DR JOSÉ G B DERRAIK (Orcid ID : 0000-0003-1226-1956)

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Nulliparity is associated with subtle adverse metabolic outcomes in overweight/obese mothers and their offspring

Sumudu N Seneviratne[1,2], José G B Derraik[1,4], Yannan Jiang[5], Lesley M E McCowan[6], Silmara Gusso[1], Janene B Biggs[1], Graham K Parry[5], Valentina Chiavaroli[1], Wayne S Cutfield[1,3], Paul L Hofman[1]*

[1] Liggins Institute, University of Auckland, Auckland, New Zealand

[2] Department of Paediatrics, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka

[3] A Better Start – National Science Challenge, University of Auckland, Auckland, New Zealand

[4] Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden.

[5] Department of Statistics, University of Auckland, Auckland, New Zealand

[6] Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand

*Author for correspondence: Liggins Institute, University of Auckland, Private Bag 92019, Auckland, New Zealand; email: p.hofman@auckland.ac.nz; ph: +64.9.923.6453; fax: +64.9.373.8763

Running head: Nulliparity and metabolism in mothers & offspring

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ABSTRACT

Background: We aimed to evaluate metabolic outcomes in overweight/obese nulliparous and multiparous women and their offspring.

Study design: Seventy-two overweight and obese women who participated in a randomized controlled trial of exercise in pregnancy were included in the study, comparing 18 nulliparous and 54 multiparous women and their singleton offspring. Women were assessed at 19 and 36 weeks of gestation. Fetal growth was measured using standard obstetric ultrasound techniques. Cord blood was collected at birth. Maternal and offspring body composition was assessed using DXA ~2 weeks after delivery.

Results: Nulliparous women had higher HbA1c in the third trimester of pregnancy than multiparous women (5.48 vs 5.29%; p=0.002), and were more insulin resistant based on the surrogate marker sex hormone binding globulin (354 vs 408 nmol/l; p=0.047). Nulliparous women also had higher levels of the inflammatory marker TNF-α (4.74 vs 3.62 pg/ml; p=0.025). At birth, the offspring of nulliparous women were on average 340 g (p=0.013) and 0.69 SDS (p=0.026) lighter than those born of multiparous women. Cord blood data showed lower IGF-II (p=0.026) and higher IGFBP-1 (p=0.002) levels in the offspring of nulliparous women. In addition, a less favourable metabolic profile was observed in the offspring of nulliparous women, as indicated by higher triglyceride (p<0.001) and interleukin-6 (p=0.039) concentrations.

Conclusions: Infants born of nulliparous overweight and obese women appear to be exposed to a less favourable metabolic environment *in utero*, with evidence of subtle adverse metabolic outcomes at birth compared to infants of overweight/obese multiparous women.

INTRODUCTION

There is increasing evidence that nulliparity is associated with adverse outcomes for mothers and the offspring[1]. Nulliparous mothers have an increased risk of obstetric complications (including pregnancy-induced hypertension, pre-labour rupture of membranes, and postpartum haemorrhage)[2], with a systematic review also showing an increased risk of giving birth to small-for-gestational-age infants[3].

Higher rates of accelerated infant growth (weight gain) and increased risk of childhood overweight have been observed in the offspring of nulliparous women compared to peers born of multiparous mothers[1]. Maternal nulliparity has also been reported to be associated with adverse cardiometabolic outcomes in the offspring[4,5]. In addition, pre-pubertal children who were first-borns were found to have lower insulin sensitivity and higher daytime blood pressure than later-borns[6]. These adverse cardiometabolic effects seem to persist into adult life. A small New Zealand study on middle-aged overweight men showed that first-borns had lower insulin sensitivity and greater body mass index (BMI) than second-borns[7]. Similarly, a large study on 26,812 sisters in Sweden observed that first-borns had greater BMI and were more likely to be overweight or obese than second-borns[8].

The mechanisms underpinning the phenotypic differences among first-borns are unclear, and may be associated with a degree of under-nutrition *in utero* or with changes in placentation[9,10]. As a result, it is likely that there are changes in maternal and offspring metabolism in subsequent pregnancies in comparison to the first pregnancy. Thus, following a randomized controlled trial of exercise in overweight and obese pregnant women[11], we conducted a secondary analysis to evaluate the effects of parity (one of the trial stratification factors) on study outcomes. More specifically, we assessed whether there were metabolic differences in nulliparous and multiparous women and their respective offspring.

METHODS

Ethics approval

Ethics approval for this study was obtained from the Health and Disability Ethics Committee (Ministry of Health, New Zealand; 12/NTB/24). The original trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN 12612000932864). Written and verbal informed consent was obtained from all participants.

Participants

The IMPROVE (Improving Maternal and Progeny Obesity via Exercise) trial was a parallel two-arm randomized controlled trial conducted in Auckland (New Zealand) in 2013–2014[11]. The study protocol has been previously published[11]. Briefly, participants were women aged 18–40 years with a BMI $\geq$25 kg/m^2 and a singleton pregnancy less than 20 weeks of gestation. Exclusion criteria were ongoing smoking, multiple pregnancy and pre-existing contraindications to antenatal exercise. Eligible women were randomly allocated to intervention or control group in a 1:1 ratio, and stratified

by parity (nulliparous/multiparous) and ethnicity (Maori/Pacific/New Zealand European/Other). Parity was defined as the number of times a woman gave birth to an infant with a gestational age of at least 24 weeks.

Clinical assessments

Mothers were assessed at baseline (19 weeks of gestation) and post-intervention (36 weeks of gestation). Maternal weight and height were measured at baseline, when socio-demographic data were also obtained. At baseline and mid-intervention (32 weeks of gestation), maternal physical activity was assessed by the Pregnancy Physical Activity Questionnaire (PPAQ)[12], while dietary intake was evaluated using 3-day food intake records, and analysed using FoodWorks software (Xyris Software Pvt Ltd, Australia). Maternal weight gain was defined as weight gained between recruitment and 36 weeks.

Maternal venous blood was collected at baseline (~19 weeks gestation) and post-intervention (~36 weeks gestation) in a non-fasting resting state during scheduled study assessments. Cord blood was collected at birth by delivery staff. Metabolic markers measured in maternal and cord blood included total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, insulin-like growth factor I (IGF-I), IGF-II, IGF binding protein 1 (IGFBP-1), as well as the inflammatory markers interleukin-6, tumour necrosis factor alpha (TNF-α), and highly-sensitive C-reactive protein (hsCRP). Maternal glycaemic status was assessed by glycated haemoglobin (HbA1c), while sex hormone binding globulin (SHBG) concentrations were used as a surrogate marker of maternal insulin resistance[13]. SHBG has been identified as one of the maternal biomarkers for early prediction of gestational diabetes mellitus[14], and concentrations can be reliably measurable in non-fasting conditions such as during pregnancy[15]. Indeed, lower SHBG concentrations have been found in pregnant women with gestational diabetes mellitus compared to corresponding controls[16].

Fetal growth, birth weight, and other perinatal outcomes were obtained from clinical records. Measurements of fetal growth were performed at 24, 28, 32, and 36 weeks of gestation using standard obstetric ultrasound techniques by a single investigator at a single medical institution in Auckland (Middlemore Hospital, Counties Manukau District Health Board). All scans were performed with the same Philips IU22 ultrasound scanner (Philips Ultrasound, Bothell, USA), using the Philips curvilinear array (C5-1, Philips Ultrasound, Bothell, USA) and a mechanical curved-array 3D abdominal ultrasonic transducer (3D 6-2, Philips Ultrasound). Measurements were taken as described in our published protocol[17]. In brief, growth measurements followed Australasian Society for Ultrasound in Medicine protocols[18]. Estimated fetal weight was calculated by the Hadlock 4

equation[19]. Soft tissue growth was assessed as per Lee et al.[20]. Volume analysis was done offline using Philips QLABTM software version 6. Measurements of fetal fat mass were calculated as described by Larciprete et al.[21].

Post-natal offspring body composition was assessed approximately 2 weeks after delivery by whole-body dual-energy absorptiometry scans (DXA, Lunar Prodigy 2000, General Electric, Maddison, USA). Scans were performed and analysed by a single investigator, using Encore 2007 software v.11.40.004 (GE Corp., Madison, WI, USA).
Birth weight standard deviation scores (SDS) were calculated using based on Niklasson et al.[22]. Augmentation of labour was defined as requiring an oxytocin infusion during labour.

Assays

Maternal HbA1c was measured on venous whole blood at time of venipuncture, using a glycohaemoglobin analyser (Seimens/Bayer DCA 2000+ analyser). Highly-sensitive CRP was measured by particle-enhanced immunoturbidimetric assay and plasma lipids by colorimetric enzymatic analysis using an automated clinical chemistry analyser (Roche Hitachi 902, Roche Diagnostics, Germany). Maternal plasma sex hormone binding globulin (SHBG) and cord plasma insulin were measured by an automated electro-chemiluminescence immune-analyser (Elecsys 2010 immuno-analyser, Roche Diagnostics). Serum adipokines, IGFs, and IGFBPs were measured in duplicate using a laser-based fluorescent analytical test instrumentation (MAGPIX 4.2, Luminex Corporation, Austin, TX, USA) and customised commercially available MILLIPLEX® map magnetic bead-based multianalyte panels (Magnetic Bead Panel #HADK2MAG-61K, #HIGFMAG-52K, and #HIGFBMAG-53K, respectively; Millipore Corporation, Billerica, MA, USA), according to manufacturer instructions.

Statistical analyses

Demographic, lifestyle characteristics, and pregnancy outcomes were compared between nulliparous and multiparous women using two-sample statistical tests appropriate to the distribution of variables, namely one-way analysis of variance, Kruskal-Wallis, and chi-square or Fisher's exact tests. Maternal metabolic outcomes were analysed using generalised linear regression models based on repeated measures, adjusting for maternal age, BMI, ethnicity, treatment allocation, and physical activity levels at baseline. Fetal anthropometry in the two groups was also compared using repeated measures (adjusting for ethnicity, sex, gestational age, and treatment allocation), while models examining changes in fetal measurement from 24 to 36 weeks of gestation also adjusted for the respective value at 24 weeks in addition to those parameters. Offspring outcomes were analysed using generalised

linear regression models adjusting for ethnicity, gestational age, and treatment allocation, as well as maternal BMI and physical activity levels at baseline. Univariable analyses were performed in Minitab v.16 (Pennsylvania State University, State College, PA, USA), while multivariable analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-sided, and missing data were not imputed. No adjustment was made for multiple testing and statistical significance was maintained at $p<0.05$.

RESULTS

Seventy-five eligible participants who completed baseline assessments were randomised into the IMPROVE trial, one participant withdrew from the trial while two babies died *in utero* (Figure 1). Thus, we studied 18 nulliparous and 54 multiparous overweight and obese women, with a similar proportion of participants in each group randomly allocated to the treatment arms (Figure 1), noting that there were no treatment effects on birthweight or clinical outcomes in the original trial[11]. Baseline characteristics in the two groups were similar (Table 1). There were also no statistically significant differences in dietary intake throughout the study (data not shown) or reported levels of physical activity at baseline (Table 1).

Maternal blood

Nulliparous women had higher HbA1c in the third trimester of pregnancy (5.48 vs 5.29%; p=0.002) in comparison to multiparous women (Table 2). In addition, there was evidence that nulliparous women were more insulin resistant, as indicated by lower SHBG concentrations (354 vs 408 nmol/l; p=0.047) (Table 2). Among inflammatory markers, nulliparous women had levels of TNF-α that were 31% higher than those of multiparous women (p=0.025; Table 2).

Pregnancy outcomes

Pregnancy outcomes were similar in nulliparous and multiparous mothers (Table 3). The exception was a rate of augmentation of labour that was nearly 4-fold higher among nulliparous women without pre-labour caesarean section (Table 3). There was no statistically significant difference in maternal weight gain in nulliparous and multiparous women (Table 3).

Fetal growth

Repeated measures analyses on ultrasound data showed no differences in fetal parameters in the last trimester of pregnancy between the two groups (Table S1). There were also no observed differences in fetal growth from 24 to 36 weeks of pregnancy between the offspring of nulliparous and multiparous women, noting in particular that the estimated change in fetal weight was virtually identical in the two groups (+2.32 vs +2.31 kg, respectively; Table S1).

Neonatal parameters

At birth, the offspring of nulliparous women had birth weight that was on average 340 g lighter (p=0.013) and 0.69 SDS lower (p=0.026) (Table 4). However, there was no significant difference in ponderal index between groups (p=0.32) (Table 4).

Cord blood and body composition

Analyses of cord blood indicated that the offspring of nulliparous women had higher concentrations of triglycerides (+60%; p<0.001) and interleukin-6 (p=0.039) concentrations (Table 4). Further, the offspring of nulliparous women had lower IGF-II levels (p=0.026) but IGFBP-1 concentrations that were 3-fold higher (p=0.002; Table 4).

At 2 weeks of age, DXA scans showed no statistically significant differences in body composition in the offspring of nulliparous and multiparous women (Table 4).

DISCUSSION

This study provides evidence that nulliparity is associated with potentially adverse metabolic *in utero* parameters in overweight and obese women compared with multiparous counterparts. Although subtle, these perturbations support the recently suggested link between maternal nulliparity and persistent cardiometabolic outcomes in the offspring[1].

Dysregulation of metabolic and inflammatory pathways has been previously reported among overweight and obese pregnant women[23,24]. Although insulin sensitivity decreases by approximately

50% from pregravid status to late pregnancy in both normal-weight and overweight/obese individuals, the latter have lower insulin sensitivity compared with lean counterparts[16]. Maternal obesity has been also associated with up-regulation of inflammatory markers[17]. Higher concentrations of interleukin-6 and CRP have been detected in obese pregnant women compared to lean individuals, reflecting a low-grade state of chronic systemic inflammation induced by adiposity[17]. However, these studies have not explored the potential effects of parity on pregnancy outcomes.

To our knowledge, this is the first study to investigate the impact of parity on metabolic and inflammatory markers in overweight and obese pregnant women. Our observations that nulliparous overweight/obese pregnant women had higher HbA1c, were more insulin resistant, and had higher TNF-α compared to multiparous women suggest some additive or synergistic interaction between maternal adiposity and nulliparity, increasing the likelihood of pregnancy complications (such as pre-eclampsia and placental insufficiency) in overweight or obese nulliparous mothers. Interestingly, the other markers of inflammation (interleukin-6 and hsCRP) were not found to be higher in the nulliparous group compared to multiparous women. While obesity is associated with increases in all three markers, there was a specific increase in TNF-α in nulliparous women. Although the reasons for this observation are unclear, TNF-α is specifically elevated in adipocytes while the other markers of inflammation measured are mostly secreted from non-adipocyte cells in fat and blood, suggesting there might be a particular alteration in adipocyte function in obese nulliparous women during pregnancy[25].

In utero exposure to metabolic dysregulation resulting from maternal obesity is hypothesised to lead to adverse fetal programming effects, including a higher risk of obesity and metabolic complications in the offspring[26]. Infants born of obese women show evidence of metabolic perturbations as early as the newborn period[27]. Catalano *et al.* showed that the offspring of obese mothers had increased body fat, higher levels of inflammation, and greater insulin resistance at birth than offspring born of lean women[27]. Later in life, first-born daughters born of obese women have risk of developing adult obesity that is nearly 5 times higher than those born to lean women[28].

Nulliparity has also been recognized as playing an important role in shaping offspring cardiometabolic profile[4,5]. We observed that infants born of nulliparous mothers showed a less favourable metabolic profile than offspring of multiparous women; namely higher interleukin-6 and triglyceride levels, but a trend towards lower HDL-C concentrations. Gaillard *et al.* also reported an adverse lipid profile (higher total cholesterol and LDL-C levels) in 6-year-old children born of nulliparous mothers[1]. Further, Ayyavoo *et al.* found that, first-born children displayed adverse changes in metabolism (including reduced insulin sensitivity) compared to subsequent children, even though they were taller and slimmer[6]. In adulthood, there is evidence of increased BMI and obesity

risk amongst first-born men[7] and women[8], with males also displaying lower insulin sensitivity than later-borns[7]. These numerous findings indicate that maternal nulliparity may have long-lasting effects on offspring health.

Of note, first-born infants were considerably lighter than later-borns. Maternal parity is an independent determinant of infant birth weight and is positively correlated with neonatal adiposity[23,29]. We did not observe differences in adiposity between groups, which might have been due to limitations with statistical power. A longitudinal cohort study of women with consecutive pregnancies showed a continuous increase in birth weight up to the third offspring and then appeared to stabilize, with the greatest increase between the first- and the second-born[30]. Surprisingly, we observed no detectable differences in fetal growth (as per ultrasound measurements) during pregnancy between nulliparous and multiparous women. Thus, the reasons underlying the differences in birth weight are unclear. It is possible that the deviation in growth rates between first- and later-borns occurs at the very end of pregnancy (i.e. beyond 36 weeks of gestation). It is also possible that weight estimation with ultrasounds may not be sensitive enough to detect differences in a small sample size.

Several biological mechanisms have been proposed as responsible for the phenotypic alterations in first-borns[30]. Uterine cavity appears to be larger during subsequent pregnancies compared to the first pregnancy, with favourable effects on placentation and fetal growth[31]. Furthermore, first pregnancies are characterized by a reduced utero-placental blood flow, leading to reduced oxygen and nutrient supply to the fetus[30,32]. An enhanced inflammatory state has been reported in both nulliparous and overweight/obese women[17], which may also exert a negative impact on fetal growth and development. Thus, a combined effect of maternal nulliparity and adiposity is likely to occur, with adverse cardio-metabolic consequences for the offspring.

There are limitations to our study as it consisted of secondary analyses of data from a prospective randomised controlled trial. As a result, our sample size was relatively small and there was an unbalanced number of nulliparous and multiparous women that could have confounded our results. In addition, we did not have a normal-weight group of mothers for comparison or access to maternal pre-pregnancy data. Further, data on previous miscarriages at less than 24 weeks of gestation were also not recorded, which might have been also a confounder unaccounted for. Nonetheless, our study examines a high-risk obstetric population with very little data on the associations between parity and maternal and neonatal metabolic outcomes.

In conclusion, infants born of nulliparous overweight/obese women are likely to be exposed to metabolic perturbations *in utero*, which translate into subtle adverse metabolic outcomes at birth when compared to the offspring of multiparous overweight/obese mothers. Further elucidation of the

mechanisms underlying metabolic programming in first-borns is required. Given the impact of obesity, pre-conceptional weight reduction in overweight and obese women should be strongly encouraged prior to the first pregnancy.

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Table 1. Baseline characteristics of overweight and obese mothers who were nulliparous or multiparous. Data are mean ± standard deviation for continuous variables or n (%) for categorical variables.

	Nulliparous	Multiparous	P-value
n	18	54	
Intervention group (exercise)	8 (44%)	28 (52%)	0.59
Age (years)	29.8 ± 4.9	32.1 ± 4.7	0.08
Ethnicity (New Zealand European)	12 (67%)	29 (54%)	0.33
Weight (kg)	91.6 ± 15.6	90.8 ± 16.7	0.85
Height (cm)	165.1 ± 6.6	165.8 ± 6.0	0.69
BMI (kg/m^2)	33.6 ± 5.7	32.9 ± 5.2	0.63
Obese (BMI ≥30 kg/m^2)	14 (78%)	36 (67%)	0.56
Physical activity (MET-h/week)	226 ± 86	311 ± 182	0.06

Table 2. Metabolic markers during pregnancy in overweight and obese mothers who were nulliparous or multiparous. Data are means (95% confidence intervals) adjusted for ethnicity, BMI, age, treatment allocation, and physical activity levels at baseline; data for all parameters are from repeated measures analyses based on measurements at 19 and 36 weeks of gestation, except for insulin that was only measured at 19 weeks. P-values that are statistically significant at $p<0.05$ are shown in bold.

	Nulliparous	Multiparous	P-value
n	18	54	
Glucose (mmol/l)	5.11 (4.72–5.53)	5.16 (4.94–5.39)	0.83
Insulin (uU/ml)	18.1 (11.1–29.4)	20.4 (15.5–26.8s)	0.67
SHBG (nmol/l)	354 (313–399)	408 (381–435)	**0.047**
HbA1c (%)	5.48 (5.38–5.59)	5.29 (5.23–5.35)	**0.002**
Interleukin-6 (pg/ml)	2.50 (1.71–3.67)	2.30 (1.86–2.84)	0.70
TNF-α (pg/ml)	4.74 (3.91–5.57)	3.62 (3.16–4.08)	**0.025**
hsCRP (mg/l)	3.46 (2.62–4.56)	4.28 (3.66–5.00)	0.19

Table 3. Pregnancy outcomes in overweight and obese mothers who were nulliparous or multiparous. Where appropriate data are mean ± standard deviation; other data are n (%). P-values that are statistically significant at $p<0.05$ are shown in bold.

	Nulliparous	Multiparous	P-value
n	18	54	
Maternal weight gain (kg) †	13.9 ± 5.7	12.0 ± 5.6	0.25
Gestational age (days)	273 ± 14	277 ± 9	0.22
Preterm birth	1 (6%)	1 (2%)	0.44
Length of hospital stay (hours)	86 ± 49	77 ± 65	0.62
Gestational diabetes mellitus	2 (11%)	4 (7%)	0.64
Gestational hypertension	1 (6%)	0	0.25
Pre-eclampsia	1 (6%)	1 (2%)	0.44
Induction of labour *	6 (40%)	7 (18%)	0.15
Augmentation of labour *	10 (67%)	7 (18%)	**0.001**
Caesarean section	7 (39%)	23 (43%)	0.78
Severe postpartum haemorrhage	0	7 (13%)	0.18
Perineal tears *	3 (17%)	12 (22%)	0.75
Apgar score at 1 minute	8.2 ± 1.1	8.4 ± 1.6	0.56
Apgar score at 5 minutes	9.4 ± 0.6	9.4 ± 1.3	0.82
Admission to HDU/ICU	1 (6%)	5 (9%)	0.99
Hypoglycaemia	2 (11%)	5 (9%)	0.99
Respiratory distress	0	7 (13%)	0.18
Sex ratio (boys)	9 (50%)	29 (54%)	0.79

†Weight gain between 19 and 36 weeks of gestation.

*Only including participants without pre-labour caesarean section (nulliparous n=15, multiparous n=39).

Table 4. Neonatal and cord blood parameters in the offspring of overweight and obese mothers who were nulliparous or multiparous. Data are presented as model-adjusted means (95% confidence intervals) adjusted for ethnicity, corrected gestational age, and treatment allocation, as well as maternal BMI and physical activity levels at baseline. P-values that are statistically significant at $p<0.05$ are shown in bold.

		Nulliparous	Multiparous	P-value
Birth outcomes	n	18	54	
	Birth length (cm)	50.9 (49.8–51.9)	51.9 (51.3–52.5)	0.10
	Birth weight (kg)	3.31 (3.07–3.54)	3.65 (3.52–3.78)	**0.013**
	Birth weight SDS	-0.06 (-0.59–0.46)	0.63 (0.34–0.91)	**0.026**
	Ponderal index (g/cm^3)	25.5 (24.3–26.7)	26.2 (25.5–26.9)	0.32
	Placental weight (g) [a]	642 (543–742)	681 (630–732)	0.49
	Placenta weight / birth weight [a]	0.19 (0.17–0.21)	0.19 (0.17–0.20)	0.55
	Occipito-frontal circumference (cm)	34.6 (33.9–35.3)	35.3 (34.9–35.7)	0.08
Cord blood parameters	n	13	43	
	IGF-I (ng/ml)	48 (31–66)	60 (51–70)	0.22
	IGF-II (ng/ml)	232 (160–304)	325 (287–364)	**0.026**
	IGFBP-1 (ng/ml)	68 (37–124)	22 (16–31)	**0.002**
	Interleukin-6 (pg/ml)	9.3 (4.8–18.2)	4.2 (3.0–5.8)	**0.039**
	TNF-α (pg/ml)	5.2 (3.7–7.3)	4.9 (4.1–6.0)	0.77
	Total cholesterol (mmol/l)	1.74 (1.49–2.03)	1.70 (1.57–1.85)	0.81
	HDL-C (mmol/l)	0.58 (0.47–0.72)	0.73 (0.65–0.82)	0.063
	LDL-C (mmol/l)	0.58 (0.48–0.74)	0.55 (0.49–0.63)	0.73
	Triglycerides (mmol/l)	0.64 (0.51–0.80)	0.40 (0.35–0.45)	**<0.001**
Body composition at 2 weeks	n	17	49	
	Weight (kg)	3.81 (3.57–4.06)	4.04 (3.89–4.18)	0.13
	Total body fat (%)	7.2 (6.0–8.3)	8.1 (7.4–8.8)	0.18
	Total body fat (g)	274 (216–332)	329 (296–363)	0.11
	Lean mass (kg)	3.49 (3.29–3.70)	3.66 (3.54–3.78)	0.17

[a] The number of placentae weighed was 12 among nulliparous and 43 among multiparous women.

Figure 1. Flow of participants in the IMPROVE trial and the numbers of nulliparous and multiparous mothers and offspring who were studied.