006; Mackenzie 1995). This hypothesis is supported by experimental evidence in animals, especially rodents (Moritz, Singh, 2009).

Given the importance of adequate fetal growth for renal development, it is surprising that there was no association in our study between birthweight Z-score and glomerular filtration rate, either in the whole cohort or after exclusion of children born at term. This could be due to estimated renal function not being sufficiently sensitive to reflect total nephron mass in children. Indeed, one study found that measured glomerular filtration rate was reduced in extremely low birth weight children who were born small for gestational age compared to those with appropriate birthweight for age (Bacchetta 2009). Moreover, in infants born at 28 to 36 weeks’ gestation, those with low birthweight for age had reduced renal volume for height (Drougia 2009). By early adulthood, a positive association between birthweight standard deviation score and estimated glomerular filtration rate was seen in a large cohort of very low birthweight subjects, as well as an inverse association with microalbuminuria (Keijzer-Veen, Schrevel, 2005). Nevertheless, when a subgroup of this cohort underwent more detailed investigation, there were no differences between those born small and those born of normal birthweight for gestational age in renal volume and measured glomerular filtration rate, although the group with prenatal growth restriction appeared to have more
microalbuminuria (Keijzer-Veen 2007; Keijzer-Veen, Devos, 2010). Thus, fetal growth restriction is likely to influence later renal function in preterm subjects but the association is not straightforward.

Because blood pressure is easily measured, its relationship to fetal growth and preterm birth has been more extensively studied. In animals, long-term elevations in blood pressure have been surprisingly easy to induce with various prenatal manipulations that affect fetal growth, including maternal dietary restriction, utero-placental insufficiency and glucocorticoid exposure (McMillen 2005; Vehaskari 2005). A large number of epidemiological studies in children, adolescents, and adults have also demonstrated modest increases in blood pressure with lower birthweight, of around 2 mmHg per kg for systolic pressure, and this relationship is intensified with age and obesity (Huxley 2000). However, these studies probably underestimate the effect of lower birthweight on cardiovascular reactivity to stress (Kajantie and Raikkonen 2010), which is a strong predictor of future overt hypertension (Matthews 2004). Disturbances in autonomic function and heightened blood pressure response to psychological stress are being increasingly recognised in low birthweight subjects (Feldt 2011; Johansson 2007; Jones 2007; Jones 2008; Phillips 1997; Spassov 1994; Ward, Moore, 2004). When combined with altered renal development, this may lead to development of hypertension in the long-term. In addition, low birthweight has been associated with altered vascular development, including reduced vascular elastin content (Martyn 2001) and endothelial dysfunction (Leeson 1997; Martin 2000; Skilton 2011).

At a population level, most of the risk of high blood pressure attributable to low birth weight, at least in the current elderly, is due to diminished fetal growth (Bonamy 2008; Eriksson 2000). However, several large studies that included a number of moderate or late preterm subjects have also shown an inverse relationship between gestation length and adult blood pressure (Cooper, Atherton, 2009; Crump 2011; Dalziel 2007; Jarvelin 2004; Johansson 2005; Leon 2000; Pharoah 1998; Siewert-Delle 1998), although this was not seen in all studies (Bonamy 2008) and did not extend to term gestations in others (Dalziel 2007; Siewert-Delle 1998). The effect of preterm birth on blood pressure appears to be stronger and more consistent in adolescents and young adults born very preterm or with very low birthweight. A growing number of studies have reported increases in systolic pressure in those born preterm compared to term controls in the order of 5 to 6 mmHg (Bonamy 2005; Doyle 2003; Edstedt-Bonamy 2008; Evensen 2009; Hack 2005; Hovi 2007; Irving 2000; Kistner 2000; Rotteveel 2008; Vohr 2010), with few exceptions
(Rogers 2005; Singhal 2001), and this has been confirmed with ambulatory blood pressure monitoring (Doyle 2003; Hovi 2010; Keijzer-Veen, Dulger, 2010; Kistner 2005). The fact that differences in blood pressure tend to be stronger when measured in clinic than with ambulatory monitoring would also suggest that very preterm birth is associated with exaggerated cardiovascular responses to stress (Kajantie and Raikkonen 2010; Pyhala 2009).

In our study, shorter gestation length tended to be associated with increased daytime ambulatory blood pressure Z-scores, but only after adjusting for the lower body mass index of children born at earlier gestations, presumably due to lower relative fat mass. Whether or not this represents a true effect on vascular function is unclear, but it suggests that any future catch-up in weight in the most preterm children may be associated with relatively higher blood pressure. Perinatal factors associated with programming of later blood pressure would be expected to have a relatively small effect in childhood, as changes to organ structure and function are likely to be compensated for over many years until a point at which homeostasis can no longer be maintained (Barker 2006). Indeed, results of previous studies comparing blood pressure in very preterm children with controls have been mixed, with some showing a marginal increase in systolic pressure (Bonamy 2007; Mikkola 2007) and others showing no difference (Bracewell 2008; Johansson 2007). However, there seems to be a clear effect of very preterm birth on blood pressure by late adolescence. Thus, the trend to higher blood pressure for height and body mass with shorter gestation length in our cohort may prove to be more significant over time.

Although fetal growth restriction may exacerbate the effects of preterm birth on renal development, it does not appear to have any additional effect of blood pressure in subjects born very preterm, at least into early adulthood. In studies that compared very preterm subjects born small or appropriate for gestational age, no differences were seen in blood pressure (Johansson 2007; Keijzer-Veen, Dulger, 2010; Mikkola 2007) and risk of high blood pressure (Vohr 2010). Furthermore, in three studies that assessed the continuous effect of birthweight standard deviation scores, there was no linear relationship with blood pressure in young adults born with very low birthweight (Doyle 2003; Hovi 2010; Keijzer-Veen, Finken, 2005). In keeping with these findings, a large population-based study of Swedish young adult males found that being born small for gestational age increased the risk of high blood pressure from 33 weeks’ gestation onwards but not before. In a small study of preterm children, nocturnal ambulatory blood pressure was increased in those born small for gestational age but the distribution of gestational ages extended to 36 weeks.
In our study, there was no evidence that birthweight Z-score was inversely associated with blood pressure, which is consistent with the above literature. Instead, there was a trend towards a positive relationship, although this was abolished by adjustment for body mass index. This may reflect the lower lean mass of children with poorer fetal growth, as blood pressure is positively correlated with lean mass (Brion 2007).

There is strong evidence that birthweight has a continuous and graded inverse relationship with risk of type 2 diabetes, although in some populations there may also be a modest increase in risk with high birthweight, largely driven by gestational diabetes-induced macrosomia (Harder 2007; Newsome 2003; Whincup 2008). Moreover, birthweight accounts for a substantial proportion of the lifetime risk of type 2 diabetes independently of adult body size, with a 20% decrease in risk for every kilogram increase in birthweight (Whincup 2008). Animal models of fetal undernutrition have identified multiple disturbances of glucose regulation that could lead to long-term impairment of insulin action and glucose tolerance, including decreased beta cell mass and secretory capacity, upregulation of hepatic gluconeogenesis, and decreased skeletal muscle glucose uptake, glycolysis and glycogenesis (Gatford 2010; McMillen 2005). Similarly in humans, being born small compared with normal birthweight for gestational age has been associated with both insulin resistance and reduced compensatory beta cell secretion, although the association with insulin resistance is more consistent (Choi 2000; Hofman 1997; Jensen, Storgaard, 2002; Mericq 2005; Ong 2004; Veening 2003). Thus, the relationship between lower birthweight and diabetes is likely to be explained to a significant degree by impaired fetal growth.

However, several population-based studies have also shown an independent association between shorter gestation length and risk of type 2 diabetes, possibly with an increased risk after term (Kaijser 2009; Kajantie, Osmond, 2010; Lawlor 2006; Pilgaard 2010). Consistent with this, in a cohort of young adults that included subjects born moderately and late preterm, there was an inverse relationship between gestation length and insulin secretion following oral glucose challenge, suggesting insulin resistance (Dalziel 2007). Glucose regulation has been studied more extensively in very preterm or very low birthweight subjects and most studies have shown that these groups have reduced insulin sensitivity compared to normal birthweight term controls, in childhood and early adulthood (Hofman 2004; Hovi 2007; Rotteveel 2008). One study of young adults born from 27 to 36 weeks’ gestation found no difference in minimal model insulin sensitivity compared to subjects born at term, but the control group was also at risk of insulin resistance as many
had short stature or intrauterine growth retardation (Willemsen 2009). Another study of preterm adolescents born at a mean gestational age of 31 weeks found no difference in insulin resistance compared to term controls, but this was on the basis of a surrogate marker, 32-33 split proinsulin (Singhal, Fewtrell, 2003). Overall, it would seem that individuals born preterm, especially at very early gestations, are at risk of insulin resistance, perhaps to a similar degree to term subjects with intrauterine growth retardation (Hofman 2004; Willemsen 2008).

The mechanisms underlying the effect of preterm birth on later insulin sensitivity have not been closely investigated, but could involve disruption of the normal development of insulin signalling and transduction pathways (Blanco 2010), due to factors such as postnatal hyperglycaemia, altered protein and energy intake (Hofman, Regan, Jefferies, 2006; Singhal, Fewtrell, 2003), and relative hyperoxia (Bandali 2003). However, preterm birth has so far not been associated with long-term effects on beta cell function (Hofman 2004; Kajantie 2011), and the emergence of glucose intolerance may depend on other risk factors, especially increased intra-abdominal fat mass (Cnop 2002; Hanley 2009; Kahn 2006). Although there is some evidence that fetal growth restriction may increase the risk of insulin resistance and diabetes in preterm subjects (Bazaes 2004; Kaijser 2009), in several preterm cohorts no differences in glucose regulation were found between those born smaller or larger for gestational age (Finken 2006; Hofman 2004; Hovi 2007; Rotteveel 2008).

The finding in this study that insulin sensitivity showed no relationship with gestation length appears somewhat inconsistent with the current literature. One possible explanation is that the effect of preterm birth on insulin sensitivity may be more categorical than graded, in which case a difference would only be seen in comparison to a normal birthweight term control group. In this cohort, relatively few children were born at term and it is possible that these children also have abnormal insulin sensitivity due to the various antenatal exposures for which their mothers were recruited to the ACTORDS trial. There are currently no published data on minimal model insulin sensitivity in healthy prepubertal children born at term which are derived from the same test protocol as used in this study, but extrapolation of data from a tolbutamide-modified protocol would suggest that this value should be around $14 \times 10^{-4} \text{min}^{-1}/[\text{mIU/L}]$ (Hofman 2004; Saad, Steil, Kades, 1997). Thus, in our cohort even children born at term may have a degree of insulin resistance, thereby obscuring any possible effect of preterm birth. Although the majority had normal birthweight, adverse in utero conditions may alter offspring metabolism.
without affecting size at birth. For example, adults exposed to poor nutrition in early gestation during the Dutch famine of 1944 to 1945 had reduced glucose tolerance despite normal birthweight (de Rooij 2006).

In our mostly preterm cohort, poorer fetal growth was not associated with any adverse effect on glucose and insulin metabolism. Although there was a trend to increased first phase insulin secretion with lower birthweight Z-score, this was dependent on adjustment for body mass index and did not appear to be a compensatory response for reduced insulin sensitivity. This is in agreement with studies that have shown no additional effect of fetal growth on glucose metabolism after preterm birth, although these studies have generally looked at insulin resistance across all preterm subjects relative to a term control group.

Altered hypothalamic-pituitary-adrenal axis function is another potential mechanism by which fetal growth restriction may affect the long-term risk of cardiovascular and metabolic disease. Animal experiments have shown that maternal undernutrition can result in increased fetal exposure to maternal glucocorticoids (Lesage 2001), permanent changes in the expression of central glucocorticoid receptors and corticotropin-releasing hormone (Go 2001; Lingas 2001), and altered adrenocortical function of offspring in adult life (Bloomfield 2003; Lingas 2001). Epidemiological studies in several populations found that lower birthweight was associated with elevated fasting cortisol concentrations in early and later adulthood (Kajantie 2002; Levitt 2000; Phillips 1998; Phillips 2000) and increased cortisol response to ACTH stimulation (Levitt 2000; Reynolds 2001). However, other studies in non-stressed conditions did not show any relationship between size at birth and daily cortisol production (Fall 2002; Honour 2007; Kajantie 2004; Koupil, Mann, 2005) or basal hypothalamic-pituitary drive (Ward, Syddall, 2004). This has led some authors to speculate that lower birthweight is primarily associated with an exaggerated cortisol stress response, and the raised plasma cortisol concentrations seen in earlier studies may reflect participant stress to fasting and venesection (Kajantie and Raikkonen 2010; Phillips 2006). This hypothesis is supported by two recent studies in children and young adults that found that those with lower birthweight had higher cortisol responses to psychological challenge (Jones 2006; Wust 2005). However, this effect may change over time, as in late adulthood there was an inverse U-shaped relationship between birthweight and cortisol response using the same stress test (Kajantie 2007). There is also increasing evidence of sexual dimorphism in stress systems, and males born with low birthweight appear to have greater reactivity of cortisol whereas sympathoadrenal responses seem to predominate in females (Kajantie and Raikkonen 2010).
Transient hypocortisolism is common in infants born very preterm due to immaturity at both the hypothalamic-pituitary and adrenal levels (Bolt 2002; Ng 2000), but there are few data on the long-term effects of preterm birth on hypothalamic-pituitary-adrenal axis function. A study of 18-month-old infants found that those born extremely preterm, but not those born from 29 to 32 weeks’ gestation, had higher morning salivary cortisol concentrations compared with those born at term (Brummelte 2011). Similarly, in later childhood, subjects born preterm had elevated salivary cortisol concentrations after awakening compared with term controls, but appeared to have a blunted salivary cortisol response to psychosocial stress (Buske-Kirschbaum 2007). However, these studies were relatively small and may be susceptible to selection bias. In contrast, in the present study there was no association between gestation length and basal salivary cortisol concentration. This is consistent with two studies in young adults that found that preterm birth did not affect fasting plasma cortisol concentration, although these cohorts included few very preterm subjects (Dalziel 2007; Walker 2002). There is little information on the effect of fetal growth restriction in preterm subjects, but in a population-based cohort of very preterm or very low birthweight subjects there was no association between birthweight standard deviation scores and fasting serum cortisol concentrations in early adulthood (Meuwese 2010).

Our cohort study has several limitations. First, in the absence of a normal term control group the absolute effect of preterm birth on cardiovascular risk indicators cannot be determined. However, a continuous relationship with gestation length would be expected for outcomes that are substantially affected by preterm birth. Despite this limitation, the associations examined in this study are clinically relevant. For example, it is important to know whether fetal growth restriction exerts an additional influence on long-term outcomes in preterm subjects, as this may influence neonatal management strategies and the type of long-term follow-up that is required. The relative influence of gestation length and fetal growth on long-term outcomes is also important in deciding the optimal time to deliver a compromised fetus.

Second, birthweight for gestational age based on a population reference is a crude measure of in utero growth as some newborns may be growth-restricted despite having a birthweight within the normal range while others are small but have normal fetal growth for their genetic potential (Verkauskiene 2007). Thus, birthweight Z-score may underestimate the true effect of fetal growth on long-term outcomes. Accurate identification of intrauterine growth retardation requires direct measures of fetal growth.
velocity, supported by other indicators of placental and fetal health (Zhang, Merialdi, 2010). However, in epidemiological research, such information is usually not available and the degree to which the fetus has attained optimal size has to be ascertained from birth anthropometric data. It has been proposed that customised birth percentiles, which adjust for maternal and pregnancy characteristics, improve the identification of pathologically versus constitutionally small babies (Gardosi 2006). But customised standards have only been validated against perinatal outcomes and not life-course morbidity (Zhang, Merialdi, 2010), and hence may inappropriately normalise the size of the infant for environmental influences that could affect long-term outcomes (Hutcheon 2011). Furthermore, the clinical benefit of customised standards may be primarily due to adjustment of preterm birth size for intra-uterine reference values, as a result of backwards calculation from the term target weight (Hutcheon 2011). This emphasises that in preterm populations, reference data based on actual birthweight may underestimate the growth trajectories of healthy growing fetuses at equivalent gestational ages (Cooke 2007). Pregnancies that result in preterm birth are often associated with factors that may impair fetal nutrition and growth, such as placental pathology or maternal stress.

Neonatal body proportions, such as relative thinness or head sparing, may provide additional information as to whether fetal growth has been optimal. However, the relationship between these measures and later outcomes can vary by sex, disease, and population. For example, in the Helsinki birth cohort, coronary artery disease was strongly related to thinness (ponderal index) at birth in males (Eriksson 1999) but shortness in females (Forsen 1999), whereas in the United Kingdom coronary artery disease was related to either shortness or thinness in males (Martyn 1996). Also, the risk of stroke is increased by low birthweight relative to head size (Martyn 1996). Thus, birthweight for gestational age, although imperfect, is probably still a good single measure by which to explore associations between fetal growth and disease risk factors. Additionally, it is important to note that an imbalance in macronutrients or short periods of fetal undernutrition may have long-term effects on cardiovascular disease risk without changing size at birth (Bloomfield 2003; de Rooij 2006; Painter 2006; Reynolds 2007).

Third, although this cohort included a broad distribution of gestational ages, it may not be representative of the entire preterm population as only women who remained pregnant more than 7 days after an initial course of antenatal glucocorticoids were recruited to the ACTORDS trial. These women may have different perinatal risk factors compared with those who give birth within seven days of glucocorticoid treatment. For example, one
study found that women who gave birth at <34 weeks’ gestation and within seven days of glucocorticoid treatment compared with those who gave birth after seven days were more likely to have spontaneous preterm labour, hypertension or pre-eclampsia (McLaughlin 2002). Nevertheless, about 40% of all women presenting with complications of pregnancy, for which glucocorticoids are indicated, deliver more than seven days after treatment. Thus, our cohort does represent a large proportion of infants born to mothers with higher risk pregnancies.

8.7 Summary
In this cohort of 264 school-aged children, born from 24 to 41 weeks’ gestation, there was no clear linear relationship between gestation length and cardiovascular risk factor variables, including ambulatory blood pressure, insulin and glucose metabolism, body composition, basal salivary cortisol and renal function. However, children born at earlier gestations tended to have reduced relative fat mass and estimated glomerular filtration rate. Fetal growth, as determined by birthweight Z-score, was significantly associated with lean mass for body size, presumably reflecting muscle mass, and this relationship extended throughout the gestational age range. The reduced lean mass seen in children with poorer fetal growth may be a risk factor for future excess fat gain and metabolic disease. There were no other significant adverse effects of impaired fetal growth on cardiovascular risk factors in this cohort.
9 Summary and conclusions

9.1 Summary of findings
This is the first report from a human randomised trial of the effects of repeat doses of antenatal glucocorticoids on physiological risk factors for cardiovascular and metabolic disease at early school age, including growth and body composition, glucose and insulin metabolism, blood pressure, renal function, and hypothalamic-pituitary-adrenal axis function. This study was performed in New Zealand children whose mothers participated in the ACTORDS trial, which randomised women at risk of very preterm birth, 7 or more days after an initial course of glucocorticoids, to an intramuscular injection of either 11.4 mg betamethasone (7.8 mg betamethasone sodium phosphate and 6 mg betamethasone acetate) or saline placebo. The allocated treatment was repeated each week that a woman remained at ongoing risk of preterm birth at <32 weeks’ gestation.

At 6 to 8 years’ corrected age, 308 children in the New Zealand arm of the ACTORDS trial were recruited to a follow-up study of neurological and general health outcomes, representing 94% of infants alive at randomisation and 94% of children presumed to be alive and residing in New Zealand at the time of follow-up. Additional investigation of physiological function was performed in 264 of these children (88% of those eligible). Data have been presented on body size and growth in 301 children, minimal-model indicators of glucose and insulin metabolism in 182, ambulatory blood pressure and heart rate in 222, body composition in 186, basal salivary cortisol concentrations in 212, stimulated plasma cortisol concentrations in 182 and estimated glomerular filtration rate in 189. Results for other outcomes of New Zealand children in the neurological and health follow-up study have not been included in this thesis, as a combined analysis of the Australian and New Zealand arms of the study is planned. The physiological investigations reported in this thesis were performed in only the New Zealand children.

9.1.1 Effect of repeat antenatal betamethasone on physiological risk factors for cardiovascular and metabolic disease
At 6 to 8 years’ corrected age there were no significant differences between children exposed to repeat doses of antenatal betamethasone or placebo in somatic growth, glucose and insulin metabolism, fat and lean mass, ambulatory blood pressure, estimated glomerular filtration rate, and basal diurnal salivary cortisol concentrations. For several outcomes, confidence limits were very narrow, making clinically relevant effects very unlikely. These included estimated glomerular filtration rate (95% CI ≤5 mL/min/1.73m²),...
ambulatory blood pressure (95% CI ≤1 to 2 mmHg) and total body lean mass (95% CI ≤5% change in geometric mean). Confidence intervals for other physiological outcomes were a little wider and potentially encompassed modest treatment effects. For basal morning salivary cortisol concentration and total body and regional fat mass, this included an increase in geometric mean values of up to 20%, but confidence intervals were symmetrical and point estimates for the ratio of geometric means were close to one. For insulin sensitivity and disposition index, the lower confidence limit extended to a decrease in geometric means of 25%. However, other studies in groups at risk of type 2 diabetes have generally demonstrated larger reductions in minimal model insulin sensitivity, even in childhood. Therefore, this study has been able to exclude clinically significant effects of repeat antenatal betamethasone treatment on cardiovascular and metabolic function, that would substantially increase the risk of future disease.

In addition, treatment groups had similar childhood growth in weight and height, and similar bone mass at early school age. This provides further evidence that treatment with repeat antenatal betamethasone is unlikely to affect metabolic function in the long term.

There was some evidence that children exposed to repeat antenatal betamethasone had slightly reduced stimulated plasma cortisol responses to mild hypoglycaemia. However, this is of uncertain clinical significance, as metabolic and cardiovascular disease is generally associated with hyperactivity of the hypothalamic-pituitary-adrenal axis. While hypocortisolism is implicated in a number of stress-related somatic disorders, blunted stress responses are usually accompanied by low morning cortisol concentrations, which were not evident in this study. The stimulated plasma cortisol results may represent a type 1 error and need to be confirmed by more detailed investigation of hypothalamic-pituitary-adrenal axis stress reactivity.

9.1.2 Effect of preterm birth and fetal growth on risk factors for cardiovascular and metabolic disease

The effect of gestation length and fetal growth, explored as birthweight Z-score, on physiological risk factors at early school age was assessed in the 264 children who participated in the physiological study. In this cohort, gestation length ranged from 24 to 41 weeks, although the majority of children were born from 28 to 34 weeks’ gestation. Shorter gestation length and poorer fetal growth were both associated with smaller body size at 6 to 8 years’ corrected age. Height and weight for age decreased by approximately 0.1 standard deviation per week decrease in gestation length and by 0.3 to 0.5 standard
deviation per standard deviation decrease in birthweight for gestational age. In addition, preterm children who had poorer fetal growth were lighter for their height at early school age.

The main finding of this cohort analysis with regard to physiological risk factors for cardiovascular and metabolic disease was that poorer fetal growth was associated with persisting deficits in lean mass for height, most likely due to reduced skeletal muscle tissue. This may explain why preterm children with lower birthweight Z-scores were lighter for their height. A positive association between birth size and later lean mass has been widely documented in term or near term populations, but previous studies in preterm populations have yielded conflicting results. This study suggests that pre-natal growth is important for long-term lean tissue and muscle mass regardless of gestational length. Lean mass for height is thought to be an important marker of long-term metabolic capacity, and relatively low muscle mass may predispose to insulin resistance and increased relative fat gain. However, poorer fetal growth was not associated with altered insulin and glucose metabolism or relative fat mass at early school age. There were no other significant associations in this cohort between birthweight Z-score or gestation length and other physiological outcomes.

### 9.2 Discussion

A central concern regarding repeat doses of antenatal glucocorticoids is whether they increase the risk of adult cardiovascular and metabolic disease. There is now extensive epidemiological and animal experimental evidence that adverse environmental conditions during early development contribute to the pathogenesis of adult chronic diseases, especially ischaemic heart disease, hypertension, stroke, type 2 diabetes, and metabolic syndrome (Barker 2004; McMillen 2005; Wells 2009). Because organ structure and capacity, and the neuroendocrine systems that determine homeostasis, are largely established before birth (Hales 1992), disruption of normal fetal growth and development can have far-reaching effects on physiological function throughout the lifespan. Fetuses that are undernourished and stressed undergo various metabolic and cardiovascular adaptations to conserve energy and redistribute fuels to critical tissues. If prolonged, these changes may permanently alter metabolic and cardiovascular development, leading to a so-called thrifty phenotype (Hales 1992). This is characterised by reduced muscle, pancreatic beta cell and nephron mass, decreased insulin sensitivity, a tendency to central adiposity, heightened stress responses, higher blood pressure and arterial stiffness (Wells 2011); all factors that predispose to cardiovascular and metabolic disease.
The fetus responds to two main maternal and environmental signals, nutrient supply and glucocorticoids, both of which have been implicated in developmental programming of disease. Placental nutrient transfer is the major determinant of fetal growth and influences the production of several fetal hormones, such as insulin and IGF. Glucocorticoids also have an important role in regulating fetal tissue growth and maturation, promote the expression of a wide range of developmentally regulated genes and influence fetal HPA axis activity. Animal experiments have shown that fetal undernutrition or fetal over-exposure to glucocorticoids can restrict fetal growth and cause similar long-term adverse effects on cardiovascular and metabolic function, especially elevated blood pressure and insulin resistance (McMillen 2005). Clinically, both mechanisms may contribute to developmental programming effects. For example, many causes of fetal undernutrition, such as maternal illness or dietary restriction, would be expected to cause maternal stress and HPA axis activation. Furthermore, placental hypoxia, inflammation and protein insufficiency reduce the activity of placental $\beta$-HSD2, thereby increasing the passage of maternal corticosteroids to the fetus.

An important implication of the developmental origins of disease hypothesis is that individuals at risk of cardiovascular and metabolic disease due to fetal programming are likely to show altered physiological function from an early age. Indeed, children with low birthweight, a proxy for impaired fetal growth, have been shown to have reduced lean mass (Chomtho 2008; Hediger 1998; Murphy 2006; Rogers 2006; Singhal, Wells, 2003; Wells, Hallal, 2005), higher plasma triglyceride concentrations (Murphy 2006), reduced renal volume and glomerular function (Chan 2010; Konje 1997; Schmidt 2005; Silver 2003; Simonetti 2008; Spencer 2001), higher blood pressure (Feldt 2011; Huxley 2000; Jones 2008), endothelial dysfunction and increased arterial thickness (Martin 2000; Skilton 2011), increased cortisol responses to stress (Jones 2006), and decreased insulin sensitivity (Hofman 1997; Mericq 2005; Veening 2002). These associations strengthen over time, and are even more apparent in early adulthood. Therefore, although cardiovascular and metabolic disease does not usually present until later adulthood, pathophysiological changes are generally evident quite early on in individuals at risk.

Given the purported role of glucocorticoids in mediating the effects of an adverse uterine environment on the fetus, and the extensive animal literature showing that exposure to synthetic antenatal glucocorticoids can affect cardiovascular and metabolic function, it is perhaps somewhat surprising that this study found no evidence of altered physiological function after exposure to repeat doses of antenatal betamethasone. About half of the
infants in the ACTORDS trial were exposed to two or more repeat treatments and so an equivalent proportion in the active arm would have had at least 3 weeks of increased glucocorticoid exposure before birth, albeit intermittently. However, it seems unlikely that the results of this study represent a type 2 error, as there was adequate power to detect clinically important treatment effects, at least in terms of changes that would substantially increase the risk of future cardiovascular and metabolic disease. In addition, this study included a number of highly accurate investigations that measured both basal and dynamic function.

Although increased cardiovascular disease risk in subjects exposed to repeat antenatal glucocorticoids cannot be completely discounted, it seems unlikely given that the treatment groups were virtually identical for several outcomes, especially ambulatory blood pressure and body composition. Longitudinal studies have also shown that by mid-childhood there is modest tracking of major physiological parameters into adulthood, such as blood pressure (Chen 2008) and insulin sensitivity (Nguyen 2010). Furthermore, the results of this study are consistent with those of the Auckland Steroid Trial, which showed that a single course of antenatal betamethasone did not substantially alter cardiovascular risk in early adulthood (Dalziel, Walker, 2005).

These two human studies, ACTORDS and the Auckland Steroid Trial, suggest that glucocorticoid-mediated developmental programming may not be as widespread in humans as would be expected from the animal experimental data (Bloomfield 2006). This may be because the dose of betamethasone clinically used in humans is quite low. Importantly, fetal glucocorticoid bioactivity is increased only several fold above baseline and is equivalent to the stress responses that normally occur in sick preterm infants (Ballard 1975; Ballard 1980). In contrast, the doses used in many animal studies are likely to result in greater fetal glucocorticoid bioactivity. Differences among species in gestation length and fetal development also make it difficult to extrapolate data from animals to humans (Aghajafari, Murphy, Matthews, 2002). Glucocorticoids may still have a role in mediating cardiovascular and metabolic programming effects in humans, but higher doses or very prolonged courses may be required (de Vries 2007).

It is also interesting to note that there was no effect on physiological function at early school age, even though betamethasone-exposed infants in the ACTORDS trial were slightly smaller at birth, as demonstrated by lower Z-scores for weight (Crowther, Haslam, 2006). There has been considerable concern about the effect of repeat doses of antenatal
glucocorticoids on fetal growth because of the widespread association in epidemiological studies between smaller size at birth and risk of adult disease. However, this study illustrates that size at birth is an imperfect proxy for developmental programming, and that a mild and transient slowing of growth does not necessarily affect later cardiovascular and metabolic function. At clinical doses, the effect of antenatal glucocorticoids on fetal growth is similar to what would normally occur in many species with the late gestational rise in endogenous cortisol. Furthermore, it is likely that glucocorticoid-induced maturational changes in the gut and liver facilitate better enteral feeding and growth after birth (Battin 2011). In contrast, infants that have impaired in utero growth due to poor fetal nutrition often have reduced capacity for postnatal growth (Hay 2008).

A potential limitation of this study is that several cardiovascular risk factors were not measured, including plasma lipid concentrations and arterial structure and function. However, because of the close physiological relationship between lipid metabolism, insulin sensitivity and fat mass, it is likely that effects on plasma lipid concentrations would be associated with changes in insulin sensitivity and fat mass. Similarly, abnormalities in arterial structure and function are likely to be associated with altered ambulatory blood pressure, especially during sleep.

There are two important caveats to this study that must be borne in mind. First, these results apply to the use of betamethasone and cannot necessarily be extended to dexamethasone. Although these two drugs have broadly similar effects, subtle differences in pharmacokinetics and potency may alter fetal glucocorticoid exposure, which could influence long-term outcomes. For example, in guinea pigs, betamethasone and dexamethasone had different long-term effects on the expression of brain corticosteroid receptors and reproductive function (Dunn 2010).

Second, these results may not apply to treatment protocols that use larger doses of betamethasone. Most of the repeat dose trials administered 24 mg betamethasone per treatment, whereas women in the ACTORDS trial received only 11.4 mg betamethasone per repeat treatment. Furthermore, in the ACTORDS trial the need for ongoing treatment was evaluated week by week. Thus, only 20% of women received four or more repeat treatments, whereas in some trials this proportion was considerably higher (Guinn 2001; Wapner 2006).

While most research on the developmental origins of disease has focused on fetal growth restriction, there is increasing recognition that preterm birth may be an important stimulus.
for metabolic programming (Hofman, Regan and Cutfield 2006). Preterm infants, especially those born very preterm, must complete organ growth and development in a vastly different postnatal environment, which is often compromised by poor nutrition and acute illness. There have been a number of reports suggesting that preterm birth may result in a phenotype similar to that of growth restricted fetuses, including higher blood pressure (Norman 2010), reduced insulin sensitivity (Hofman 2004; Hovi 2007), decreased renal function (Rodriguez-Soriano 2005) and elevated salivary cortisol concentrations (Buske-Kirschbaum 2007). However, there was no evidence in this cohort that preterm birth, assessed by gestation length as a continuous variable, affected physiological function at early school age.

There are several possible explanations for this apparent discrepancy. First, in the absence of a very large sample, the effects of preterm birth on later physiological function may only be apparent when categorical comparisons are made between preterm and healthy term subjects. Indeed, in this cohort, there was a trend towards a positive association between gestation length and estimated glomerular filtration rate in the whole cohort but not when term children were excluded from the analysis. Second, all children in this cohort were exposed to at least one course of antenatal glucocorticoids, which could confound results. However, data from the Auckland Steroid Trial have shown that a single course of antenatal betamethasone does not adversely affect cardiovascular risk factors into early adulthood. Third, variation in local neonatal intensive care practices may contribute to differences in outcomes.

Within this largely preterm cohort there was no evidence that fetal growth affected physiological outcomes at early school age, with the exception of lean mass. This seems inconsistent with the wider data from term or near term populations. However, it is possible that the influence of fetal growth on developmental programming is less straightforward in the context of preterm birth. For example, several studies in very preterm subjects have not shown any association between birth size and later blood pressure, whereas an inverse relationship has been widely observed in term populations. It is important to note that the birthweight standards used for preterm infants are different from those for term infants, and the extent of fetal growth restriction may be underestimated compared with healthy growing fetuses who remain in utero (Cooke 2007).
9.2.1 Implications for clinical practice

Despite significant advances in neonatal practice in recent decades, preterm infants, especially those born very preterm, continue to have high morbidity. A single course of antenatal glucocorticoids substantially reduces neonatal complications associated with preterm birth, but even with optimal exposure, the maturational response is incomplete. For example, the incidence of respiratory distress syndrome is reduced, at best, by only 50% (Roberts 2006). Furthermore, infants born after remote glucocorticoid exposure are at increased risk due to the diminishing effect of treatment over time. Control infants in the ACTORDS trial, two-thirds of whom were born at <34 weeks’ gestation, experienced substantial morbidity. Moderate or severe neonatal lung disease developed in 38% and 26% had one or more serious non-respiratory complications (Crowther, Haslam, 2006).

The ACTORDS trial showed that administration of repeat doses of betamethasone to women at ongoing risk of very preterm birth, 7 or more days after an initial course of glucocorticoids significantly reduced neonatal morbidity. This may be due to re-induction of tissue maturation after reversal of the effects of glucocorticoid treatment, or a stepwise increase in maturation in fetuses with an incomplete response to the first course of treatment, or both. In terms of absolute benefit, the number needed to treat was 14 (95% CI 8 to 50) for respiratory distress syndrome, 14 (95% CI 9 to 29) for severe neonatal lung disease, and 18 (95% CI 11 to 129) for composite serious morbidity (Crowther, Haslam, 2006). These absolute benefits for respiratory disease are similar to those associated with a single course of antenatal glucocorticoids (Roberts 2006). The results of the ACTORDS trial are supported by the findings from our systematic review involving 10 trials, which found that repeat antenatal betamethasone treatment significantly reduced the incidence of respiratory distress syndrome and combined serious infant outcome, with numbers need to treat of 17 (95% CI 11 to 32) and 33 (95% CI 20 to 83), respectively (McKinlay 2012). Thus, there is evidence that repeat doses of antenatal betamethasone have important short-term neonatal benefits. Given that there are currently few antenatal interventions with proven benefit for improving outcomes related to preterm birth, the finding of reduced serious morbidity is particularly important.

These short-term benefits must be weighed up against the possibility of harmful effects, especially as repeat antenatal glucocorticoid treatment reduces neonatal morbidity but not mortality. Follow-up studies at two to three years of age from four of the randomised trials, including ACTORDS, have shown that repeat doses of antenatal glucocorticoids do not cause any major long-term harm. This includes effects on neurosensory development,
cognitive function, behaviour, growth, respiratory illness, general health and blood pressure (McKinlay 2012).

With the advent of the developmental origins of disease paradigm, clinicians also now have to consider whether perinatal interventions have any long-term effects on the risk of adult chronic disease. It is difficult to weigh up the relative risks and benefits of a treatment across the lifespan, and clinicians currently have little experience in making such judgements. Because the effects of developmental programming may not be fully apparent until later adulthood, it is necessary to rely on surrogate outcomes, namely, physiological markers of risk. However, a detailed assessment of important physiological parameters is likely to identify major programming effects. This study has shown that the risk of cardiovascular and metabolic programming after exposure to repeat doses of antenatal betamethasone is low.

Therefore, current evidence indicates that repeat treatment of women at risk of very preterm birth 7 or more days after an initial course of glucocorticoids is associated with net benefit; neonatal morbidity is reduced without any evidence of long-term harm. Clinicians should consider using repeat doses of antenatal betamethasone because of the potential to reduce complications associated with preterm birth. Until further information is available about optimal treatment dose and frequency, it would seem prudent to follow the treatment protocol used in the ACTORDS trial. This regimen uses a relatively low dose, has been shown to achieve significant absolute benefits and now has the most extensive long-term safety data of all the repeat dose trials. However, because ovine data has suggested that induction of surfactant components may diminish after more than 3 weeks of betamethasone exposure (Ballard 1997), it may be advisable to limit treatment to two or three repeat doses. If delivery has not occurred during this time then the likelihood of very preterm birth should be questioned.

9.2.2 Recommendations for future research
The most important determinant of the absolute benefit associated with repeat antenatal glucocorticoid therapy is the proportion of treated women who give birth at <32 weeks’ gestation, as this is when the risk of neonatal complications is greatest. One study compared the characteristics of women who gave birth at <34 weeks’ and within 7 days of a single course of antenatal glucocorticoids with those who gave birth at <34 weeks’ and more than 7 days after glucocorticoids (McLaughlin 2002). However, there is no direct information about the characteristics of women who are eligible for repeat dose treatment
and who give birth before or after 32 weeks’ gestation. This information could be used to generate guidelines to help clinicians target those infants most likely to benefit and to minimise the unnecessary exposure of fetuses delivering near or at term. These data will have been collected as part of the repeat dose trials but are not currently available. An individual patient data meta-analysis of the main repeat dose trials is in progress to investigate how to maximise the benefits of repeat dose therapy by assessing factors relating to the women and her pregnancy (http://adelaide.edu.au/arch/research/res_synthesis/#IPD). It will be important that units perform regular audits of who is treated with repeat antenatal glucocorticoids to ensure that high-risk groups are being appropriately targeted (Polyakov 2007).

Our systematic review of repeat dose trials showed that there was heterogeneity for some outcomes. This was not readily explained by differences in treatment protocols, although the analysis was limited to a comparison of subgroups based on intended dose and frequency of glucocorticoid administration (McKinlay 2012). It seems more likely that this heterogeneity of effect is due to differences in the study populations in the trials. It is possible that glucocorticoid effect is modified by various pregnancy and fetal factors, and such interactions will hopefully be elucidated by the individual patient data meta-analysis.

It is notable that significant benefits were achieved in the ACTORDS trial and yet each repeat treatment consisted of only a single betamethasone injection rather than a full course. Similarly, substantial benefits were observed in the trial by Garite et al (2009) although women received only a single repeat course of betamethasone rather than serial repeat treatments. This suggests that it may be possible to minimise glucocorticoid exposure by limiting the number or frequency of doses but still maintain clinical benefits. Again, the individual patient data meta-analysis should help to clarify this issue. However, caution is required, as even small changes in dosing protocols can have unexpected effects (Jobe 2006). Ultimately, all treatment regimens should be validated in randomised trials.

Animal studies have raised concern that antenatal glucocorticoids may impair brain growth and development, but in humans there have been no adverse effects on adult cognitive function after exposure to a single course of antenatal betamethsone (Dalziel, Lim, 2005; Dessens 2000), or on neurodevelopment in early childhood after exposure to repeat doses (McKinlay 2012). Nevertheless, neurological follow-up of children from the repeat antenatal glucocorticoid trials at school-age is important to exclude effects on higher cognitive function. The neuro-psychological data collected as part of the ACTORDS 6 to 8
year follow-up will help to define more clearly the overall benefit-to-risk ratio of repeat antenatal betamethasone treatment. However, it seems unlikely that this will change substantially. Neurological follow-up of children from the MACS trial at five years of age is in progress (http://www.utoronto.ca/miru/macs-5/).

There has also been concern that repeated acceleration of pulmonary maturation could reduce total alveolar number and peripheral lung growth. Therefore, effects on later lung function should be monitored. Spirometry was performed as part of the ACTORDS 6 to 8 year follow-up and results are awaited. However, detection of abnormal alveolar function may require specialised testing, such as measurement of diffusion capacity.

This study has shown that the risk of major cardiovascular and metabolic programming effects after exposure to repeat antenatal betamethasone is low, at least with the treatment regimen used in the ACTORDS trial. Thus, there would seem to be little value in repeating the physiological assessments again in childhood. However, given the potential importance of glucocorticoids in the developmental origins of disease and the limited availability of human experimental data, further investigation of cardiovascular and metabolic function in adulthood is warranted.

9.3 Conclusion
Clinicians should consider using repeat doses of betamethasone in women at risk of very preterm birth, 7 or more days after an initial course of glucocorticoids, in view of the associated neonatal benefits, namely, a reduced incidence of respiratory disease and combined serious morbidity. There is no evidence of major adverse health effects in early childhood, and our study has provided further reassurance that exposure to repeat doses of antenatal betamethasone does not alter physiological risk factors for future cardiovascular and metabolic disease.
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