In Vivo Isotopic Studies of the Metabolic Effects of Recombinant Human Growth Hormone and Insulin-like Growth Factors I and II

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Abstract

The continued loss of protein from patients with severe sepsis, trauma or cancer cachexia, which occurs despite the provision of adequate calories and nitrogen, remains a challenge for surgeons and intensivists involved in their care. Prolonged loss of protein may impair cardiopulmonary and immune function and wound healing, and so its effective treatment may represent a considerable clinical advance. In this thesis, studies have been performed in both patients and laboratory animals which investigate the potential of human growth hormone and insulin-like growth factors to slow or reverse the accelerated protein catabolism associated with severe surgical illness.

In the patient studies, the following issues have been addressed:

i) The effects of a short course of recombinant human growth hormone (rhGH) on the rate of net protein loss, whole body protein turnover, glucose kinetics, free fatty acid and glycerol concentrations and total carbohydrate and fat oxidation have been measured in septic and injured patients in both postabsorptive and parenterally fed states.

ii) The effects of a short course of recombinant human growth hormone on the rate of protein synthesis has been determined in tissues removed from patients with cancer.

In the animal studies the following issues have been addressed;

i) The effects of acute infusions of recombinant human insulin-like growth factor I (rhIGF-I) on the rate of net protein loss, whole body protein turnover, tissue protein synthesis, glucose kinetics and free fatty acid and glycerol concentrations have been measured. The actions of rhIGF-I infusion were then compared to those of an insulin infusion of equivalent

hypoglycaemic potential and to an equimolar infusion of recombinant human insulin-like growth factor II (rhIGF-II).

ii) The difficulty in achieving nitrogen accretion in patients with critical surgical illness or cancer cachexia may result from metabolic derangements caused by release of inflammatory mediators such as tumour necrosis factor (TNF). In order to determine whether recombinant human insulin-like growth factor I (rhIGF-I) preserved its protein sparing effects in the face of high plasma TNF concentrations, rhIGF-I was infused into lambs which were simultaneously receiving infusions of recombinant human tumour necrosis factor (rhTNF), and the change in the rate of net protein loss compared to control animals who received rhIGF-I alone.

The major findings of the human studies were as follows:

i) In parenterally fed patients, rhGH treatment almost halved the rate of net protein loss, although a positive nitrogen balance was not achieved. The rate of whole body protein turnover was not altered, suggesting that the reduction in net protein loss was due to an increase in the rate of protein synthesis rather than reduced catabolism. In the post absorptive group, rhGH treatment increased the rate of appearance and oxidation of free fatty acids and reduced the rate of protein oxidation, from which it was concluded that fat was being oxidized in preference to protein.

ii) The rate of protein synthesis in skeletal muscle tissue removed from patients with cancer undergoing resection to whom a short course of rhGH had been administered was significantly greater than that of a matched group of control patients, however the rate of protein synthesis in the tumour *per se* was not increased. It appears that rhGH favourably alters host tissue protein kinetics without accelerating the rate of tumour protein synthesis.

The important results from the animal studies were as follows:

i) Infusion of rhIGF-I reduced the rate of net protein loss by both increasing the rate of protein synthesis and reducing the rate of protein catabolism. The rate of protein synthesis was increased by a greater extent in the heart and in the diaphragm than in other skeletal muscles. High rates of rhIGF-I infusion which saturated the plasma IGF-I binding capacity lowered the blood glucose concentration by enhancing peripheral glucose uptake. Infusion of an equimolar dose of rhIGF-II did not influence protein kinetics but did result in a small increase in the rate of glucose clearance. Insulin infused at a dose which resulted in the same degree of hypoglycaemia as the rhIGF-I infusion caused a similar reduction in the rate of net protein loss but did not accelerate the rate of protein synthesis in any of the tissues examined.

ii) Recombinant human TNF infusion resulted in pyrexia and increased the plasma glucose, cortisol, glucagon and insulin concentrations. The reduction in the rate of net protein loss following rhIGF-I infusion in rhTNF infused lambs was identical to that seen in control animals infused with rhIGF-I alone. We conclude that as rhIGF-I preserves its protein anabolic action in the face of high rhTNF levels, further investigation into a possible clinical role for rhIGF-I in severe surgical illness is warranted.

In general terms, these studies demonstrate that rhGH administration to septic or injured patients significantly reduces their rate of loss of protein, and that rhIGF-I infusion similarly conserves protein in an animal model of sepsis.

These findings present the challenge to determine whether the favourable changes in protein kinetics brought about by the administration of anabolic peptide hormones will realize tangible improvements in the clinical course of severely ill surgical patients.

Preface and Acknowledgements

The studies presented in this thesis represent three years of full time research performed in the Department of Surgery of the University of Auckland which I completed in October, 1990. The motivation to undertake this work was provided by Associate Professor James Shaw. His example encouraged my interest in clinical surgery, and he impressed upon me the potential of research experience to enhance clinical competence. I am grateful to the time he spent with me discussing hypotheses, protocols and results and his advice in the preparation of manuscripts.

I am also grateful to Professor Graham Hill, in whose department the patient studies were performed, for his encouragement and guidance of my surgical career. I am indebted to Professor Peter Gluckman for the provision of animal research facilities and his invaluable advice in the performance of the animal experiments, and I gratefully acknowledge the assistance of Dr Bernhard Breier, from whom I learnt a great deal about scientific experimentation.

I would like to acknowledge the role of the Medical Research Council of New Zealand, which provided financial support for me and also funded the majority of my research expenses, and of Kabi Vitrum Peptide Hormones, Stockholm, Sweden for donating the recombinant human growth hormone and insulin-like growth factor-I used in my studies

Many technical staff provided a great deal of energy and expertise in the derivation of samples. Of these, I would like to express special thanks to Mrs Linda Stubbs and Mrs Lynn Thomas for their analysis of isotope specific activities, to Dr Anthony Haystead for the performance of isotope ratio mass spectrometry measurements, to Mr Brian Gallaher who performed determinations of the molecular weight forms of the insulin-like growth factors, to Miss Christine Gibson who performed the insulin-

like growth factor-I radioimmunoassays and to Mr Terry Wilson who performed the growth hormone and cortisol radioimmunoassays.

I would also like to express my gratitiude to my parents for their support and encouragement.

I began working in the laboratory of Associate Professor James Shaw as he was completing a four year project during which he employed isotopic tracer methodology to define the metabolic profiles of the major types of surgical illness: sepsis, trauma, cancer and pancreatitis. From these many studies it became clear that these broad groups shared similarities in protein kinetics. Critically ill surgical patients, largely irrespective of the aetiology, were found to have a marked increase in the rate of protein catabolism, which was not completely compensated by the associated increase in the rate of protein synthesis. Whereas providing parenteral nutrition to a purely depleted patient enabled that patient to accrue body protein, the severely septic or traumatized patient continued to lose nitrogen, albeit at a reduced rate. From these observations developed the idea to use anabolic hormones to encourage protein synthesis in severely ill surgical patients.

This thesis takes the form of two introductory chapters in which the metabolic problems associated with surgical illness are defined, and the isotopic studies of performed in the Department of Surgery of Auckland Hospital integrated with the existing literature on this topic. The subsequent two chapters describe patient studies in which the effects of administration of recombinant growth hormone to surgical patients are investigated. The protein anabolic effects of growth hormone are largely mediated via its stimulation of production and release of insulin-like growth factor-I. Recombinant human insulin-like growth factor-I and -II have recently become available in pharmacological quantities, and the following three chapters describe studies of the metabolic effects infusions of

these hormones in an animal model. A model which replicates many of the hormonal changes seen in sepsis was developed, and the protein conserving properties of rhIGF-I investigated in this model, in order to determine whether rhIGF-I is likely to preserve its anabolic effects in the clinical setting of sepsis.

Much of the material presented in this thesis has been used for the preparation of six manuscripts which are published or presently undergoing editorial review (24 November, 1990).

Douglas RG, Shaw JHF. The metabolic response to sepsis and trauma. *Br J Surg* 1989;**76**:115-122.

Douglas RG, Shaw JHF. The metabolic response to cancer. *Br J Surg* 1990;**77**:246-254.

Douglas RG, Humberstone DA, Haystead, Shaw JHF. The metabolic effects of biosynthetic human growth hormone. *Br J Surg* 1990;**77**:785-790.

Douglas RG, Gluckman PD, Ball K, Breier B, Shaw JHF. The effects of infusion of IGF-I, IGF-II and insulin on glucose and protein metabolism in fasted lambs. Submitted *J. Clin. Invest.*

Douglas RG, Gluckman PD, Breier BH, McCall JL, Parry B, Shaw JHF. The effects of recombinant IGF-I on protein and glucose metabolism in rTNF infused lambs. Submitted *Am. J. Physiol.*

Douglas RG, Gallagher B, Breier BH, Shaw JHF, Gluckman PD. The distribution of infused rIGF-I across IGF binding proteins and subsequent effects on glucose production. Submitted *Diabetologia*.

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List of Abbreviations

AEC	Acid Ethanol Extraction
APE	Atom Percent Excess
BCAA	Branch Chain Amino Acids
BSA	Bovine Serum Albumin
cpm	Counts Per Minute
dpm	Decays Per Minute
ECF	Extracellular Fluid Volume
f	Rate of Isotope Infusion
FFA	Free Fatty Acid
FPLC	Fast Protein Liquid Chromatography
FSR	Fractional Rate of Synthesis
GH	Growth Hormone
IGFBP	Insulin-like Growth Factor Binding Protein
IGF-I,-II	Insulin-like Growth Factor-I,-II
im	Intramuscular
ISS	Injury Severity Score
IU	International Unit
iv	Intravenous
IVN	Intravenous Nutrition
NPL	Net Rate of Protein Loss
NSILA	Non-suppressible Insulin-like Activity
PBS	Phosphate Buffered Saline
PEG	Polyethylene Glycol
Ra	Rate of Appearance
rh	Recombinant Human
RIA	Radioimmunoassay
RME	Resting Metabolic Expenditure

ROx	Rate of Substrate Oxidation
RQ	Respiratory Quotient
SA	Specific Activity
sc	Subcutaneous
TNF	Tumour Necrosis Factor
VCO_2	Minute Volume of CO_2
VO_2	Minute Volume of O_2
VLDL	Very Low Density Lipoprotein

Chapter 1

The Metabolic Response to Sepsis and Trauma

1.1 Introduction

The accelerated breakdown of skeletal muscle following significant injury was first identified by Cuthbertson more than fifty years ago[77]. He divided the response into a shortlived 'ebb' phase, corresponding to the period of hypovolaemia and sympathetic activity immediately subsequent to injury and a more prolonged 'flow' phase which is characterized by a negative nitrogen balance. A catabolic state similar to the flow phase of trauma also occurs in patients with established sepsis, and many parallels can be drawn between the metabolic profile of the severely injured and septic patient. This chapter is a review of the present knowledge of the metabolic responses to trauma and sepsis. Contention persists over some aspects of the nature of these responses and the factors which mediate them, and data and interpretations of the polemical issues will be presented.

The consequences of a prolonged catabolic response are now seen more frequently as intensive care facilities prolong the survival of multiply injured and severely septic patients. Although modern techniques of enteral and parenteral nutrition are able to reduce the rate of consumption of protein and energy reserves, it is not yet possible to achieve a positive nitrogen balance in severely stressed patients[325]. The last section of this review discusses the impact of intravenous nutrition on the metabolism of septic and trauma patients and summarizes the results of experimental work with agents which promote protein conservation in this setting.

1.2 Mediators of the Metabolic Response to Sepsis and Trauma

1.2.(i) *Introduction* It is well established that the neuroendocrine reponse to sepsis and trauma effects many of the observed changes in metabolism. More recent work has suggested that inflammatory mediators released from the wound itself or from a septic focus may also play a role in these changes[364]. Despite extensive investigation there remains much conjecture about the nature of the link between tissue insult and the resultant metabolic response.

1.2.(ii) *The Neuroendocrine Response* Injury is followed by an outpouring of sympathetic activity and a clearly positive relationship between the Injury Severity Score[13] (ISS) and the plasma concentration of adrenaline, nor-adrenaline and dopamine has been established[82,111]. The plasma catecholamine levels are maximal shortly after injury, but this response is short lived and the plasma levels have usually returned to the normal range within 24 hours[19]. Although it is possible to relate plasma hormonal levels to the ISS score we have recently demonstrated that there is no correlation between the ISS score and the degree of metabolic abnormality seen in patients following blunt trauma[313]. The metabolic response in these patients appears to be an 'all or none' response - the patient with an ISS of 15 is metabolically similar to the patient with a score of 50.

At the same time as the sympathetic nervous system response, the hypothalamic-pituitary axis is activated. However, the relationship between injury severity and plasma cortisol is not direct; cortisol levels peak at an ISS of 12 and then become lower with more severe injury[17]. In addition, the cortisol response is transient and the plasma cortisol level

falls to normal within a few days of injury. Further, the correlation between hormonal changes and metabolic abnormalities is difficult to show; for example we have found no correlation between the plasma cortisol level and the degree of alteration in either glucose or protein kinetics[313].

The plasma concentration of growth hormone is raised for 24 hours post injury[55], as is prolactin. Following thermal injury antidiuretic hormone levels have been reported to be ten times control values[243]. Aldosterone is increased[194] and the renin-angiotensin system is activated[320] and glucagon levels rise markedly about 12 hours after major injury[228].

Thus, the ebb phase of injury is associated with increased sympathetic activity and an outpouring of counter-regulatory hormones. However, during the stage of maximal nitrogen loss during the flow phase (about 7 to 10 days post-injury) the catecholamine, glucagon and cortisol plasma levels are not raised[320]. This is, however, the time when the plasma insulin level rises to a peak[110].

Although the catabolic response during the post injury flow phase and established sepsis is similar, there exist differences in the hormonal milieu between these two conditions; the counter-regulatory hormones remain high in septic patients and the elevation of insulin levels is not a consistent feature[320].

There has been a recent attempt to simulate the hormonal responses associated with injury in normal volunteers to elucidate the role hormones play as mediators of the metabolic changes seen following trauma[23]. Nine volunteers received a continuous 74 hour infusion of the three 'stress' hormones (cortisol, glucagon and adrenaline) at a rate which produced plasma levels similar to those seen following mild to moderate injury. The infusion resulted in a significantly raised basal metabolic rate, hyperglycaemia, hyperinsulinaemia and insulin resistance, and a negative nitrogen balance. Further studies with single hormone infusions indicated

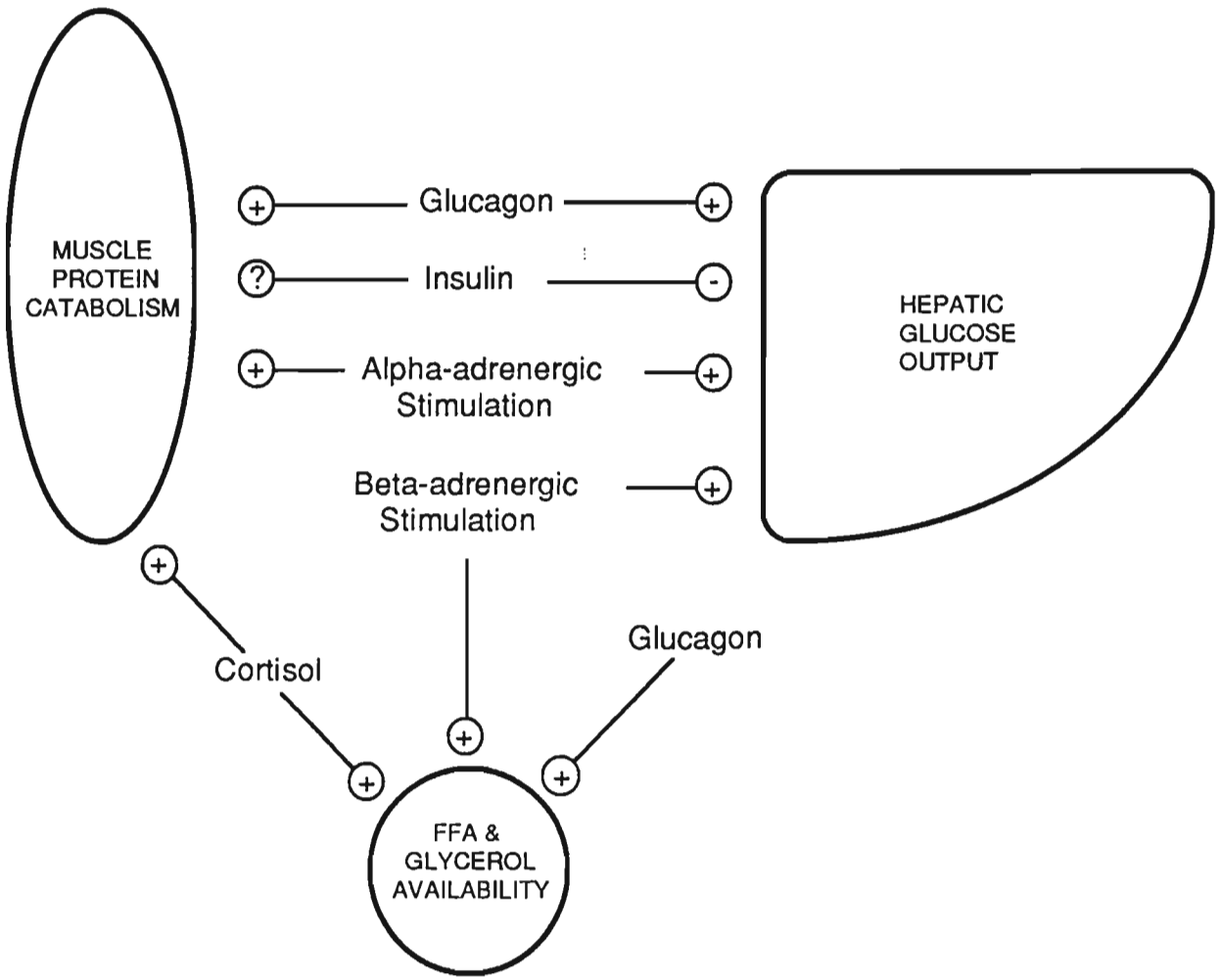


Figure 1.1 *An overview of the important hormonal regulatory factors in the regulation of metabolism in septic and injured patients.*

that there was a synergistic interaction between the three hormones. The researchers concluded that the simultaneous release of stress hormones may be reponsible for mediating the metabolic response to injury.

1.2.(iii) *Inflammatory Mediators* It is not possible to explain the complete spectrum of metabolic changes seen in septic and injured patients by the neuroendocrine response as it is presently understood. This has provoked the search for other mediators. Recent research has suggested that substances such as tumour necrosis factor (TNF) and interleukin-1, released by macrophages at sites of inflammation, play a role in the afferent link between damaged tissue and the central nervous system. This new work is further discussed below in relation to protein metabolism and in Chapters 3 and 7. In order to further define the metabolic effects of inflammatory mediators Wilmore has recently described a synergism in the interaction of inflammatory and endocrine mediators and concluded that both are integral factors of the metabolic response to sepsis and trauma[364].

1.3. Energy Production

1.3.(i) *Extent of Hypermetabolism*

Cuthbertson's appealingly simple description of a hypometabolic ebb phase and hypermetabolic flow phase[78] has undergone substantial modification in the light of more contemporary research. There is little evidence from clinical studies to suggest that heat production is reduced during the ebb phase[201], and it is likely that the increase in metabolic rate during the flow phase is not as large as previously held[320]. Nonetheless, a modest increase in resting energy expenditure in the flow phase is commonly observed, and in the extreme case of extensive burn injury this increase may be as great as 100%[203]. The maximal increase in resting energy

expenditure coincides with the maximal rate of protein catabolism and occurs around one week post injury.

1.3.(ii) *Pathogenesis of Hypermetabolic Response*

Numerous factors have been proposed to account for the extra heat production such as the increased oxygen consumption of the injured tissues, increased energy expenditure by the heart, the Q_{10} effect of raised body temperature, the thermic effect of accelerated protein breakdown and the heat of evaporation lost from burn surfaces[79,320]. The matter has been the subject of a concise review by Little who concludes that the underlying mechanism is probably a resetting of the hypothalamic regulatory control[200]. Yet a further explanation has recently been advanced: it is known that in trauma patients there is an increased rate of substrate recycling in which triglyceride is hydrolysed and then re-esterified, and glucose and its glycolytic intermediates are recycled. As there is no net production of free fatty acid or glucose during these cycles but ATP is hydrolysed, such recycling represents an energy drain. Wolfe has recently quantified the energy dissipated by such substrate recycling and although his methodology grossly underestimates the extent of these processes, he concludes that it is possible they provide the principal biochemical explanation for the increased heat production seen in trauma patients[381].

1.4. Glucose Metabolism

1.4.(i) *Changes in Glucose Concentration*

The post traumatic ebb phase and the earliest stage of sepsis are both characterised by hyperglycaemia[384]. This rapidly established response is initially the result of enhanced glycogenolysis[321], and later a consequence of increased glucose production coupled with reduced peripheral utilization[384]. Sympathetic activity and circulating adrenaline provide the stimulus for

hepatic glycogenolysis and encourage glucagon release while simultaneously inhibiting insulin release from the endocrine pancreas[1,274,283]. The release of cortisol probably plays a facilitatory role in this response[363]. The plasma glucose levels subsequently fall during the post traumatic flow phase to less elevated or normal levels[112,377].

However, the plasma glucose level gives little indication of glucose turnover and this has been the subject of much recent investigation. It is generally agreed that in septic and flow phase trauma patients glucose turnover is increased and that gluconeogenesis is enhanced despite freely available plasma glucose, but there is controversy over changes in glucose oxidation in these situations[309].

1.4.(ii) *Gluconeogenesis*

In health gluconeogenesis is effectively inhibited by an increase in blood glucose levels. However, hepatic glucose production is maintained at normal or elevated rates during the high flow response to trauma or sepsis despite the hyperglycaemia characteristic of such severely ill patients[168,309,367]. The suppression of gluconeogenesis by glucose infusion is very much less effective in septic and trauma patients than in normal volunteers[205,308,373]. This reduction in the suppressibility of gluconeogenesis is probably caused by the increased availability of gluconeogenic substrates occurring in a favourable hormonal milieu[283,373,383]. In severely stressed patients, muscle glycogenolysis and the metabolism of hypoxic tissues produces lactate, glycerol is released from adipose tissue, and plasma alanine levels are increased as a result of enhanced proteolysis[109,367]. In burn patients it has been determined that lactate is quantitatively the most important gluconeogenic substrate[383]. We have previously demonstrated an increase of 40% in the rate of appearance of alanine and a100% increase in the rate of appearance of glycerol and

availability of lactate in trauma patients[313]. On the basis of two moles of either alanine, glycerol or lactate being required to produce one mole of glucose, it is likely that this substantial increase in three carbon substrates would be adequate to explain the observed increase in basal glucose appearance seen in these patients.

However, recent work by Wolfe[171] in burn patients in which both insulin and glucagon concentrations were lowered simultaneously by infused somatostatin, hepatic glucose production decreased despite an increase in the delivery of alanine to the liver. These results suggest that glucose production is controlled at the liver and not by precursor supply.

1.4.(iii) *Glucose Oxidation*

Although most investigators report that glucose oxidation is increased during the high flow phase and in sepsis in absolute terms, there remains disagreement over whether glucose is oxidized as efficiently as in health. The Manchester Trauma Unit researchers have found an increase in glucose oxidation in relation to plasma glucose in injured patients, which they interpreted as a consistent response of substrate mobilization and oxidation[112]. However, the situation is far from clear: there exists a considerable body of data derived from isotopically labelled substrate studies which suggests that glucose is oxidized less efficiently in septic and trauma patients[301,302,309,309,313,377]. The recently reported reduced activity of the pyruvate dehydrogenase complex in septic rats suggests that intracellular derangements in enzymatically controlled pathways may account for the observed reduced efficiency of glucose metabolism[341].

It is germane to the interpretation of the data reported on glucose kinetics that the rate of glucose clearance from the plasma is not related to the rate of glucose oxidation or even to the percentage of glucose uptake

oxidized[385]. Therefore, it is unlikely that reduced glucose oxidation is simply a consequence of the prevailing insulin resistance.

1.4.(iv) *Role of Insulin*

Although changes in insulin release and responsiveness in septic and trauma patients have been well documented[267,338,343] the role of this hormone in the metabolic changes associated with these conditions remains to be clearly defined.

The sympathetic discharge following severe burns has been associated with an inhibition of insulin release[1] although a low plasma insulin level is not a consistent feature of the immediate post injury period[110]. Plasma insulin subsequently rises to reach a peak several days after injury of up to three fold higher than basal levels[284]. This coincides with the period of maximal catabolism

During the flow phase, the plasma insulin level is inappropriately high for the plasma glucose concentration[321] with the pancreas showing a normal or augmented response to glucose infusion[32,136]. However, this phase of the metabolic response is characterized by insulin resistance. The high levels of insulin fail to suppress glucose production, and there is a reduction in glycogen storage[377], lipolysis and fat oxidation[7]. Frayn has demonstrated a highly significant correlation (r=0.97) between the plasma insulin concentration and nitrogen loss in trauma patients and concludes from this finding that protein turnover is resistant to the normal anabolic effect of insulin[110]. However, Wolfe has found in burn patients that insulin acts to conserve protein by restraining the release of amino acids from peripheral tissues[171]. Wilmore has similarly demonstrated a protein anabolic effect of insulin despite profound insulin resistance to carbohydrate in the skeletal muscle of injured patients[365]. The nature of the changes in insulin receptors have not been accurately characterized although it is likely

that there are changes at both the receptor and post receptor level[144]. Experimental animal models most clearly implicate glucocorticoids as a cause of insulin resistance[18] although the mechanism of this response is yet to be fully elucidated.

1.4.(v) *The Adaptive Value of Fuel Store Mobilization*

The mobilization of fuel reserves in trauma and sepsis is common to several species of laboratory animal studied and is likely to be of adaptive value. Infusion of hypertonic glucose has been shown to decrease mortality in pigs following severe haemorrhage[326] and this manoeuvre has also resulted in a rapid increase in blood pressure in recently injured battle casualties[226]. It has been suggested that the observed pressor effect is due to a mass action effect of glucose increasing myocardial glucose uptake and hence the availability of glucose for anaerobic glycolysis[226]. Hyperglycaemia will also compensate for intravascular fluid losses.

The increase in gluconeogenesis and its reduced suppressibility during exogenous glucose administration during the post trauma flow stage and in sepsis may reflect the influence of the damaged and reparative tissues on the rest of the body[366]. The cells involved in inflammation and wound repair rely on glucose as a primary fuel which they predominately metabolize anaerobically. The wound may be looked upon as a privileged organ whose glucose demands can account for most of the approximate doubling in glucose turnover seen in severely burned patients. Wilmore concludes that the increased glucose turnover provides essential fuel for inflammatory and reparative tissues which optimize host defenses and ensures wound repair[365].

1.5 Fat Metabolism

1.5.(i) *Introduction*

The complex changes in the mobilization and oxidation of fat in sepsis and trauma have not been as fully unravelled as those in carbohydrate metabolism. Again it is appropriate to discuss the post trauma changes in the ebb and flow phase, and again there are parallels to be drawn between the flow phase and established sepsis.

1.5.(ii) *Lipolysis*

Lipolysis is enhanced immediately post injury by the stimulation of of the sympathetic innervation of adipose tissues and by raised plasma adrenaline, glucagon and cortisol levels[108]. Growth hormone may also play a role in this response[113]. The accelerated lipolysis occurs despite the prevailing hyperglycaemia and raised plasma insulin[58]. There is, however, little correlation between plasma free fatty acid levels and the severity of the trauma[321]. This is probably a result of the reduced perfusion of adipose tissues which often follows severe trauma, so that the supply of albumin carriers for released free fatty acids is inadequate[323]. In addition, the plasma free fatty acid level is further lowered by the lactic acidosis of systemic hypoxia which encourages re-esterification[238].

1.5.(iii) *Fat Oxidation*

Septic patients have a lower respiratory quotient (RQ) than non-septic controls, and worsening sepsis is frequently accompanied by progressive falls in RQ. These findings have been corroborated by isotopic studies and suggest that increased fat oxidation may be an important feature of the altered metabolism seen in sepsis[249,309,322]. During the first few days after severe injury the RQ rises from a value of close to 0.7 to one that indicates that carbohydrate is the major fuel[320]. Glucose infusion in normal

volunteers inhibits fat oxidation and the RQ rises above one as the excess glucose is deposited as triglyceride. However, in patients with severe sepsis, although the RQ rises following glucose infusion it does not reach a value of1.0 indicating that fat continues to be the main substrate for oxidation[32].

Under most circumstances the rate of uptake of free fatty acid is directly proportional to their plasma concentration[345]. However, the increase in fatty acid oxidation in sepsis and trauma is not 'substrate led' as the plasma levels of free fatty acid are often quite low[112]. This suggests that there are changes in the intracellular metabolism of fat in these patients[109].

The preference for fat as an energy substrate is more pronounced in septic than in trauma patients[322]. Using the sepsis severity scale developed by Elebute and Stoner, it has been shown that there exists a positive relationship between fat oxidation and sepsis severity, whereas there is a negative relationship with glucose oxidation[96].

1.5.(iv) *Triglyceride Metabolism*

Less is known about changes in triglyceride metabolism. It has been reported that the concentration of triglyceride in plasma is elevated in Gram negative sepsis[119] and after injury[110]. There is presently no isotopically labelled triglyceride suitable for infusion into patients, but studies in septic animals have revealed that the increase in triglyceride concentration is due to an accelerated rate of production of triglyceride, and in particular very low density lipoprotein (VLDL), rather than the response being secondary to a decrease in triglyceride clearance[305,387]. The increase in activity of adipose tissue lipoprotein lipase is consistent with the postulated increase in VLDL turnover[278].

1.5.(v) *Ketone Metabolism*

Simple starvation is attended by ketosis. As ketone bodies can serve as alternative energy substrates for many tissues, they reduce whole body glucose demand and therefore gluconeogenesis from protein. However, in severe sepsis and, to a lesser extent, trauma there is a blunting of the adaptive ketonaemic response and its subsequent nitrogen conservation[31,347]. It is likely that the increased insulin levels seen in stressed surgical patients are responsible for the impaired production of ketone bodies[250], although reduced activity of hepatic acyl carnitine transferase has also been implicated[348].

1.6. Protein Metabolism

1.6.(i) *Introduction*

The negative nitrogen balance described by Cuthbertson[77] has been similarly demonstrated in septic patients[387]. When this response is prolonged, the resultant protein depletion plays a major role in the pathogenesis of multiple organ failure which develops in severely injured or septic patients[143]. A detailed understanding of the nature and mechanism of the protein loss is important to provide a basis for providing more effective nutritional support and offers the alluring possibility of metabolic manipulation in such cases.

1.6.(ii) *Whole Body Kinetics in Sepsis and Trauma*

A net protein loss may result from a relative decrease in whole body protein synthesis or increase in catabolism, or a combination of both mechanisms. The varied responses reported following elective surgical procedures, musculoskeletal trauma, burns and sepsis are summarised in table 1.1. The trauma of elective surgery results in an inhibititon of protein

Table 1.1 *Protein Synthesis and Catabolism Rates in Sepsis and after Surgery and Trauma*

Reference	Subjects	Methodology	Synthesis	Catabolism	Comment
Crane *et al.* 1977 [76]	Elective orthopaedic $n = 11$	Oral ^{15}N glycine	↓	No change	
O'Keefe *et al.* 1974 [258]	Elective abdominal operations $n = 4$	Infused ^{14}C leucine	↓	No change	Reduced postoperative calorie intake 2 cancer patients
Kien *et al.* 1978 [183]	Skin construction in children $n = 6$	Infused ^{15}N glycine	↓	No change	Postoperative intake maintained
Rennie and Harrison 1984 [275]	Abdominal surgery $n = 11$	Skeletal muscle efflux of 3-methylhistidine and tyrosine	↓↓	↓	Postoperative all on IVN, 11 cancer patients
Kien *et al.* 1978 [182]	Burns, children $n = 11$	Infused ^{15}N glycine	↑	↑↑	Controlled nutritional support
Birkhan *et al.* 1980 [31]	Multiple trauma $n = 6$	Infused ^{14}C leucine	↑	↑↑	
Shaw and Wolfe 1989 [313]	Trauma $n = 43$	Infused ^{15}N lysine and ^{14}C urea	↑	↑↑	Some on IVN
Shaw *et al.* [303]	Sepsis $n = 22$	Infused ^{15}N lysine and ^{14}C urea	↑	↑↑	Net protein catabolism continued despite IVN

synthesis, whereas catabolism proceeds at an unchanged rate[76,183,258]. However, in severe trauma involving multiple fractures or burns or in septic patients, both synthesis and catabolism are increased, with the rate of protein degradation outstripping that of production (fig 1.2). The cause of this disparity in response is not clear, but it is likely that the post operative changes primarily reflect the effect of starvation rather than the response to trauma *per se*. It is noteworthy that the acute metabolic response to severe injury and sepsis is the direct reverse of that seen in starvation in which both protein synthesis and degradation are reduced[185,237].

The preceding description of the relative changes in the relationship between net protein synthesis and catabolism oversimplifies the situation as it exists in the clinical setting as it does not include the influence of protein intake. This deficiency is rectified in the model developed by Clague and his colleagues[63], in which they propose that the protein breakdown in response to trauma is largely obligatory, whereas synthesis increases with substrate availability. Accordingly, provision of adequate protein can reduce net nitrogen loss after trauma.

1.6.(iii) *Changes in Skeletal Muscle*

Skeletal muscle is the major site of nitrogen storage and of nitrogen loss, although the contribution of other tissues such as skin, gut and lungs may not be insubstantial[275]. The rate of release of 3-methylhistidine, an amino acid derived exclusively from actin and myosin and excreted unchanged, has been used as an index of the rate of skeletal muscle breakdown[391]. Considerable increases in the rate of production of 3-methylhistidine have been seen in severely traumatised and septic patients[334], whereas no increase occurs following orthopaedic operations and minor injuries[362]. Although recent doubts have been voiced about the specificity of 3-methylhistidine as an indicator of skeletal muscle

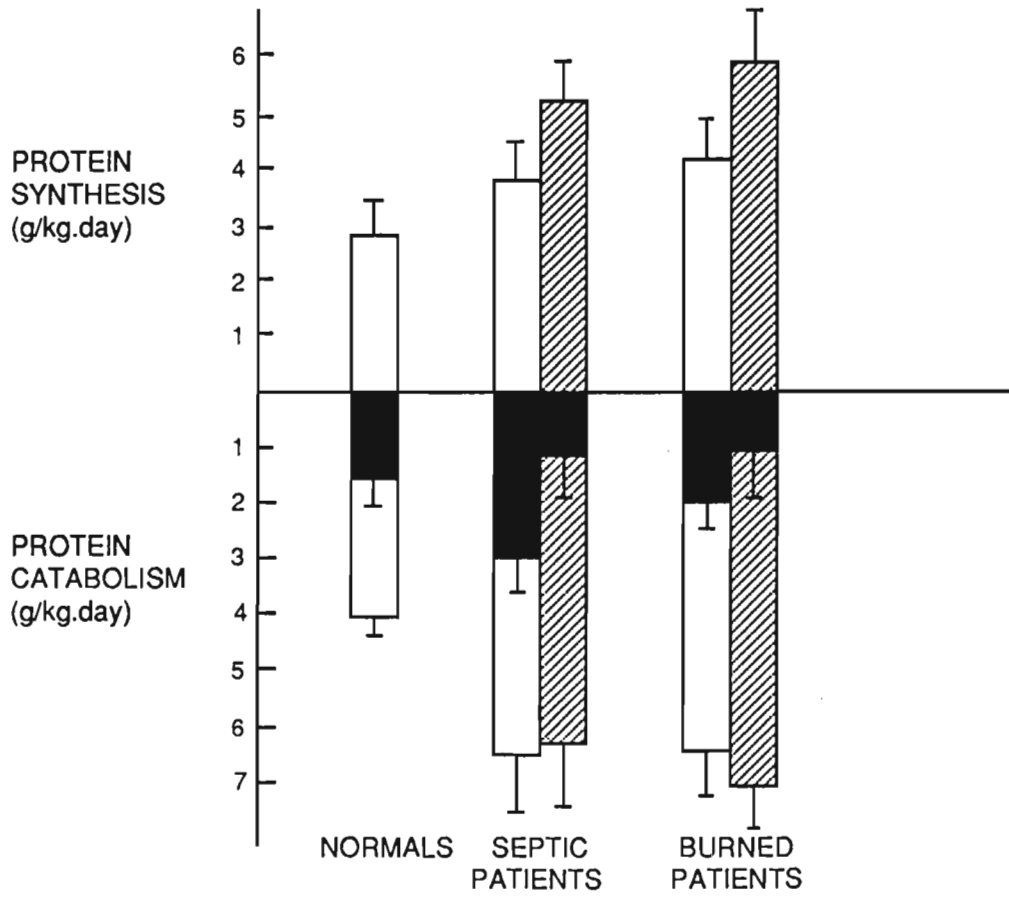


Figure 1.2 *Rates of total protein synthesis, total protein catabolism and net protein catabolism in normal volunteers, septic patients and in burned patients. In septic and burned patients the rate of net protein catabolism is significantly greater ($p<0.05$) than in volunteers. Providing IVN reduces net protein catabolism by significantly increasing total protein synthesis. The rate of total protein catabolism remains unchanged (modified from Shaw and Wolfe (309)).*

□ *Basal* ▨ *IVN* ■ *Net Protein Catabolism*

breakdown[276] other investigators have provided sound arguments for its continued use14.

In injury the increased rate of protein release from skeletal muscle does not simply represent the degradation of damaged tissue; there is increased loss of 3- methylhistidine and changes in skeletal metabolism in muscle undamaged by trauma[273,333].

The technique of percutaneous needle biopsy of human muscle has enabled investigation of the intracellular metabolic derangements[21]. The changes in intracellular amino acid concentration following elective surgery, trauma and in sepsis are similiar, suggesting a common response to varying types of insult[9,10,190]. The response consists of an increase in essential amino acids, particularly branched chain amino acids, and reduction in non-essential amino acids largely as a result of a 50% decrease in the level of intracellular glutamine[10]. The cause of these changes remains a matter for conjecture[9,190,256].

1.6.(iv) *Hepatic Protein Metabolism*

The data of studies of whole body protein metabolism represent the summation of response of the body tissues, but fail to reflect variation in kinetics between different tissues. In both severe trauma and sepsis, whereas skeletal muscle catabolism exceeds synthesis there is a net increase in the production of hepatic structural and secretory proteins[104,340].

The teleological explanation of the autocannibalism of muscle tissue in response to stress is that this mechanism allows greater than normal quantities of amino acids to be available to the liver for gluconeogenesis, oxidation and the synthesis of proteins involved in immune defense and wound healing[281,283,367]. This increased hepatic synthesis occurs at a time of depressed efficiency of other hepatic cellular functions[54,65], reflecting the high priority of this function in sepsis and trauma[260].

1.6.(v) *Mechanism of Protein Loss*

The catabolism of muscle protein reserves to provide substrate for increased hepatic synthesis to defend against infection and promote wound healing is an appealing explanation of the changes in protein metabolism seen in sepsis and trauma. This presents the challenge of delineating the neuroendocrine changes which mediate this response.

It is known that physical rest, fever, starvation, glucocorticoids and insulin resistance[104,143,185] are associated with an increased net rate of protein breakdown but that none of these stimuli induces protein catabolism to the degree seen in sepsis and trauma[260]. Glucocorticoids cause an increased amino acid release, increased oxidation of leucine by skeletal muscle and favour hepatic conversion of amino acid to glucose. However, it is generally believed that glucocorticoids have primarily a permissive role and are not responsible for the overall protein catabolism seen in sepsis and trauma[283]. We have demonstrated that the glucagon plays a role in accelerating protein breakdown in trauma patients[312].

Recent work has sugested that factors produced by leucocytes may be central in promoting the changes in protein metabolism in sepsis and trauma. Interleukin-1, a polypeptide synthesized by macrophages as part of the inflammatory response, causes fever and leucocytosis[22]. Rat muscle incubated with interleukin-1 showed a stimulated rate of proteolysis but the protein synthesis rate was unaffected[15]. These effects may be mediated by protaglandin E_2 as the interleukin-1 increased protaglandin E_2 production and the catabolic catabolic effects of interleukin-1 can be abolished by the addition of indomethacin. When injected into the peritoneal space of rats interleukin-1 increased the rate if synthesis of hepatic secretory and structural proteins[202,328].

The evidence attributing an important role in the mediation of the septic response to tumour necrosis factor has been recently reviewed by Michie[233], and is summarized in the introduction to Chapter 7, in which a study is described in which TNF is infused into an animal model to simulate some of the hormonal and metabolic changes seen in sepsis. TNF is a peptide released by macrophages in response to tissue inflammation, and it appears that the release of TNF at the onset of a significant septic episode may may the event which triggers many other elements of the systemic response to sepsis, such as elevations in the plasma levels of counterregulatory hormones and other inflammatory mediators.

1.6.(vi) *Consequences of a Prolonged Catabolic State*

The breakdown of skeletal muscle that occurs in septic and trauma patients may be of adaptive advantage as the convalescing animal is provided with adequate energy substrates and amino acids for hepatic protein synthesis. However, if this catabolic response is prolonged the loss of body protein can pose a threat to survival by resulting in pulmonary and cardiovascular insufficiency and impaired immune function and wound healing[283]. It is for these reasons that attempts have been made to reverse or at least slow down protein catabolism in critically ill surgical patients.

1.7. The Effects of Nutritional Support

1.7.(i) *Energy Requirements*

The optimal nutritional support of severely injured and septic patients has been the subject of intensive investigation. As such patients have a basal metabolic expenditure elevated 20% or more above normal[309] and as they can lose as much as 40 g of nitrogen a day[189], they exhaust their energy and protein reserves much more rapidly than non-stressed patients.

Although normal hospital food is the most efficient means of providing calories and protein to these patients their gastrointestinal system is frequently unable to cope and intravenous nutritional support is indicated.

Septic and injured patients require approximately 40 kcal/kg.day[151] and this energy requirement has traditionally been supplied as glucose. Glucose infusion has been demonstrated to be a simple and effective means of conserving body protein in severely stressed patients[301]. However, there has been considerable interest in the role of fat as a major and perhaps preferred energy substrate in septic patients and this debate has recently been reviewed by Long[204]. We have used isotopic tracer methodology to demonstrate that lipolysis and fatty acid oxidation are enhanced in sepsis[302,306,311], but whether fat has a greater protein sparing effect than glucose in this setting is yet to be resolved[295].

1.7.(ii) *Failure to Achieve Protein Anabolism*

Despite recent advances in intravenous nutritional support there are several reports indicating that a positive nitrogen balance cannot be achieved in septic or severely traumatized patients [275,325]. Intravenous nutrition reduces the net rate of loss of protein by increasing the rate of protein synthesis. However, the capacity to increase synthesis by providing substrate falls short of the increased protein catabolism which proceeds uninfluenced by nutritional support[303,313].

The failure of standard IVN regimens to achieve nitrogen retention in severely stressed patients has led to the investigation of more exotic mixtures. The use of branched chain amino acids to encourage protein anabolism has received much recent attention. Branched chain amino acids play an important role in the regulation of muscle synthesis[49,255] and muscle biopsies of injured and septic patients have shown significant increases in the intracellular concentration of BCAA's[7]. It has been

postulated that this may provide a stimulus for protein synthesis97. Series in which post operative patients have been infused with BCAA's alone[114], with BCAA enriched IVN[59] or with alpha keto-isocaproate, the ketoacid breakdown product of leucine[285], have shown improved nitrogen balance, presumably on the basis of increased muscle synthesis. As yet there are no data from prospective trials to support the routine use of such modified IVN mixtures.

1.8. Pharmacological Manipulation

1.8.(i) *Hormonal Blockade*

Several approaches have been taken in attempts to attenuate the catabolic response of severe surgical illness by hormonal blockade. Epidural anaesthesia abolishes the post operative release of catecholamines and cortisol and has been shown to improve the cumulative nitrogen balance over a five day post operative period in women following hysterectomy[39]. Using isotopic tracer methodology we have recently studied the effects of epidural anaesthesia in 23 patients who had recently undergone extensive surgical procedures[297]. There was a 20% decrease in the rate of production of glucose with a parallel reduction in glucose oxidation, and net protein catabolism as measured by the rate of appearance of ^{14}C urea was reduced by11%.

The sympathetic response may also be attenuated with specific pharmacological antagonists. Sympatholytic drugs have been demonstrated to reduce the degree of hypermetabolism in burn patients[368]. In our laboratory we have studied the effects of alpha and beta blockade produced by infusions of phentolamine and propranolol respectively in stressed patients receiving intravenous nutrition[299]. Our data demonstrate that the role of the sympathetic nervous system in the promotion of endogenous glucose turnover in such patients is primarily a beta adrenergic

effect whereas the promotion of protein catabolism is mainly an alpha adrenergic effect.

Considerable interest in the role of prostaglandins as effectors of the stress response has been aroused by the recent work on interleukin-1. However, decreasing prostaglandin synthesis with cyclo-oxygenase inhibitors does not reduce muscle catabolism in septic or thermal injury models in animals[64,142] or in septic patients[312], and their use exposes patients to side effects such as peptic ulceration and impaired haemostasis.

1.8.(ii) *Anabolic Hormones*

The use of anabolic hormones to attenuate the rate of breakdown of body reserves is an appealing proposition. Insulin has been shown to improve nitrogen balance in trauma patients[388], but was found to have no effect on the gain of body nitrogen or fat when used as an adjuvant with a two week course of IVN[217]. Similarly, anabolic steroids have improved the nitrogen balance in post operative patients[339] but have not been demonstrated to improve the efficacy of IVN[392].

Interest in the therapeutic role of human growth hormone has increased since 1985 when gene recombinant technology enabled its manufacture on a scale which makes feasible its use as a pharmacogical agent. Several early investigators reported improved nitrogen balance in burn patients to whom growth hormone was administeredand more recently other groups have demonstrated a favourable influence of growth hormone on protein kinetics in postoperative and parenterally fed patients (table 1.2). The potential therapeutic role of recombinant human growth hormone is presented in much greater detail in Chapters 4 and 5.

1.9 Summary

1.9.(i) The increased release of hormones, particularly adrenaline, glucagon and cortisol, mediate many of the metabolic changes seen in sepsis and trauma. The role of interleukin-1 is yet to be precisely defined, but there is increasing evidence that substances released from damaged tissue are at least partly responsible for the accelerated proteolysis seen in severe surgical illness.

1.9.(ii) Glucose turnover is increased in sepsis and trauma, but glucose is oxidized with reduced efficiency. There is evidence to suggest that fat is the preferred energy substrate in septic and, to a lesser degree, injured patients.

1.9.(iii) In critical surgical illness the rates of both protein synthesis and catabolism are increased. However, the increase in catabolism is of greater magnitude resulting in a net breakdown of protein and, if prolonged, muscular wasting, cardiopulmonary insufficiency and immune compromise.

1.9.(iv) The provision of sufficient energy substrates and nitrogen does not reduce the catabolic response but encourages protein synthesis, decreasing net catabolism. Numerous substrate, hormonal and pharmacological manipulations have been tried to improve the nitrogen balance in severely ill sugical patients. To date, the clinical efficacy of none has been conclusively proven.

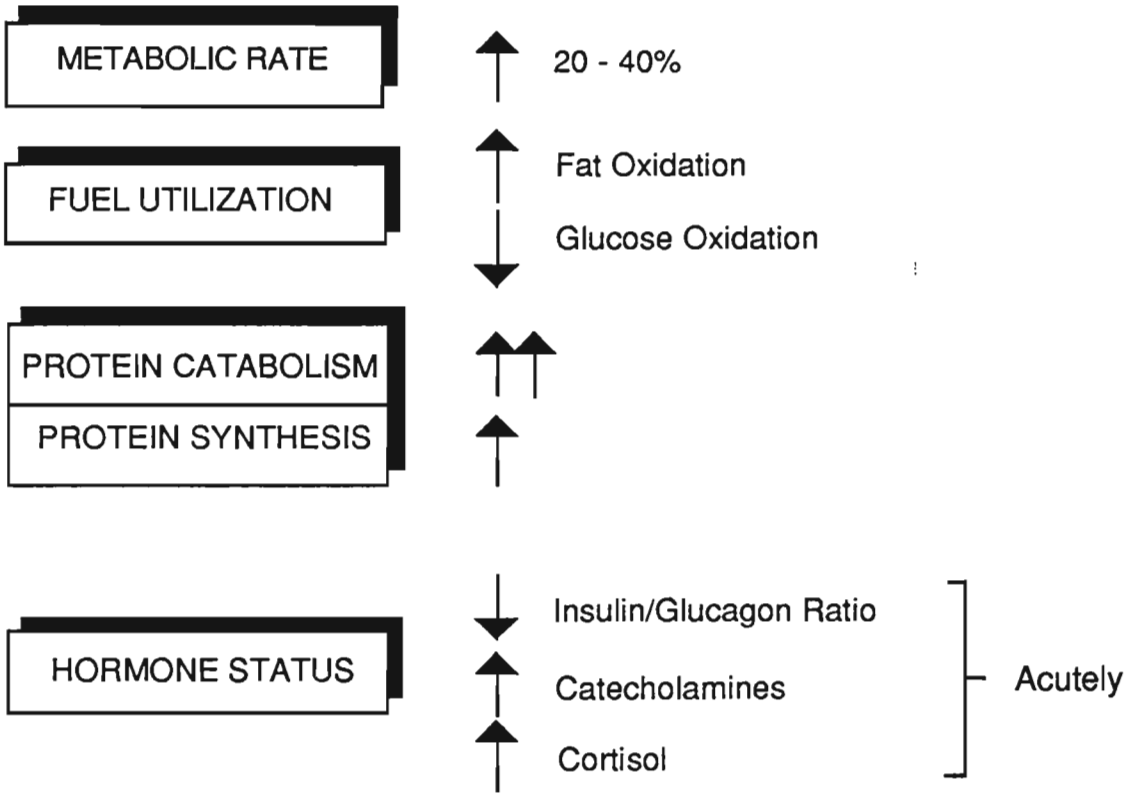


Figure 1.3 *Summary of the metabolic changes associated with critical surgical illness (modified from Shaw and Wolfe, 1987 31).*

Table 1.2 *Summary of Patient and Volunteer Studies of Metabolic Effects of Growth Hormone.*

Authors	Patients	Growth Hormone	Methodology	Nutrition	Results	Comments
Prudden *et al.* 1956 [268]	Severely burned studied on multiple occasions18 to 124 days post-injury, $n = 4$	Dose and species of origin not specified, probably bovine, given for 5 day periods.	Oral nitrogen intake and urinary nitrogen excretion measured	Unrestricted oral diet, large range of nitrogen intakes	When administered to catabolic patients, increased N loss, but reduced N loss in anabolic patients	Efficacy of bovine GH in this setting is open to doubt, small n, data are not convincing
Liljedahl *et al.* 1961 [198]	Severely burned patients studied 12 to 32 days post-injury $n = 5$	Human pituitary derived, 10 mg i.m. mane. for 7-9 days, 1 patient 10 mg b.d. for 8 days	Oral nitrogen intake and urinary nitrogen excretion measured	Unrestricted oral intake, 1900 - 3200 kcal/day	hGH reduced urinary nitrogen loss by 0.8 - 9.4 g/24 hours, albumin synthesis also increased	hGH stimulated appetite
Wilmore *et al.* 1974 [368]	Severely burned patients studied 14 to 74 days post-injury, $n = 9$, with one patient studied twice own controls	Human pituitary derived, 10 IU i.m. daily for 7 days	Urinary nitrogen excretion	Enteral or parenteral feeding, hypercaloric diet	Urinary nitrogen excretion decreased in 9 out of 10 studies	Protein conserving action of hGH manifest during catabolic phase

Authors	Patients	Growth Hormone	Methodology	Nutrition	Results	Comments
Manson and Wilmore, 1986 [220]	Normal volunteers, $n = 4$, subjects own controls	rhGH, 10 mg s.c. for 7 days	Urinary nitrogen excretion	Hypocaloric IVN (566 kcal/m^2.day)	Positive N balance achieved despite hypocaloric feeding	Possibility of hypocaloric peripheral parenteral feeding in conjunction with IVN.
Ward *et al.* 1987 [350]	Postop major GI surgery, $n = 7$ rhGH, $n = 7$ placebo, beginning on day of operation	rhGH, 0.1 mg/kg.day for 7 days	Urinary enrichment of isotopically labelled tracers, indirect calorimetry	Very hypocaloric i.v. fluids, no nitrogen given	rhGH reduced nitrogen losses but N balanve remained negative	Fat oxidation increased, protein oxidation reduced suggesting a switching of fuel sources
Fong *et al.* 1980 [107]	Normal volunteers. $n = 10$	rhGH, 2 ug/kg.hr i.v. for 6 hours	Forearm arterio-venous difference before and after infusion	n = 5 post-absorptive (PA), n = 5 on 10th day of hypercaloric IVN	rhGH in PA subjects had no effect on amino acid flux, but increased FFA flux, rhGH in IVN subjects reduced aa flux, no effect on FFA flux	Evidence that effects of GH are influenced by nutritional state, may be better anabolic agent in fed rather than starved subjects

Authors	Patients	Growth Hormone	Methodology	Nutrition	Results	Comments
Ponting *et al.* 1988 [266]	Postoperative major GI surgery, $n = 6$ rhGH, $n = 5$ placebo	rhGH, 0.1 mg/kg.day i.m. for 7 days	Urinary enrichment of isotopically labelled tracers, indirect calorimetry	IVN, hypercaloric, approx 60/40 CHO/fat ratio, 7 g nitrogen	Patients receiving rhGH were in a positive nitrogen balance, placebos were in a negative nitrogen balance	Randomized, prospective. Increase in both protein synthesis and breakdown
Ziegler *et al.* 1988 [396]	Malnourished, stable surgical patients, $n = 11$, patients own controls	rhGH 10 mg s.c. daily for at least 7 days, and up to maximum of 25 days	Urinary nitrogen excretion	Hypocaloric, isonitrogenous IVN	Positive N balance achieved despite hypocaloric diet	Significant N retention observed in some patients for up to 25 days of rhGH therapy
Jiang *et al.* 1989	Postop, major abdominal surgery, $n = 9$ rhGH, $n = 9$ placebo randomized prospective study	rhGH 0.15 IU/kg i.m. daily for 7 days beginning immediately postoperatively	Urinary ^{15}N enrichment following ^{15}N glycine infusion	Hypocaloric, isonitrogenous IVN	Positive N balance in rhGH treated patients, negative N balance in controls	Postop reduction in grip strength seen in controls prevented in rhGH treated patients

Chapter 2

The Metabolic Response to Cancer

2.1 Introduction

Cachexia is commonly the cause of death in cases of advanced malignancy[352], and cancer patients who have lost a significant percentage of their body weight prior to surgical treatment are subject to a much greater risk of postoperative mortality and morbidity[70,150,229]. There is no doubt that reduced oral intake resulting from anorexia or obstruction of the gastrointestinal tract plays a very significant role in the development of the cancer cachexia syndrome. However, whereas the metabolic response to uncomplicated starvation acts to limit the consumption of host reserves, in the cachectic cancer patient there is often an accelerated mobilization and oxidation of energy substrates and loss of nitrogen[41,310,311]. These changes are a consequence of alterations in intermediary metabolism associated with cancer bearing[178].

Understanding the metabolic response to cancer has become increasingly important over the last two decades with the introduction of effective and safe parenteral nutrition techniques[92]. It is now possible to provide sufficient calories and nitrogen to all cancer patients, but the metabolic milieu associated with advanced cancer may retard the restoration of lean body mass[315]. In the following chapter the manner in which malignant tumours effect host metabolism will be presented, and the effectiveness of the available therapeutic options discussed.

One consistent feature of data from metabolic studies in cancer patients is the range of response between individuals, even when comparing those with the same diagnosis and stage of disease[161]. The interpretative difficulties are compounded by many reports comparing small

heterogeneous groups of cancer patients with equally small groups of controls, which may well be poorly matched for age or weight loss. To overcome some of these problems laboratory models have been developed in which malignant cells of identical genotype are transplanted into genetically uniform animals[121]. However, the growth dynamics and tumour to host weight ratios frequently do not bear resemblance to those observed in patients, and this chapter will by in large present data from patient studies.

2.2 Changes in Energy Metabolism

2.2.(i) *Effect of Cancer on Energy Expenditure* The hypothesis that tumour bearing increases energy expenditure and results in a cumulative negative energy balance and progressive weight loss has been exhaustively investigated, and there now exists a substantial body of supportive evidence. Bozzetti and colleagues studied a heterogeneous group of patients with advanced tumours and found a highly significant correlation between the resting metabolic expenditure (RME) and the magnitude of weight loss[38], and other groups of researchers have similarly found elevated RME's in patients with cancer cachexia[140,196,216,351]. There are a few anecdotal reports of cases in which successful anti-neoplastic therapy has reduced energy expenditure in hypermetabolic patients[6,351], suggesting that the presence of the tumour itself is capable of elevating the RME. However, hypermetabolism is not an invariable finding in cancer patients who have lost weight, with large series having been reported recently which have failed to demonstrate a significant increase in the resting metabolic rate of cachectic cancer patients when compared to patients with weight loss of a similar magnitude or to weight stable cancer patients[100,141]. In a series consisting of 200 patients with a variety of tumour types 29% had a resting metabolic expenditure that was 10% higher than that predicted by

the Harris-Benedict equation, 31% were found to be hypometabolic using the same criterion and no relationship was demonstrated between RME and weight loss or tumour burden[185].

Although some of the disparity in the findings of these studies is no doubt a reflection of differences in experimental material and methodology, it is likely that they are reflecting a true heterogeneity of response to the tumour bearing state. It is now clear that cancers arising from certain tissues eg sarcomas[300], leukaemias[164] and bronchial carcinomas[314] frequently provoke a hypermetabolic response, whereas patients with pancreatic and hepatobiliary tumours tend to be hypometabolic[85].

Many cancer patients with advanced disease have a reduced caloric intake. In normal man or in patients with benign disease, semistarvation is attended by a reduction in RME[122,131], so in an undernourished cancer patient even a normal metabolic rate represents a failure of this adaptive response[6,99].

2.2 (ii) *Mechanism of Effect of Cancer on Energy Expenditure* The mechanism by which malignant tissue alters the energy expenditure of the host is not clear. It is unlikely that increased energy consumption by the tumour itself is responsible in human tumours as it is rare for tumours to account for more than 5% of body weight[38]. More plausible is the hypothesis that mediators are released by some cancers which alter host metabolism[331-2], and some of the changes which may be effected in this manner are discussed in subsequent sections.

2.3 Changes in Glucose Metabolism

2.3.(i) *Introduction* There are many reports describing an increased rate of endogenous glucose production in cancer patients [146,161,164,213,353] (fig 2.1), and considerable research effort has been directed toward determining the mechanism and significance of this occurrence. It is clear that the magnitude of the increase in glucose turnover is influenced by tumour stage[186,310] and histology, and that it is associated with cancer cachexia[62]. In this section, some of the observations made in cancer patients of changes in glucose metabolism will be summarized and the implications that these have on energy balance discussed.

2.3.(ii) *Gluconeogenesis* Shaw and Wolfe[310] have defined glucose kinetics in a group of patients with early (limited to the gut wall) and advanced gastrointestinal malignancies. Whereas the rate of glucose turnover in the group of patients with early lesions was indistinguishable from from that seen in normal volunteers, glucose production was significantly increased in patients with advanced lesions. Similarly, tumour histology has also been demonstrated to influence the extent of increase of glucose production. The glucose turnover rates in sarcoma[300] and leukaemia[164] patients have been reported to be respectively two and nearly three times the value determined in normal volunteers, whereas the glucose turnover rate in lymphoma patients does not differ significantly from normal[164]. Other researchers have studied the effect of weight loss on glucose turnover. Holroyde has reported that weight stable cancer patients have rates of glucose production similar to normal volunteers, however those with progressive weight loss have markedly elevated rates[158]. This is a particularly significant finding as progressive

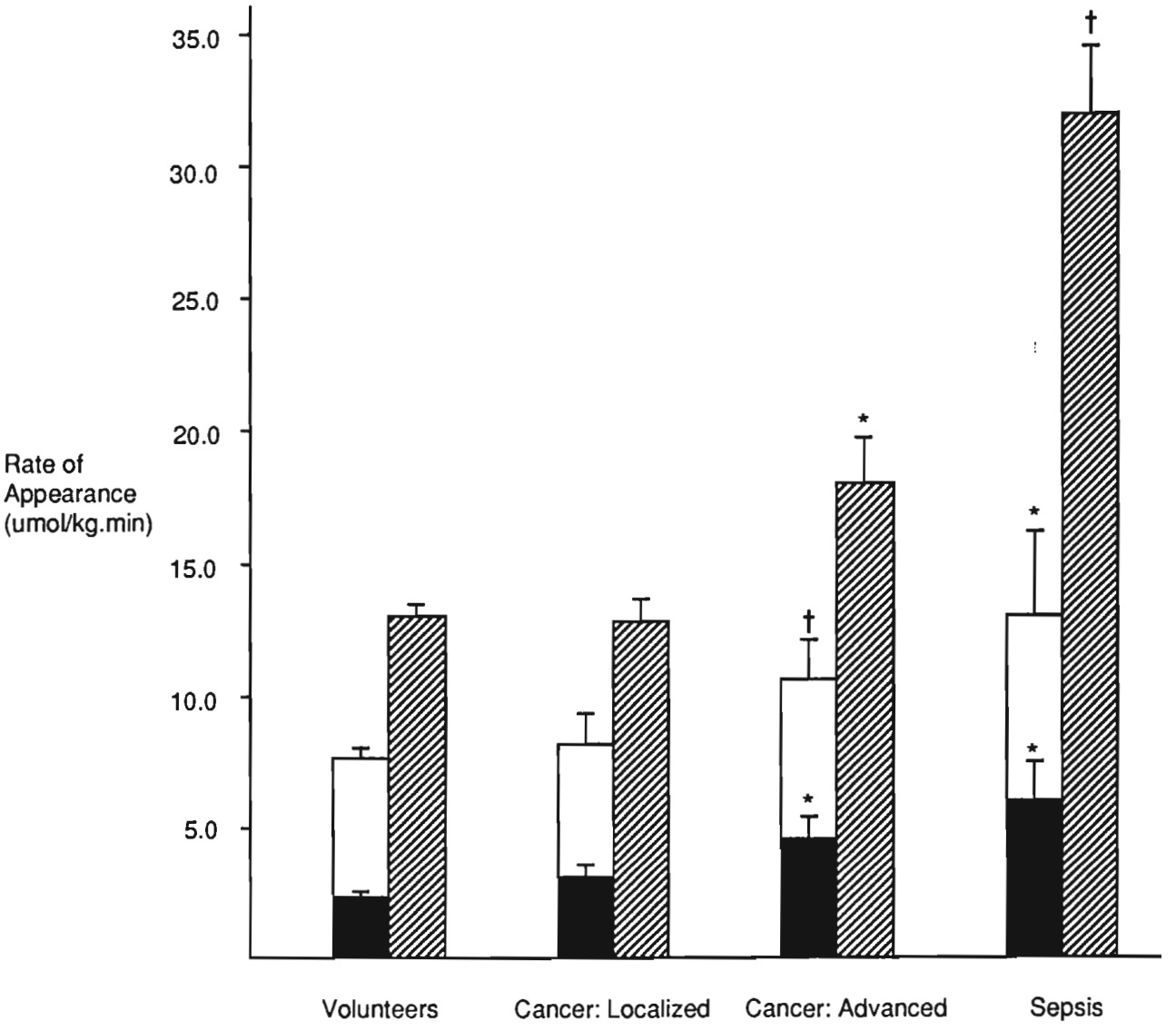


Figure 2.1 *The influence of localized or non-weight losing cancer, advanced or weight losing cancer and sepsis on the rate of production of glycerol (■), free fatty acids (□), and glucose (▨) (* $p<0.01$, † $p< 0.05$). Modified from Shaw and Wolfe, 1986 (308) and Shaw and Wolfe, 1986 (310).*

weight loss secondary to uncomplicated starvation is attended by a reduction in glucose turnover[160].

The hepatic production of glucose becomes less sensitive to the usual homeostatic regulating mechanisms in some patients with cancer. If a normal volunteer is infused with glucose at a rate of 4 mg/kg.hour (the dose of a typical total parenteral nutrition regimen) the suppression of endogenous glucose production will approach 100%[206]. In patients with advanced gastrointestinal cancer, there is a 70% reduction in endogenous glucose production[310], whereas in sarcoma and leukaemia patients hepatic glucose production is reduced by less than one third[164,300].

The cause of elevated hepatic gluconeogenesis and its reduced suppressibility in patients with malignant tumours is unclear. The plasma levels of the hormones involved in glucose homeostasis (insulin, cortisol, growth hormone) are not consistently deranged in cancer patients[62] and are unlikely to play a significant role, although insulin receptor insensitivity would be consistent with increased gluconeogenesis. The increased availability of the gluconeogenic substrates lactate, alanine and glycerol presents a plausible mechanism, and of these, lactate is probably the most quantitatively important. Warburg described more than fifty years ago the dependence of malignant cells on anaerobic glycolysis and the resultant release of lactate[349]. Indeed, lactic acidosis has been reported in some cancer patients, particularly in those with disseminated haematological malignancy[33] and a greater rate of hepatic synthesis of glucose from lactate has been reported by several research groups[214,353]. Increased gluconeogenesis from alanine,[47,356] has been described in cancer patients, which would act to accelerate wasting of body protein and which will be discussed in greater detail in a subsequent section. The contribution of glycerol toward encouraging gluconeogenesis is likely to be minor[214]. The balance of available evidence suggests that the increased rate of

gluconeogenesis is substrate led, however the isolation of induced gluconeogenic enzymes from hepatocytes of cancer bearing laboratory animals[137,139] suggests that this may not be exclusively so.

2.3.(iv) *Cori Cycling* In the Cori cycle[72], lactate released as a result of glycolysis in peripheral tissues is used as a gluconeogenic substrate by the liver. This process consumes energy, as six ATP's are required for the resynthesis of glucose from lactate whereas only two are produced by the glycolytic degradation of each glucose molecule. When the anaerobic glycolysis occurs in malignant tissue, the energy cost to the host is compounded by the loss of glucose parasitized by the tumour[48]. Accordingly, there has been considerable interest in determining the extent of Cori cycling in cancer patients as it may be one of fundamental metabolic changes causing cancer cachexia.

The rate of Cori cycling can be easily measured using ^{14}C and ^{6}H labelled glucose tracers[270]. Increased rates of cycling have been measured by Holroyde in a group of 20 patients with metastatic colorectal cancer when compared to control subjects of comparable age and sex[157]. It was inferred that tumour glycolysis was responsible for the excess lactate production, although this supposition was not confirmed by the lack of correlation between the extent of tumour burden and the increased rate of Cori cycling. Evidence for the cancer *per se* being responsible for increasing the rate of Cori cycling has been provided by the study of Eden and Edstrom[93], who compared the rate of Cori cycling in patients with cancer cachexia with a control group of patients who had suffered a similar degree of weight loss but from benign causes. The rate of Cori cycling was, in both the fasted and enterally fed states, significantly higher in the patients with malignant disease, suggesting it was the cancer *per se* that was responsible for this increase. However, Burt and colleagues have measured an increased rate

of release of lactate from the forearm of a small group of patients with localized carcinoma of the oesophagus, implying that the tumour is capable of effecting a distant influence on the metabolism of carbohydrate in host tissue[46]. It is likely, although still conjectural, that increases in both tumour and host tissue glycolysis are responsible for the observed changes in whole body lactate metabolism.

The role played by such futile cycles in the pathogenesis of cancer cachexia has been the subject of much debate. Gold has performed considerable work in this field and describes the "fundamental position of tumour glycolysis-host gluconeogenesis in the production of cancer cachexia"[127]. The rate of Cori cycling has been measured by Holroyde in two groups of cancer patients, one with progressive weight loss and the other with stable weight[158]. The rate of Cori cycling was considerably elevated in the first group but normal in the second, suggesting that the energy lost by the futile cycling was responsible for the weight loss. However, such findings have not been universally reproduced: Kokal and colleagues were unable to demonstrate any significant differences in glucose cycling rates as a function of pre-illness weight loss[186].

Eden and associates, having demonstrated an increase in glucose turnover and glucose cycling in cancer patients, estimated the potential energy cost to the cancer patient[46]. They calculated that if the incomplete oxidation of glucose were to be substituted by the complete oxidation of fat that this would lead to an increase in energy expenditure of 250 to 300 kcal per day and loss of 0.9 kg of fat per month. However, a contrary argument has been forwarded by Young who estimated that if only 15% of the total lactate production is oxidized completely and 85% is converted to glucose that "there will be maintenance of high energy phosphate balance...and it is difficult to accept therefore that changes in Cori cycle activity are a significant cause of the marked body wasting in patients with progressive

neoplasia"[390]. These conflicting but equally well considered viewpoints underscore the great difficulty in accurately determining a long term energy balance in cancer patients and, accordingly, the influence that changes in metabolic efficiency have on that balance. Nonetheless, the accelerated activity of energy wasting cycles are likely to play some role in the development of cancer cachexia.

2.3.(v) *Insulin and Glucose Uptake* Impaired glucose tolerance in patients with leukaemia, lymphoma and a variety of epithelial tumours was described in the 1950's by Marks and Bishop[221] and resistance to both exogenous and endogenous insulin has been subsequently demonstrated in cancer patients[193]. The insulin binding receptors of monocytes extracted from cancer patients are normal, implying that the defect is post-receptor in site[287]. Jasani has reported a decrease in the sensitivity of pancreatic beta cells to insulinogenic stimuli[172], while others have determined that reductions in both peripheral sensitivity and pancreatic release are responsible for the observed glucose intolerance[215]. However, the cause of the glucose intolerance in the setting of malignancy has undergone some critical reappraisal in recent years, and it has been suggested that it may be due to intercurrent factors such as weight loss, bed rest and sepsis rather than to the cancer *per se* [62,158].

For plasma glucose concentration to remain constant, the increase in glucose production observed in some cancer patients must be attended by an equal increase in the rate of clearance of glucose from the plasma compartment. This occurs despite the prevailing state of insulin resistance. Results obtained from animal tumour models have suggested that the tumour acts as a "glucose trap", consuming large quantities of glucose in the process of anaerobic glycolysis[44]. The high tumour-host weight ratio in such models (sometimes exceeding 40%) casts a shadow on their

applicability to patients; human tumours rarely exceed 5% of body weight and therefore only very substantial metabolic changes within the tumour itself would be detectable at the whole body level. However, the glucose trap concept is supported by the demonstration of increased glucose uptake across soft-tissue sarcoma bearing limbs compared to the opposite non-tumour bearing limb[253]. Interestingly, the forearm glucose uptake in patients with oesophageal cancer has been found to be significantly greater than in healthy controls by Burt and colleagues[46]. The plasma insulin levels were lower in the cancer patients so it is unlikely that this hormone mediated the observed changes. The authors speculate that increased non-suppressible insulin like activity (NSILA) may be responsible. NSILA is the likely cause of the hypoglycaemia seen with some nonislet cell tumours in man[130], and is probably elaborated by the tumour itself[175]. The wider role of tumour related NSILA remains a matter of conjecture.

2.3.(vi) *Glucose Oxidation* Although several studies have reported modest increases in the rate of glucose oxidation in cancer patients[141,158-9], the increases are not commensurate with the greater glucose availability which implies a reduction in efficiency of the oxidative process[310]. In skeletal muscle isolated from patients with cancer, the activities of enzymes regulating oxidative metabolism have been found to be reduced[213], which is consistent with data gathered from studies of whole body glucose oxidation. It is likely that in those cancer patients in whom glucose production is occurring at an accelerated rate that the extra glucose production is being consumed in Cori cycling[160].

2.4 Fat Metabolism

2.4.(i) *Introduction* In many cases of cancer cachexia the greater proportion of weight loss is due to depletion of body fat[11,73,351]. Loss of body fat with malignant disease has been confirmed by a variety of anthropometric techniques[6,69,210] and muscle biopsy samples from patients with cancer have been found to have only half the amount of fat present in normal controls[74]. Although the consumption of fat reserves in cancer patients is partly a reflection of reduced calorie intake, several changes in lipid metabolism have been described which probably result from cancer bearing itself and these will be discussed in the following paragraphs. Fat metabolism in cancer patients has been the subject of far less research effort than carbohydrate metabolism, and correspondingly fewer conclusions can be drawn.

2.4.(ii) *Fat Mobilization* Triglyceride in adipocytes, which represents the major storage form of fat, is mobilized by hydrolysis to glycerol and free fatty acids which are released into the plasma. Using stable isotopic tracers Shaw and Wolfe[310] have measured the turnover rates of glycerol and free fatty acids in weight stable and weight losing patients with gastrointestinal malignancies and compared these to normal volunteers (fig 2.1). There were no significant differences in whole body glycerol and fatty acid kinetics between the weight stable patients and the normal volunteers, however those with weight loss had significantly elevated rates of release into the plasma of both glycerol and free fatty acids. These data, which are in agreement with the work of others[95,196], suggest that the loss of fat reserves seen in patients with cancer cachexia is due to increased fat mobilization rather than decreased synthesis. However, definitve studies of the influence of cancer on lipogenesis inhuman subjects have not been

performed, so it is possible that both mechanisms are operating to reduce body fat stores.

2.4.(iii) *Lipid Clearance* Lipoprotein lipase is the enzyme responsible for the clearance of triglyceride molecules from the plasma. Although hyperlipidaemia is not a marked finding in cancer patients, it has been found in association with some tumours[88] and the proposed mechanism is a reduction in activity of this enzyme. Support for this hypothesis has recently been provided by Vlassara and colleagues who found that the plasma lipoprotein activity in a group of cancer patients was reduced and that there was a correlation between weight loss and the extent of reduction of enzyme activity[344]. The decreased lipoprotein lipase activity which occurs in uncomplicated starvation is mediated by a reduction in the plasma level of insulin, however the insulin levels in the patients in Vlassara's study were normal suggesting that this was not the mechanism resposible for the observed changes.

2.4.(iv) *Fat Oxidation* There a considerable body of data which suggests that fat is oxidized at an increased rate in cancer patients[6,75,93,196,354], although as is common in studies involving small numbers of patients with heterogenous conditions this finding is not universal[355]. Fat oxidation rates determined in a series of 70 patients with colorectal or gastric cancer by a combination of indirect calorimetry and urinary nitrogen excretion have been recently reported by Hansell and colleagues[141]. They found that the patients with cancer had significantly higher fat oxidation rates (and significantly lower carbohydrate oxidation rates) than control patients with benign disease. Patients with cancer and weight loss oxidized fat more rapidly than either patients with cancer and no weight loss or patients with weight loss caused by benign disease. Similarly, patients with

hepatic metastases had a significantly greater fat oxidation rate than patients with localized malignant disease. Others have reported that in cancer patients a greater percentage of the body's energy requirements are provided by fat than in normal volunteers, and that fat is mobilized and oxidized with at least the same efficiency as in health[196]. It is unlikely that malignant tissue *per se* is responsible for the the increased fat oxidation, but rather that the changes which are induced in the regulation of metabolic pathways occurring in normal host tissues in the cancer bearing state favour fat oxidation.

2.5 Protein Metabolism

2.5.(i) *Introduction* Loss of body protein in patients with cancer cachexia is manifest clinically as skeletal muscle atrophy and hypoalbuminaemia, and is associated with an impaired tolerance of treatment procedures[231]. Significant protein loss may occur in patients who are maintaining what would be in health an adequate intake of nitrogen and calories, implying that tumour bearing *per se* is able to exert a detrimental influence on whole body nitrogen balance. However, a negative nitrogen balance is not an inevitable accompaniment of malignancy. Nearly thirty years ago, Watkin measured nitrogen balance in a large group of cancer patients and found a range of responses from positive to very negative balances, and he insightfully related the more negative nitrogen balances with increased disease 'activity' (reflecting weight loss, increased resting energy expenditure and other factors)[360]. This concept concurs with our own observations, in which patients with aggressive metastatic disease (unpublished observations) or those with histological types frequently associated with a poor prognosis (eg sarcoma[300]) tend to lose protein significantly more rapidly than those with less aggressive disease.

2.5.(ii) *Whole Body Protein Kinetics* The rate of whole body protein turnover can be measured using isotopically labelled amino acids as metabolic tracers, and a number of such studies in cancer patients have produced a spectrum of results. A consistent 50 to 70% increase in turnover rates in large groups of patients with lung and colorectal cancer has been reported[100] and there have been similar findings in patients with small cell cancer[147] and in children with leukaemia[180-1]. Norton and coworkers found an inconsistent response in a diverse group of cancer patients[254], whereas others have found no difference between patients with cancer and age-matched normal controls[148]. Several investigators have suggested that whole body protein turnover is increased with advancing stage of disease and weight loss[56,94,147,165].

The accelerated protein turnover seen in many cancer patients contrasts with the reduction in total protein turnover observed in cases of simple starvation[280]. Recently, in order to distinguish the metabolic effects of pure malnutrition from those of cancer bearing, Jeevanandam and colleagues compared the protein kinetics of malnourished cancer patients with patients who were equally malnourished as a result of benign disease and with a group of starved normal subjects[173]. Compared to the non-cancer patients and starved normals, whole body protein turnover in the cancer patients was elevated 32 and 35% respectively. These results confirm the observations made by Brennan nearly a decade earlier that in cancer cachexia there is a maladaptation to the starved state, with a continued mobilization of protein and calorie reserves in the face of a reduced intake[41]. An example of this is the decreased efficiency with which simple substrates limit the rate of gluconeogenesis and protein flux in patients with advanced cancer[353-4,357].

Protein turnover is an energy expensive process which accounts for10 to 20% of basal metabolic expenditure[269]. The reduction in protein turnover seen in simple starvation accordingly represents an adaptive response[358], and it has been suggested that its failure to occur in some cases of malignancy is responsible for the development of cancer cachexia[45]. This hypothesis has recently been examined in substantial groups of cancer patients and normal controls[100], and although the cancer patients had a significantly higher rate of protein turnover, their resting metabolic expenditure was not increased nor was there any correlation between individual rates of protein turnover and energy expenditure. These results suggest that when protein turnover is increased in cancer patients, it is unlikely to play a major role in the development of cancer cachexia.

The influence of the cancer *per se* on whole body protein metabolism has been a matter of some conjecture. The concept of the tumour of a 'nitrogen trap' which parasitizes amino acids from healthy tissues was developed by researchers working with rapidly growing transplantable animal tumour models[209,272], but it is unlikely to be applicable to patients in whom tumour bulk is usually a much smaller percentage of body weight. Rather, it is more plausible that the tumour is releasing a humoral agent or agents which effect the observed metabolic changes. Glass and colleagues attempted to quantify the influence of tumour bearing on protein dynamics by studying a group of patients with colorectal carcinomas just before and 12 weeks after resection[124]. They were unable to demonstrate a significant difference in whole body protein metabolism after tumour excision, and concluded that the primary tumour does not alter protein kinetics. However, the study group was comprised of patients with localized lesions whose nitrogen flux was comparable to normal controls, and so it perhaps not surprising that tumour excision caused no change.

2.5.(iii) *Skeletal Muscle Metabolism* Whole body protein turnover studies reflect the sum total of synthesis and degradation rates in the individual tissues. Accordingly, it is possible for synthesis and/or catabolism to be reduced in one particular tissue while whole body turnover is increased[359]. Several investigators have attempted to determine the manner in which the protein kinetics of individual tissues are affected by cancer. Lundholm and colleagues used the rate of incorporation of ^{14}C leucine by skeletal muscle biopsies incubated *in vitro* to compare synthesis rates in a heterogeneous group of 43 cancer patients with 55 age and sex matched controls[212]. They found that the capacity of the muscle fibres removed from the cancer patients to incorporate the amino acid tracer was significantly impaired, and that having been incorporated the rate of loss of tracer was also greater in the cancer patients. The group concluded that malignant tumours provoke a decrease in protein synthesis and an increase in protein degradation. In a subsequent series of experiments the same group used arteriovenous differences of 3-methylhistidine, an amino acid which is relatively specific to skeletal muscle, and found that there was no significant difference in the rate of appearance of this marker in patients with cancer and controls who were depleted with benign disease[211]. They concluded that the effect of malignant tissue was to reduce the rate of protein synthesis. As skeletal muscle comprises the majority of the body's protein, changes in skeletal muscle protein protein kinetics are subsequently likely to be manifest at the whole body level. The results of Lundholm's group are therefore at odds with a substantial body of whole body kinetic data which suggests that whole body protein synthesis is either unchanged or increased[56,94,100,147,180]. Recently, Shaw and colleagues have determined *in vivo* fractional synthetic rates (FSR) of muscle in patients with benign disease, weight stable patients with malignant disease and patients with cancer cachexia[165]. There were no significant differences in

the rate of muscle FSR between patients with benign disease and weight stable cancer patients, but there was a significant increase in FSR in those patients with cancer cachexia. Given that these patients had lost weight (and presumably protein) this implies the occurrence of an even greater increase in the rate of degradation of muscle protein, and indeed increased activities of lysozymal enzymes isolated from skeletal muscle of cancer patients have been reported[212-3].

2.5.(iv) *Hepatic Protein Synthesis* There is a paucity of data on the rate of hepatic protein synthesis and catabolism in cancer patients. Using the same *in vitro* methodology employed in their study of skeletal muscle metabolism, Lundholm and colleagues have reported an increase in the rate of protein synthesis in liver biopsies from cancer patients[213]. These results are consistent with those from our own laboratory, in which we have demonstrated *in vivo* a significant increase in the fractional synthetic rate of protein of hepatic tissue in patients with cancer cachexia, but no difference between the hepatic FSR of weight stable cancer patients and patients with benign disease[165]. There are no data describing the rate of catabolism of structural hepatic proteins in cancer bearing man, however a recent report in which organ imaging techniques were used to determine liver size in a small number of patients with cancer cachexia suggested that there was relative sparing of visceral protein[149] which implies that the observed increase in hepatic protein synthesis is likely to be attended by an equal increase in protein catabolism.

Many patients with advanced malignancy are hypoalbuminaemic, which may result from a reduced rate of synthesis, an increased rate of breakdown or a loss of albumin from the intravascular volume. As albumin degradation rates have been demonstrated to be normal in cancer patients[319,346] and with the exception of cases of malignant effusion the

distribution of albumin is relatively unchanged, this implies that the rate of synthesis is decreased. However this is contrary to some recent data from our laboratory in which we measured the rate of synthesis of albumin using a ^{14}C leucine marker[166]. A significantly higher rate of albumin synthesis was found in those patients with cancer cachexia compared with those cancer patients who were weight stable, and to patients with benign disease. It is clear that the influence of malignancy on hepatic structural and secretory protein synthesis is yet to be clearly resolved.

2.6 Treatment of Cancer Cachexia

2.6.(i) *Nutritional Support* Following the introduction of safe total intravenous nutrition techniques nearly twenty years ago, it was hoped that the great majority of cachectic cancer patients could be repleted prior to surgical, radio or chemotherapy and that a reduction in treatment morbidity would be effected. The enthusiasm of the initial reports describing the efficacy of IVN in cancer patients[71] has not always been reaffirmed by more recent appraisal[41]. It has become clear that providing sufficient nitrogen and calories to a patient with cancer cachexia does not augment lean body mass as efficiently as can be achieved in a malnourished patient with benign disease. The issue of using IVN in cancer patients raises several questions, such as whether hyperalimentation promotes growth in malignant tissue, how the IVN prescription can be tailored to ameliorate the metabolic defects associated with cancer, and whether the provision of IVN lead to an improved patient outcome.

The concern of clinicians that the protein and energy substrates provided by IVN will be consumed preferentially by the tumour have some support from the results of experiments performed with animal tumour models, in which tumour growth is encouraged more than repletion of host tissues[53,115,133]. However, to date, stimulation of tumour growth by

IVN has not been observed in patients[282]. The most elegant evidence that IVN does not have a deleterious effect has been provided by Mullen and his colleagues who used the *in vivo* rate of incorporation of ^{15}N glycine as a measure of protein synthesis[246]. They found that the tumours of study patients who were given IVN for 7 to 10 days prior to surgery were synthesizing protein no more rapidly than the tumours of the control patients who were on an *ad libitum* oral diet. Although some caution must be exercised in the interpretation of these results as a net increase in tumour size may have resulted from a reduction in the rate of protein catabolism, it is most likely that malignant tissue is synthesizing protein at a maximal rate and that its rate of growth cannot be significantly affected by the provision of extra nutrients[192].

It is generally agreed that approximately 130% of the RME need be provided to cancer patients[86], but there are few data which clearly indicate which is the optimal caloric source. Despite some cancer patients having marked changes in intermediary metabolism, they have been demonstrated to be able to oxidize efficiently both infused glucose and fat[199]. There is some evidence from a laboratory tumour model that the provision of calories as fat retards tumour growth[51], but this has not been duplicated in other animal models[138] nor is there evidence from human studies to support these findings. Holdaway and Shaw have demonstrated that infusion of isocaloric volumes of glucose and fat (administered as *Intralipid 20*, KabiVitrum Laboratories, Stockholm) have an equal ability to spare protein at the whole body level, although lipid infusion fails to suppress endogenous glucose production[298]. Infused glucose is able to suppress gluconeogenesis in cancer patients[356], but it does not do so with the same efficiency as in healthy volunteers[311].

Although it is possible to restore the weight of a cachectic cancer patient with parenteral nutrition, it has been questioned whether the

weight gain is primarily as fat[252] or whether it represents a useful replenishment of the lean body mass. There are several reports of positive nitrogen balances being achieved cancer patients on IVN[37,47], but these studies require meticulous sample collection and may be difficult to interpret in the presence of a growing tumour. However, longitudinal studies of body composition over a one month course of IVN have shown no increase in total body nitrogen, despite increases in body fat and total body potassium[315]. These data are compatible with isotopic studies which have demonstrated an attainment of nitrogen equilibrium with IVN, but not the protein anabolism which is readily achievable in patients depleted by benign disease[296] (fig 2.2). There is evidence which suggests that leucine is central in the regulation of protein metabolism[230] and leucine enriched IVN has been provided to cachectic cancer patients in an attempt to improve the nitrogen balance[167,191], with a small improvement in nitogen balance being demonstrated.

It is likely that the difficulty in achieving restoration of lean body mass with IVN in cachectic cancer patients is partly responsible for the paucity of convincing evidence of its therapeutic efficacy[43]. Prospective randomized trials of less than one week of IVN have failed to demonstrate any advantage to the study group over the control group which fed *ad libitum*[162,239]. A recently published analysis of the pooled results of 18 randomized trials assessing the effectiveness of perioperative IVN (16 of which were comprised of cancer patients) concluded that there was little evidence supporting the routine use of perioperative IVN, but that it may have a role in supporting a subgroup of patients who are at high risk[87].

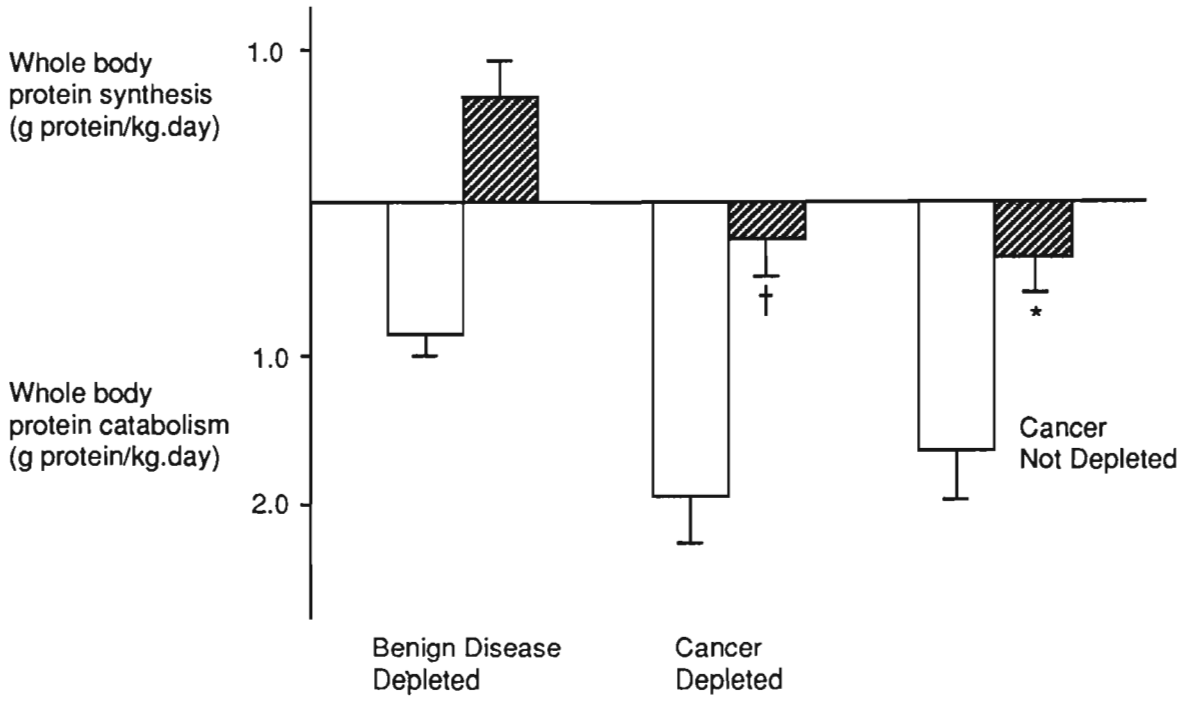


Fig 2.2 *The response to IVN* (▨) *of depleted patients with benign disease, and cancer patients with and without depletion compared with basal values* (□)

(* $p < 0.01$, † $p < 0.05$). *Modified from Shaw, 1988 (296).*

The authors of this paper were generally critical of the methodology of the trials which have been performed to date, and comment that the effectiveness of IVN may have been underestimated by inclusion of patients who were not malnourished. Certainly there are some trials which have demonstrated advantages to the patients who received IVN: Heatley and colleagues followed the postoperative course of 70 patients with gastric cancer who were randomized to receive either IVN for seven to ten days or a normal diet, and reported a significant reduction in the occurence of wound infection in the group who received IVN[145]. A preoperative course of IVN of a similar length in a group of patients with gastrointestinal malignancies reduced the incidence of complications from 19% in the control group to 11% in the treatment group, and the mortality from 11% to 3%[247]. However, any advantage attributed to IVN must be weighed against the risks of pneumothorax and catheter related septicaemia.

2.6.(ii) *Pharmacological Manipulation* As the provision of adequate calories and nitrogen does not ensure protein accretion in cachectic cancer patients, there have been several attempts to counter the adverse tumour associated metabolic changes by the administration of pharmacological agents. An example is a trial involving 101 intensively pretreated cancer patients who were randomly assigned to receive either hydrazine sulphate, an agent which inhibits a key enzyme in the gluconeogenic pathway, or a placebo[61]. The treatment group experienced significantly improved weight stabilization and glucose tolerance. Megasterol acetate, an anabolic steroid, has been recently reported to have enhanced appetite and increased weight in a group of 28 patients with breast cancer[330]. Nearly a decade ago Schein and colleagues argued lucidly that many of the cancer related metabolic derangements were a result of insulin resistance and suggested that many of these could be ameliorated by the provision of exogenous insulin[287]. This

proposal has been supported by the results obtained from animal model experimentation[60,241,245] but to date no human studies have been published. The potential role of recombinant human growth hormone in the preoperative replenishment of cancer patients is discussed in greater detail in Chapter 5.

Although these and other trials involving anticachectic agents have shed some light on the mechanisms of cancer cachexia, no agent has yet been demonstrated to meaningfully improve the clinical course of cancer patients.

2.7 Mediators of the Metabolic Response to Cancer

2.7.(i) *Cancer as a Parasite* It was long held that the metabolic changes observed in cancer patients at the whole body level were a reflection of the metabolic activity of the malignant tissue *per se*. From this supposition was born the concept of the tumour acting as an internal parasite, trapping nitrogen and energy substrates as the host tissues became progressively more malnourished[103]. Despite the alluring simplicity of this hypothesis it fails to account for the profound metabolic changes which have been documented in some patients with apparently trivial tumour burdens[244], nor does it explain the changes in metabolism detected in host tissue distant from the tumour site[46].

2.7.(ii) *Role of Humoral Mediators* An alternative theory advanced to explain these observations is that tumours release small molecular weight proteins which alter the activities of various host enzymes[332] (fig 2.3). A large number of polypeptides and other substances secreted by tumour cells have been described, such as toxohormone[248] and lipid mobilizing factor[197], to which have been attributed various roles in the

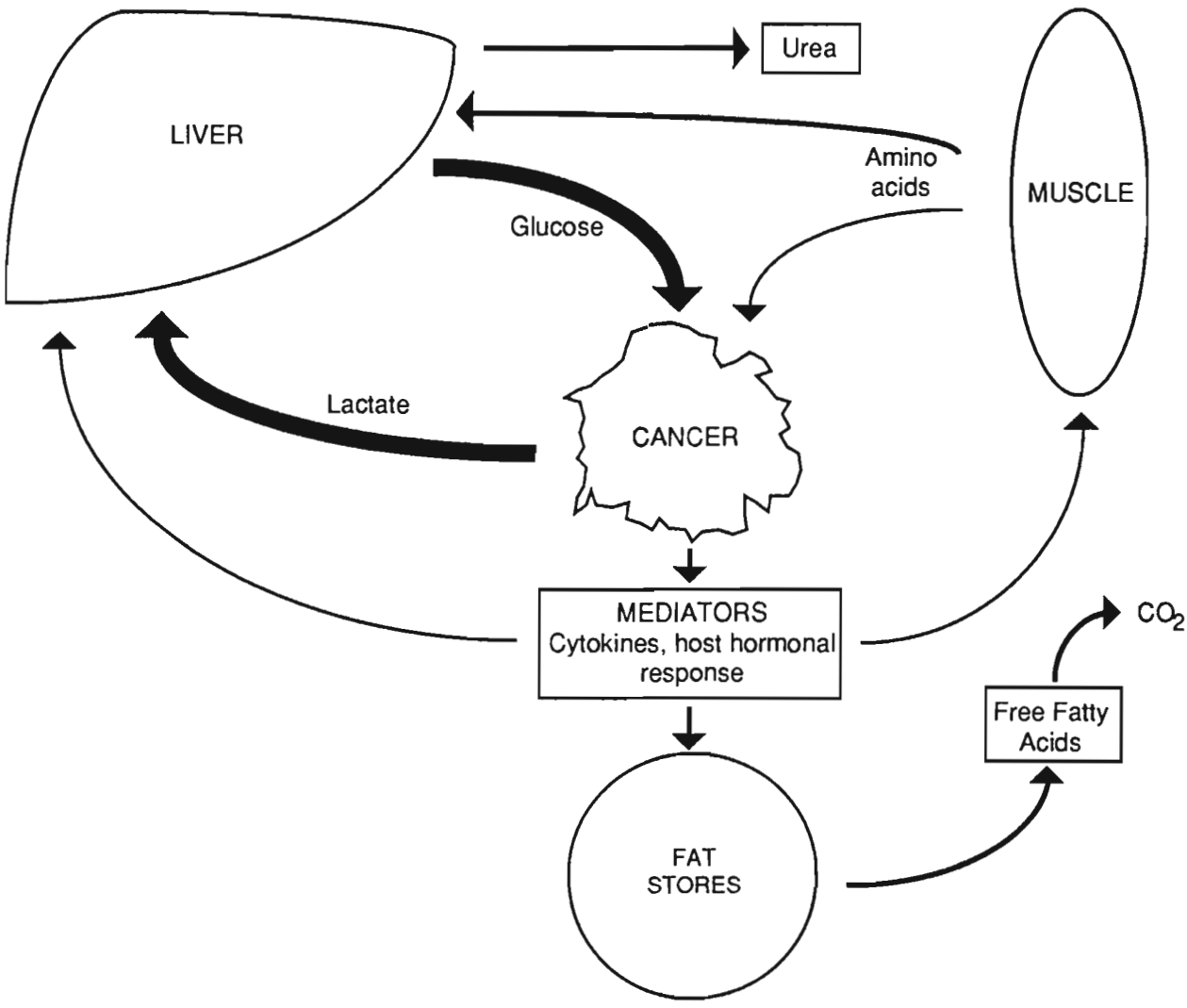


Figure 2.3 *Proposed overview of the metabolic changes associated with advanced cancer.*

causation of cancer cachexia largely on the basis of animal experiments. However, there is little evidence which convincingly relates these substances to the metabolic changes seen in cancer patients

2.7.(iii) *Similarities with Sepsis and Trauma* The similarities between the metabolic responses to sepsis and trauma to that provoked by tumour bearing have been clearly described by Brennan[41]. The loss of nitrogen and increased turnover of glucose and mobilization of fat which occur in severely septic or injured patients result from the combined influences of counter-regulatory hormone secretion and the release of inflammatory mediators from cells of the immune system (Chapter 1). It has been suggested that similar mediators released by immunocytes in response to tumour cells are responsible for the metabolic response to cancer. Cachectin, a 17 kilodalton polypeptide released by macrophages which acts as a mediator of endotoxic shock[28], has been found to share strong sequence homology with tumour necrosis factor (TNF)[26], also a macrophage product. It may be that cachectin/TNF is central to the mediation of the metabolic response to both sepsis and cancer[25]. Early studies were unable to detect measurable levels of TNF in the plasma of cancer patients, which may well have reflected the lack of a sufficiently sensitive assay. Recently, using a radioimmunoassay sensitive to 1ηg/l, a significantly higher level of plasma TNF has been demonstrated in patients with cancer cachexia when compared to patients with cancer but without weight loss (J. McCall, personal communication). Wilmore and coworkers have reported a negative nitrogen balance in cancer patients infused over a five day period with recombinant TNF, which they attributed to the anorexia induced by the TNF rather than to a cytokine specific effect on protein metabolism140. Kern and Norton have proposed a mechanism explaining the metabolic derangements of cancer cachexia in which the tumour stimulates the host's

immune cells to secrete factors such as cachectin/TNF whose primary role is cytotoxic, but which have secondary metabolic effects[178].

2.8 Summary

Malignant tumours do not have a consistent effect on the intermediary metabolism of the host. However, patients with advanced disease and/or those demonstrating cancer related cachexia typically have accelerated rates of energy substrate and protein turnover despite reduced calorie and nitrogen intake. In this manner the metabolic response to cancer cachexia is opposite to that seen in uncomplicated starvation, but rather bears many similarities to the changes described in patients with sepsis and trauma. The rates of gluconeogensis and Cori cycling, fat mobilization and oxidation and protein synthesis and degradation tend to be increased, and there is greater difficulty in replenishing lean body mass with methods of nutritional support (table 2.1). The metabolic response to cancer bearing may be largely effected by mediators released by cells of the immune system, but this matter remains conjectural. Beyond the provision of adequate calories and nitrogen and removal of malignant tissue, there are presently no metabolic therapies available which have been demonstrated to influence clinical outcome.

Table 2.1 *Metabolic change commonly associated with advanced or weight-losing cancer, severe sepsis or multiple trauma, and depletion due to benign disease (or in some cases simple starvation in normal volunteers)*

	Cancer	Sepsis/Trauma	Starvation	Reference
Carbohydrates				
Gluconeogenesis	↑	↑	↓	*158, 169, 214, 308,3 10, 313*
Glucose recycling	↑	↑	-	*93, 157, 300, 308, 313*
Insulin resistance	↑	↑	-	*32, 193, 215*
Fat				
Lipolysis	↑	↑	↑	*52, 111, 196, 311*
Fat oxdidation	↑	↑	↑	*6, 52, 141,196, 322*
Protein				
Whole body flux	↑	↑	↓	*30. 100, 269, 308, 313*
Net catabolism (NPC)	↑	↑	↑	*300, 308, 311, 313*
Responsiveness of NPC to parenteral nutrition	↓	↓	↑	*37, 296*
Energy				
Resting metabolic expenditure	↓/N/↑	↑	↓	*38, 85, 122, 131, 185, 217, 203, 217, 322*

Chapter 3

Methodology

3.1 The *In Vivo* Study of Metabolism

3.1.(i) *Introduction*

Given the complexity of the interrelationship between the disposal of metabolic substrates and the hormonal environment, the performance of dynamic *in vivo* measurements of the rates of substrate turnover is a difficult undertaking. The great advantage of *in vivo* investigations is that the homeostatic responses to the experimental perturbation are not disrupted, providing a considerable advance over *in vitro* methods in which the applicability of results to the intact animal is always open to question. However, when performing *in vivo* research the variables are myriad and control of each is impossible. Such difficulties are compounded when studies are performed in severely ill surgical patients.

The methodological difficulties of performing such studies are twofold:

1. The practical difficulty of obtaining informed consent and performing studies on patients whose clinical condition and number of invasive procedures to which they have already been subjected often lessens their enthusiasm for further tests, and

2. The difficulty of objectively grouping together patients with different conditions of differing severity, in order that valid conclusions applicable to the entire group can be made.

In this chapter, some of the methods available to measure metabolic parameters will be briefly discussed, and the principles and calculations involved in isotopic tracer techniques described. The method of classifying

severely ill septic patients will be presented and the experimental lamb model both in the basal state and during rhTNF infusion described.

3.1.(ii) *Approaches to In Vivo Metabolic Studies*

(a) Plasma Concentration. The simple measurement of the plasma concentration of substrates provides no information of the individual fluxes from which the concentration results. For example, an increased plasma concentration measured after the administration of a dose of growth hormone may be caused by a reduction in the rate of peripheral uptake, an increase glucose release, or from a combination of both these processes. Similarly, the plasma concentration may remain unchanged if the rates of substrate appearance and clearance are simultaneously increased or decreased by equal amounts.

(b) Glucose Tolerance Test. Because of the limited information afforded by simple measurement of plasma substrate concentration various techniques have been developed in order to facilitate dynamic measurements of substrate turnovers from plasma substrate concentrations. The most commonly applied of these methods is the glucose tolerance test in which a bolus of glucose is administered orally or intravenously. The interpretation of this test assumes that the administered glucose is rapidly and uniformly mixed in a single space, that all of the clearance of glucose is due to tissue uptake, and that the administration of glucose does not influence basal glucose kinetics. However, these assumptions have been determined to be invalid on theoretical and experimental grounds[4,20].

(c) Arteriovenous Differences. The measurement of arteriovenous differences has also been employed in the study of metabolic regulation. The difference in substrate concentration is multiplied by the flow rate so

that the net balance of uptake of release can be determined. The difficulty in accurately measuring the flow rate in a particular organ or limb (the error of such measurements has been estimated at 20%) presents a practical difficulty[5], as does the often very small arteriovenous difference in concentration. A theoretical limitation of the method is that it provides no information on the substrate fluxes across the tissues but only on the net balance of the fluxes.

(d) Isotopic Tracer Methodology The use of isotopic tracers to study substrate metabolism dates to the 1930's when Schoenheimer and Rittenberger used deuterium and ^{15}N labelled tracers to study fat and protein turnovers in mice[288-9]. Subsequently, isotopic tracer methodology has been developed and refined so that it is now possible to use either stable or radioisotopes to measure the rates of turnover, clearance and oxidation of the important metabolic substrates. Studies employing this methodology is particularly suited to the clinical setting because they can easily be performed at the patient's bedside over a few hours, and are safe and easily accepted by the patient.

Although the first isotopic experiments in the 1930's were performed with stable isotopes, the subsequent development of scintillation counters enabled the use of radioactive tracers in metabolic studies. The principles behind the use of stable and radio-isotopes are identical, although there exist differences in terminology and application. The ratio of labelled to unlabelled substrate is known as the percentage enrichment (or atom percent excess) when using stable isotopes. For example, an atom percent excess of 2% for ^{15}N urea implies that 2% of the nitrogen in the urea sample exists as the ^{15}N isotope. As there is a significant amount of ^{15}N urea present in the normal state, it is necessary to take plasma specimens prior to the infusion so that the baseline level of enrichment can be quantified.

This is not generally necessary in studies involving radio-isotopes in which it is assumed that the background activity is negligible. For radio-isotopes, the ratio of labelled to unlabelled molecules is described in terms of specific activity, which is a measure of the decays per minutes detected from each mmol of substrate. Radio-isotopes offer advantages over stable isotopes because they are generally less expensive and their laboratory analysis is more easily performed. However, with a rising awareness of the dangers posed by radioactivity and with the development of quadrupole mass spectrometers interfaced to gas chromatographs, there has been a resurgence of the use of stable isotopes in the last two decades.

There are two approaches to the use of isotopic tracers: they may be given either as an intravenous bolus, or as a constant infusion which may or may not be accompanied by a priming dose. Both approaches use the same fundamental principle that the rate of turnover of a substrate will be reflected by the rate of dilution of the infused tracer. Such studies assume that the isotopically labelled tracer will not be discriminated from the unlabelled substrate. Although some enzymes have been reported to selectively fractionate the isotopic tracer from the unlabelled substrate [188,316] these errors are small relative to the errors in involved in the *in vivo* measurement of substrate turnover by isotopic methodology[375].

(e) Bolus Technique This technique involves the intravenous administration of a bolus of tracer followed by the repeated taking of blood specimens at specific times. At each time, the ratio of labelled to unlabelled substrate is determined. This ratio is termed the specific activity for radioactive tracers or the degree of enrichment (atom percent excess) for stable isotopic tracers. In this manner, a curve which descibes the disappearance of the label is constructed. By determining the slope of the curve and the specific activity or the atom percent excess at $t = 0$ it is

possible to calculate the rate of turnover of the substrate. However, the decay curve is rarely described by a single expotential function. The distribution of the tracer into several different compartments or pools has been proposed as a model to account for the changing rate of decay. However, such mathematically derived pools may not describe real physiological events or entities, and there is no basis for deciding which is the appropriate slope to extrapolate to zero order in order to determine pool size. The considerable errors which have been been measured in experimental attempts to validate this approach may be reduced by performing an integral analysis of the area under the curve[4]. Despite such refinements, the bolus technique can rarely offer advantages over constant infusion methods[375].

(f) Constant Infusion Methods This technique involves the isotopically labelled tracer at a constant rate. The labelled substrate will be lost from the extracellular volume (ECF) at a rate proportional to its abundance relative to unlabelled substrate. Initially, when the concentration of the labelled tracer is low, it will leave the ECF at a rate lower than that at which it appears. Accordingly, there will be a relative rise in concentration of labelled substrate and, *pari passu*, an increase in the rate of labelled substrate leaving the ECF. Eventually, the isotope will be lost at the same rate at which it appears, and there will be no further change in the relative concentrations of labelled and unlabelled substrate while the infusion remains constant. This situation is known as isotopic equilibrium. At isotopic equilibium, the concentration of isotope and unlabelled substrate in the ECF will equal their respective concentrations in the infusate. The rate of appearance of unlabelled substrate into the ECF can be derived if the isotope infusion rate and the relative abundance of labelled to unlabelled substrate (atom percent excess or specific activity) are

known. The equations will be described in Section 3.2, although their derivation is beyond the scope of this discussion. Representative examples of specific activities of 6 ^{3}H glucose and ^{14}C leucine and ^{15}N urea atom percent excess at isotopic plateau in saline infused lambs are illustrated in figures 3.1 a&b.

Several hours may be required for isotopic equilibrium to be reached if the pool size is large relative to the rate of turnover. Attainment of isotopic equlibrium can be greatly hastened by the administration of an appropriately chosen priming dose of isotope. The priming dose is chosen so that it will almost instantaneously label the entire pool to the level of enrichment or specific activity seen at equlibrium. The required priming dose to rate of infusion ratio can be calculated by dividing the pool size of the substrate by the substrate's rate of appearance[375].

This principle may be extended to priming of the bicarbonate pool in order that measurement of the rate oxidation of a substrate by infusion of carbon-labelled isotope of the substrate may be performed[3]. In order for this to be accomplished, it is necessary to measure the specific activity of $^{14}CO_2$ in expired air, the volume rate of production of CO_2, the specific activity of the labelled substrate in the plasma and a correction factor, k, which accounts for the fact that not all of the $^{14}CO_2$ excreted at the cellular level is expired, but rather a certain percentage (0 - 25%, depending on the species) is retained in the body (see Equation 3.2.(ii)).

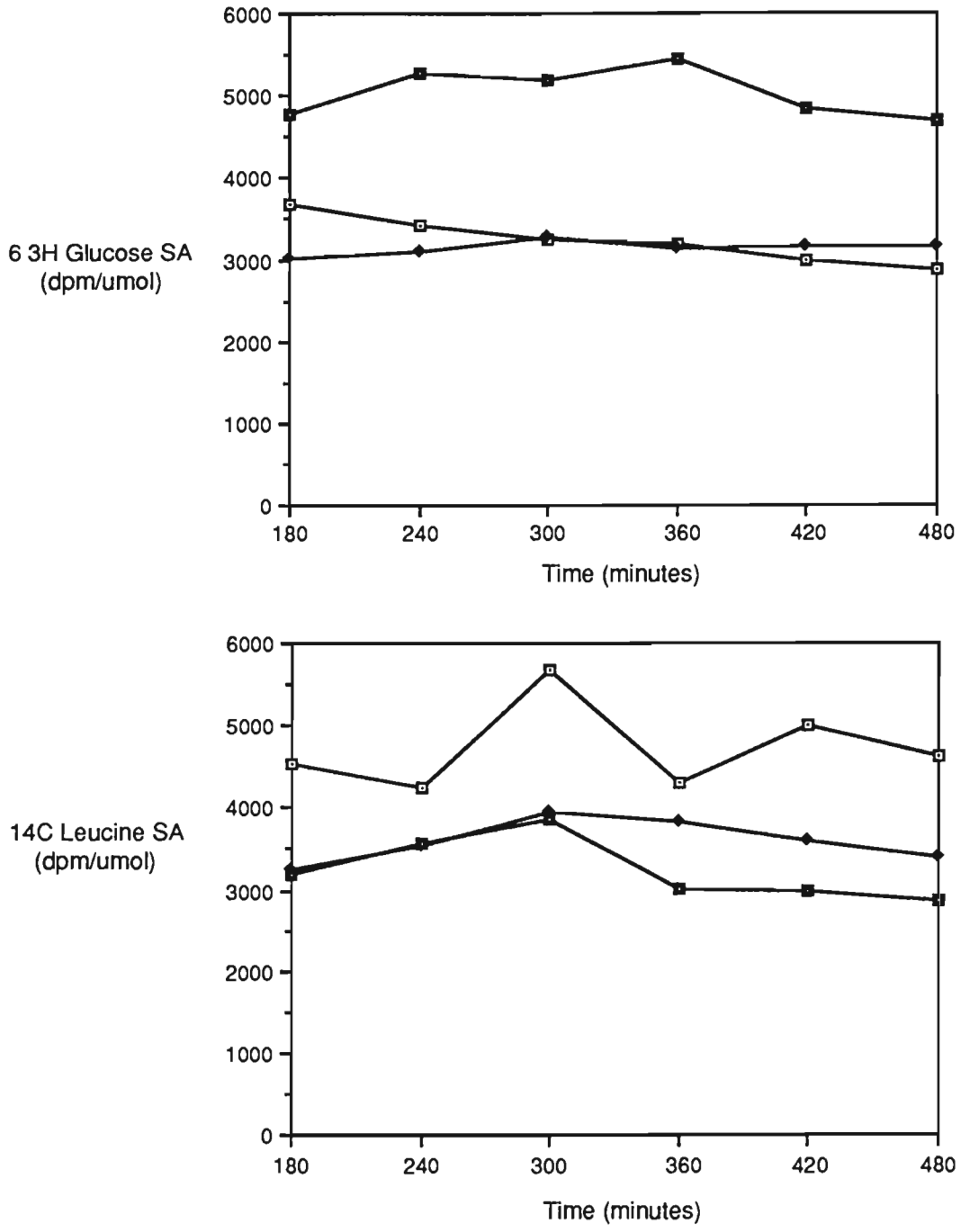


Figure 3.1a *Representative examples of 6 3H glucose and 14C leucine specific activities measured over the course of 300 minute normal saline infusions in 48 hour fasted lambs. The isotopes were infused for 180 minutes prior to specimens being collected to enable isotopic equilibrium to be reached.*

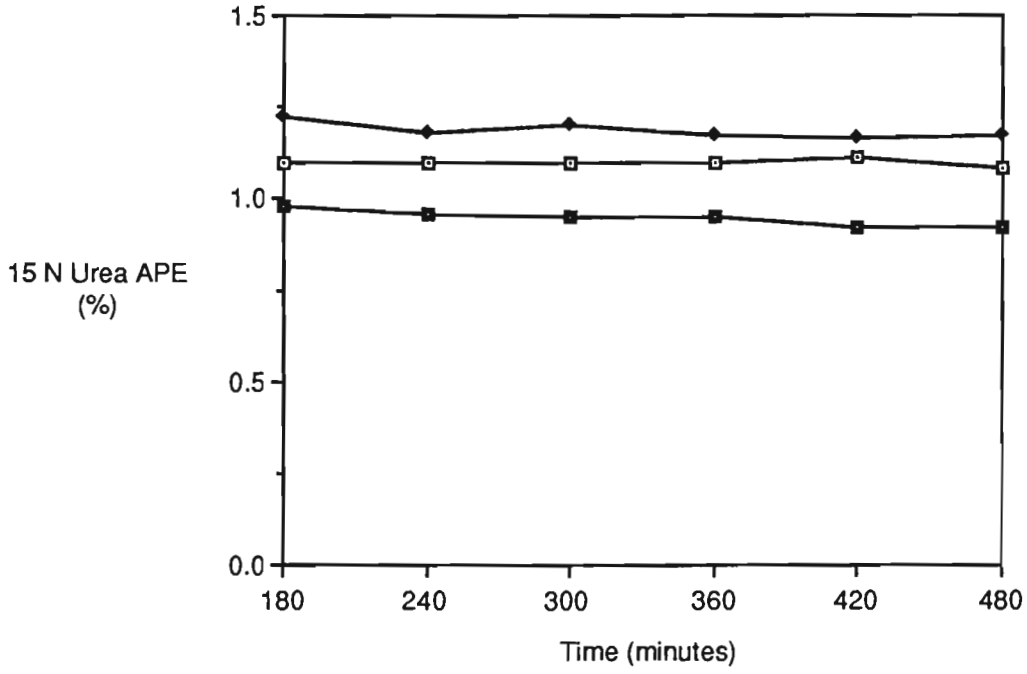


Fig 3.1b *Representative examples of 15N urea atom percent excess measured over the course of 300 minute normal saline infusions in 48 hour fasted lambs.*

3.2 Calculations

3.2.(i) *Rate of Appearance of Substrate*

a) Stable Isotopes

$$Ra = \left(\frac{\%\text{Isotopic Enrichment Infusate}}{APE} - 1\right) \times f$$

Ra = Rate of appearance (μmol/kg.min)

APE = Atom percent excess

f = Rate of isotope infusion (μmol/kg.min)

b) Radio-isotopes

$$Ra = \frac{f}{SA}$$

f = Rate of isotope infusion (DPM/kg.min)

SA = Specific activity (DPM/μmol)

3.2.(ii) *Substrate Oxidation*

$$ROx = \frac{SA^{14}_{CO_2} \times VCO_2}{SA_{substrate} \times k}$$

ROx = Rate of substrate oxidation (μmol/kg.min)

VCO_2 = Rate of ventilation of CO_2 (μmol/kg.min)

k = Bicarbonate recovery correction factor[307]

3.2.(iii) *Protein Fractional Synthetic Rate*

$$FSR = \frac{\left(\frac{\Delta SA_{tissue}}{\Delta t}\right)}{SA_{plasma}} \times 100$$

FSR = Fractional synthetic rate (%/day)

ΔSA_{tissue} = Change in ^{14}C leucine SA in tissue specimen

Δt = Time between between priming dose and taking of specimen

SA_{plasma} = Mean SA leucine in plasma over time of infusion

3.2.(iv) *Plasma Glucose Clearance*

$$\text{Plasma clearance (ml/kg.min)} = \frac{Ra_{Glucose}}{\text{Plasma glucose concentration}}$$

3.2.(v) *Net Protein Loss from Urea Ra*

NPL = Protein Intake - (Ra urea x 2 μmol N/μmol urea x 14 μg N/μmol N x 6.25 μg protein/μg N ÷ 1,000,000 g/μg x 60 min/hr x 24 hr/day) [375]

NPL = Net protein loss (g protein/kg.day)

3.2.(vi) *Frayn's Equations* [108]

a) Carbohydrate Oxidation = $(4.55\ VCO_2 - 3.21\ VCO_2 - 2.87n)1440$

b) Fat Oxidation = $(1.67\ VO_2 - 1.67\ VCO_2 - 1.92n)1440$

Carbohydrate and fat oxidation (g/day)

VCO_2 = Carbon dioxide production (l/min)

VO_2 = Oxygen consumption (l/min)

n = Nitrogen production (g/min)

3.3 Techniques for Patient Studies

3.3.(i) *Introduction* The data presented in this thesis are derived from studies in which the *in vivo* metabolic profile is defined of both surgical patients and an lamb animal model using primed constant infusions of a number of isotopically labelled substrates, often in combination with indirect calorimetry. The baseline metabolic parameters having been defined in each individual, the effects of rhGH or rhIGF administration could then be measured either at a defined time interval following injection or during continuous infusion.

3.3.(ii) *Patient Study Protocols and Techniques* The patients involved in the rhGH studies had been admitted to the General Surgical or Orthopaedic Services of Auckland Public Hospital. The protocols were approved by the Ethics Committee of this institution and signed, informed consent was obtained from the patients on the day prior to the study.

The studies were mostly performed at the patients beside, an arrangement which was found to least disturb the patients and the ward routine. The patients were asked to refrain from eating breakfast if they were on a normal ward diet but were allowed as much water as they required during the baseline study period. The patients receiving parenteral nutrition had all been established on this form of nutritional support for a minimum of three days prior to the performance of the baseline study, and their IVN prescriptions were continued unchanged during the course of the study, except for required alterations in electrolyte

additives. The standard parenteral nutrition regimen is described in Section 4.2.(ii).

A blood sample was taken in order to determine pre-infusion levels of ^{15}N urea enrichment if this isotope was being infused, and the isotopic infusions were begun at 0900 hrs. Various combinations of isotopes were employed in the studies and the priming doses used and validation studies are descibed in table 3.1. The infusions were continued for a three hour period. During the last hour, the patient was wheeled in his or her bed to the metabolic laboratory in the Department of Surgery which is adjacent to the general surgical wards. Once there, they were accustomed to the perspex hood of the calorimeter, and indirect calorimetry was performed using a hood calorimeter with associated Sybron Taylor oxygen analyzer (Hoddeson, UK) and ADC CO_2 analyzer (Hertfordshire, UK) over a period of approximately 20 minutes in a darkened room. Following the performance of indirect calorimetry, multiple samples of expired air were collected in 3 litre anaesthetic bags using a three way valve. The expired breath was then bubbled through 3 ml aliquots of a methylbenzonium/ethanol solution in scintillation vials, each trapping 1 mmol of CO_2. The samples were counted in a beta counter, and in this manner the $^{14}CO_2$ specific activity was determined. At the end of the infusion period, blood samples were taken again and the study was complete. Blood specimens were immediately placed in chilled, sterile heparinized test tubes and centrifuged in order to separate the plasma from the cells. The plasma was then frozen prior to analysis.

Table 3.1 *Isotope Prime:Infusion Ratios and Validation Studies*

Isotope	Prime:Infusate Ratio	Reference
^{14}C, ^{15}N Urea	450:1	374
^{14}C Leucine	60:1	381,293
^{3}H, ^{14}C Glucose	80:1	375,376
^{14}C $NaHCO_3$	Variable	3
^{3}H Glycerol	10:1	375
^{14}C Palmitic Acid	No prime	378

The rhGH administered to the patients was provided by KabiVitrum Laboratories, Stockholm, Sweden. This rhGH is marketed under the brandname *Genotropin* and it is an *E. coli* derived peptide identical to human growth hormone. The biological activity is 3 IU per mg substance and the purity is extremely high. On the morning of administration, the rhGH was dissolved in 1 ml of sterile water and injected subcutaneously.

Following the daily administration of rhGH or a placebo the patients were restudied with isotopic infusions and indirect calorimetry in the manner described above.

3.3.(iii) *Patient Selection* The criterion used for selection of patients to be administered rhGH (study presented in Chapter 4) was sepsis or injury of sufficient magnitude so as to be likely to induce an accelerated rate of protein loss. There have been many attempts to develop a definition of sepsis which reliably relates clinical features to physiological response, although none of these have proved to be entirely satisfactory. Probably the most reliable and easily applicable definition demands the identification, either radiologically or surgically, of a septic focus. If this is present, then it is highly likely that the metabolic response to infection will have been invoked[303], and it is this definition that was employed for selection of septic patients in the study presented in Capter 4. Two patients with severe pancreatitis (Ranson's criteria > 3) were included although neither had true abscess cavities. This was considered justified because it has been previously demonstrated that the metabolic response in patients with pancreatitis is identical to that of patients with severe sepsis, presumably because the diseased pancreatic tissue releases inflammatory mediators in much the same way as the tissues surrounding an abscess[302].

Elebute and Stoner have developed a system for the grading of sepsis which is based on the the local and secondary efects of sepsis, the presence

of pyrexia and laboratory data[96]. The system is relatively simple to apply in the clinical setting, and preliminary data suggests that it correlates well with patient outcome. This quantitative system was applied to the septic patients in the rhGH study in order to determine whether there were any changes in the response to rhGH administration with an increasing severity of sepsis.

The classification of injured patients is considerably more straightforward, as the extent of the injury can most often be clearly defined in anatomical terms and quantified by application of the Injury Severity Score of Baker and colleagues[13]. The metabolic response to injury is not linearly related to the severity of the injury, but rather after a threshold severity is reached (at an I.S.S. of 15), the degree of acceleration of net protein loss does not increase (at least until an I.S.S. of 50 is exceeded)[313].

3.4 Techniques for Animal Studies

3.4.(i) *Animal Selection and Preparation* The animal studies were performed using cryptorchid lambs. A lamb model was chosen to investigate the metabolic effects of rhIGF-I and II infusion because well validated radioimmunoassays existed in the Department of Paediatrics, Auckland School of Medicine for ovine IGF, growth hormone, insulin and prolactin. There exist a number of previous reports in which isotopic methodology has been successfully applied to sheep or lamb models[155]. The lambs were bred solely for research purposes from a crossbred flock at the Ruakura Research Centre, Hamilton, New Zealand. The lambs were of between three and five months of age and 15 to 20 kg in weight, and all had been weaned several weeks prior to delivery to the laboratory.

Upon delivery to the Large Animal Suite at the Auckland School of Medicine, the lambs were housed indoors for a minimum of one week prior to be studied. During this time they were fed *ad libitum* on hay,

cereals and sheep nuts, and they were accustomed to the Pavlov slings in which they were restrained while being studied.

The animals were fasted for 48 hours, and on the morning of the study were weighed and placed in Pavlov slings. The animals were studied in the fasted state for two reasons: i) so that the measured Ra's could be solely attributed to release and uptake of substrate by the body's tissues, and were not complicated by uptake of substrate from the gut, which would in the fed state be both variable and difficult to measure, ii) it was thought that the biological actions of rhIGF-I and II would manifest themselves more clearly in a low plasma insulin environment. There exists some data from experiments performed in starving mice which suggest that the anabolic effects of IGF-I were indeed more pronounced after a period of starvation[259].

The lambs having been placed in the Pavlov slings, a dose of diazepam (0.3 mg/kg) was administered and *Surflo* teflon catheters (Terumo, Japan) inserted into the external jugular veins. A 20 G catheter was inserted anterogradely into one external jugular vein to enable infusion of isotopes and hormones and a 16 G catheter was inserted retrogradely into the contralateral vein to facilitate the taking of blood specimens. The catheters were attached to three way taps via *Portex* manometer tubing (Hythe, United Kingdom) and the isotopes and test solutions were infused through this system by *Vial Medical* pumps (Brezins, France). The individual experimental protocols are described in greater detail in forthcoming chapters, immediately prior to the presentation of results.

3.4.(ii) *Effects of Fasting* In order to characterize the effects of 48 hours fasting on the lambs' metabolic profile, samples of plasma were taken from 11 animals at 0900 hrs on two occasions when food was freely available, and then after 24 and 48 hours of food deprivation. The results are presented in table 3.2. The fall in plasma glucose concentration and increase in FFA and

ß-OH butyrate concentration were accompanied by a consistent fall in the plasma insulin level. These changes represent previously well described starvation adaptations in which fat becomes increasingly preferred to carbohydrate as the substrate for oxidation. Surprisingly, however, the plasma level of IGF-I did not drop in response to food deprivation. IGF-I levels have been previously described in rats to respond in the same manner as insulin, falling when the availability of substrates is reduced. The reason for there being no reduction in the plasma IGF-I level in the experimental lambs is not clear - it may possibly relate to the ruminant digestive system acting as a food reservoir so that there is still some food being digested after 48 hours of starvation. However, this proposition is at odds with the other observations made of the starvation adaptation occurring in these animals. In a series of experiments I performed involving the administration of pharmacological doses of recombinant bovine GH to lambs in both the fed and fasted state, there was a marked reduction in the IGF-I response to rbGH injection after 24 hours of starvation (unpublished data). It is fascinating but not readily explicable that there should not be a reduction, therefore, in plasma IGF-I with starvation when in the same model there is GH resistance.

In contrast to the reduced level of plasma IGF-I reported in undernourished rats[218], IGF-I receptor binding has been observed to increase with starvation in various rat tissues between 30 and 100%[208]. This raises the possibility of the fasted lambs employed in our studies

Table 3.2 *Effect of Fasting on Plasma Hormone and Metabolite Concentrations*

	Fed (mean of 2 samples)	**24 hr Fast**	**48 hr Fast**	**Signif.** (paired t test, Fed *vs* 48 hr)
IGF-I *(µg/l)*	320 +/- 22	355 +/- 44	403 +/- 55	n.s.
Insulin *(µg/l)*	0.27 +/- 0.06		<0.15	$p < 0.07$
Glucose *(mmol/l)*	3.9 +/- 0.2	3.2 +/- 0.2	2.9 +/- 0.2	$p < 0.0001$
FFA *(mmol/l)*	0.66 +/- 0.10	0.98 +/- 0.16	0.97 +/- 0.14	$p < 0.01$
Glycerol *(mmol/l)*	0.38 +/- 0.04	0.42 +/- 0.04	0.51 +/- 0.09	n.s.
ß-OH Butyrate *(mmol/l)*	0.65 +/- 0.09	0.80 +/- 0.12	1.01 +/- 0.16	$p < 0.04$
Urea *(mmol/l)*	5.1 +/- 0.3	6.5 +/- 0.8	6.1 +/- 0.4	$p < 0.07$
Potassium *(mmol/l)*	4.6 +/- 0.1	4.4 +/- 0.1	4.0 +/- 0.1	$p < 0.003$

being more sensitive to the effects of exogenous rhIGF-I than they would be in the fed state.

3.4.(iii) *Model of Sepsis - Effect of rhTNF Infusion* One of the most pressing problems in the field of surgical nutrition is the continued net negative nitrogen balance in severely septic or multiply injured patients, despite their being provided with adequate calories and nitrogen. An anabolic or anti-catabolic agent which is effective in the clinical setting of sepsis or injury would have considerable clinical potential in the intensive care setting. However, as discussed previously in Chapter 1, some forms of hormonal manipulation which are unequivocally protein sparing in normal volunteers, such as insulin and androgenic hormones, have failed to demonstrate consistently an anabolic effect in severely septic patients[216]. So in order to test the protein sparing potential of such anabolic agent in its early stages of development, it is necessary to develop an animal model which accurately reproduces the endocrine and metabolic response to sepsis.

Hitherto, animal models of sepsis employed either injections of endotoxin or the infusing or implanting of live bacterial organisms. Experimental endotoxaemia has the advantage of being readily reproducible as endotoxin can be administered in a consistent and readily quantifiable manner. However the marked cardiovascular response to endotoxin makes the measurement of metabolic parameters difficult.

Induction of sepsis by live organisms is also not without difficulties. Various peritonitis models using faecal soaked foreign bodies have been developed[261-2]. However, when faecal material is implanted, reproducibility is often difficult to achieve because of the wide spectrum of organisms present in the faeces. Induction of sepsis by performing manoeuvres such as caecal ligation have been tried, but unfortunately reproducibility is a problem with this approach as well[361]. A consistent

difficulty with the abscess/peritonitis models is that several days is usually required before the sepsis becomes florid, and during this time the animals usually become anorexic and dehydrated, complicating the performance of metabolic studies. The injection of boluses of live bacteria has been used successfully to induce the septic response in a reproducible manner which closely imitates the metabolic and hormonal response seen in severely septic patients[304]. However, again there is a delay of least 24 hours prior to the induction of the septic response.

A fascinating series of studies were performed by Wilmore and colleagues[23], who infused normal volunteers with glucagon, adrenaline and cortisol and administered aetiocholanolone in an attempt to simulate the metabolic profile of the injured patient. Directly infusing at least some of the effectors of the stress response has the effect of reducing varibility of response and provides the advantage of the stress response being reproduced very rapidly.

In order to achieve a septic profile as rapidly and reproducibly as possible, we infused recombinant human tumour necrosis factor (rhTNF) into lambs. TNF, a peptide released by macrophages in response to endotoxin exposure, has been increasingly implicated as one of the most important effectors of the metabolic response to sepsis[223]. Infusion of rhTNF replicates many of the clinical and metabolic features of sepsis, including fever, hypotension, anorexia, hyperglycaemia and a negative nitrogen balance[105,236,335]. C3H/HeJ mice, which have a genetically determined inability to produce TNF, are resistant to the effects of endotoxin, and passive immunization of baboons against TNF protects them from otherwise lethal doses of endotoxin[336]. TNF probably does not have a direct action on protein metabolism[240], but rather it acts by provoking the release of the counterregulatory hormones[227,337] and inflammatory mediators such as interleukin-I and prostaglandins[234]. TNF

infusion provides a method with the potential of more rapidly and reproducibly simulating the metabolic response to sepsis than endotoxin because it acts further along the chain of events leading to that response.

In the study to be presented in Chapter 7, rhTNF was infused over an eight hour period at a dose of 1 μg/kg.hr. This dose was chosen on the basis of a pilot study in which rhTNF was infused for five hours at this dose into four 48 hour starved lambs. Infusions of 6 ^{3}H glucose and ^{15}N urea were begun three hours prior to the rhTNF infusion commencing so that isotopic equilibrium was reached, enabling a baseline measurement of metabolic parameters to be made (fig 3.2). Blood specimens were removed hourly during the rhTNF infusion. The resultant rectal temperature, hormonal and metabolic changes in these four pilot study animals are presented in fig 3.3 and table 3.3. The control animals were infused with normal saline over the same period.

The rhTNF infused animals did not appear overtly distressed during the time of the infusion, despite their experiencing a mean rectal temperature rise of 1.2 °C. There was a dramatic increase in the plasma TNF levels from close to 0 ηg/l to levels similar to those measured in baboons during lethal bacteraemia[336]. The mean plasma cortisol level increased, but this did not reach statistical significance because of the small numbers and high variance of the data.

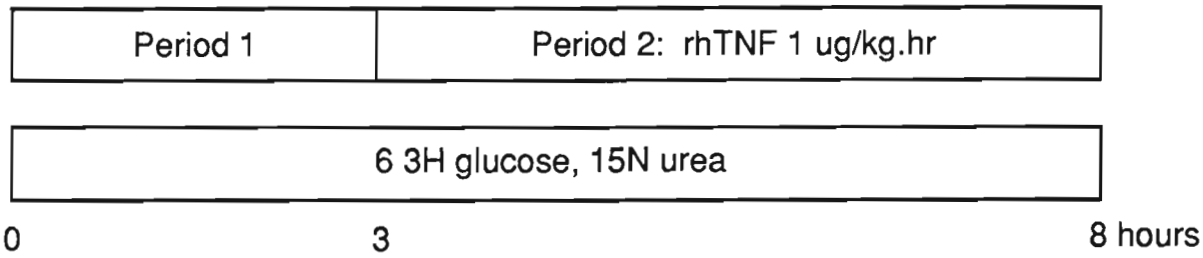


Fig 3.2 *Protocol for rhTNF pilot studies. The control animals received normal saline during the second period*

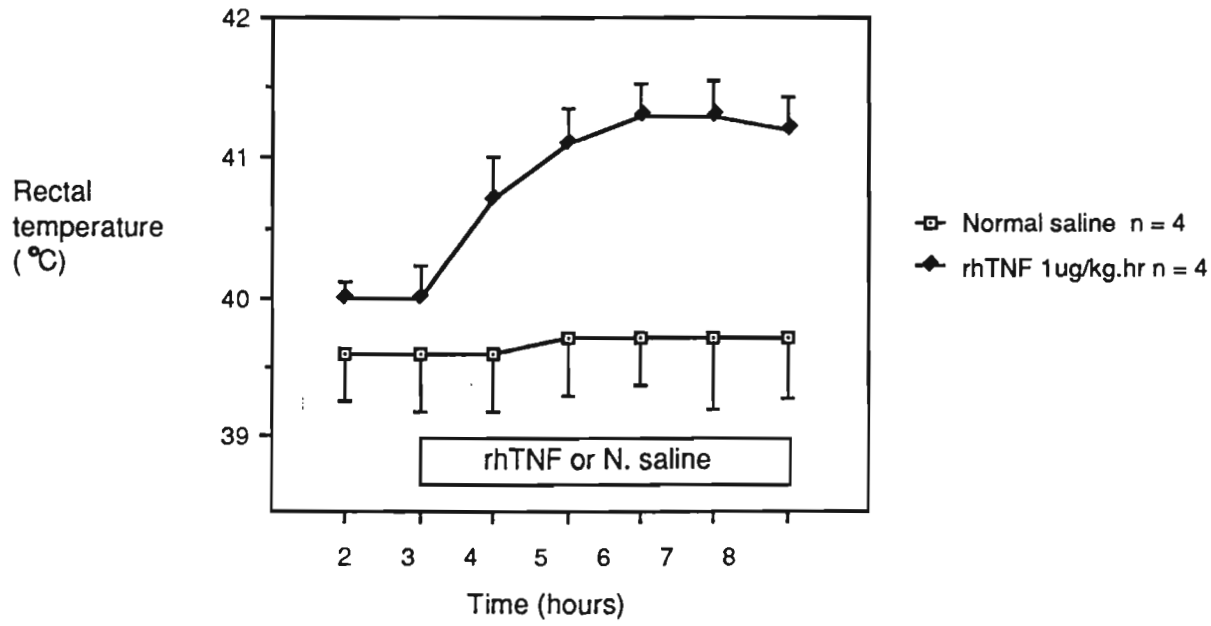


Fig 3.3 *Effect of rhTNF Infusion: Rectal Temperature*

Table 3.3 *Effect of rhTNF Infusion on Plasma Hormone Levels and Metabolite Concentration and Turnover*

	t = 0 min	t = 300 min	Significance
Plasma TNF *(ηg/l)*	3 +/- 2	10,039 +/- 674	p < 0.001
Cortisol *(ηmol/l)*	122 +/- 62	340 +/- 170	n.s.
Glucose Conc *(mmol/l)*	3.4 +/- 0.4	3.1 +/- 0.1	n.s.
Glucose Ra *(μmol/kg.min)*	13.7 +/- 0.4	15.6 +/- 0.8	p < 0.04
Glucose Clearance *(ml/kg.min)*	4.2 +/- 0.4	5.1 +/- 0.3	p < 0.06
Glycerol Conc *(mmol/l)*	0.68 +/- 0.08	0.51 +/- 0.11	n.s.
Triglyceride Conc *(mmol/l)*	0.2 +/- 0.0	0.2 +/- 0.0	n.s.
Urea Conc *(mmol/l)*	7.2 +/- 0.5	8.0 +/- 0.6	p < 0.04
Net Protein Loss *(g/kg.day)*	3.8 +/- 0.5	5.3 +/- 1.8	n.s.

3.5 Analysis of Samples

Complete descriptions of the following analytical procedures are described in Appendix 1

3.5.(i) *Glucose*

a) When ^{14}C glucose tracer was being infused, plasma glucose specific activity was determined by first precipitating the plasma proteins with $Ba(OH)_2$ and $ZnSO_4$ solutions and then passing the resultant supernatant through anion (*Dowex AGI-X8,* Serva Feinbiochemica, Heidelberg) and cation (*Dowex AG 50W-X8,* Sigma Chemical Co, St Louis) resin exchange columns. The column step was not performed when ^{3}H glucose tracer was infused. The eluate was evaorated to dryness, and reconstituted in 1 ml of distilled water. The glucose concentration of the reconstituted eluate was measured by a Hitachi 704 Autoanalyser (Hitachi, Japan), and 0.5 ml samples of the remainder were pipetted into glass scintillation vials and counted in 10 ml of *ACS II* scintillant (Amersham, United Kingdom).

b) When 2 and 6 ^{3}H glucose tracers were infused simultaneously, the specific activity of each was determined by a method described by Issekutz[169]. In brief, the samples were deproteinized and run over columns in the manner described above. Paired aliquots of the eluate were then mesaured out. One was evaporated to dryness, reconstituted and counted. This aliquot measured the counts generated by both the 2 and 6 ^{3}H glucose. The second aliquot was incubated with hexokinase and phosphoglucose isomerase, and then evaporated to dryness and redissolved in 1 ml of distilled water and counted. This process removed the ^{3}H from the 2 carbon, and so the difference between the counts of the two aliquots represented the activity of the 2 ^{3}H glucose.

3.5.(ii) *Free Fatty Acids*

Plasma free fatty acids were extracted for quantitative gas chromatography by a method described by McDonald-Gibson and Young[225]. Two hundred microlitres of chloroform were added to the extracted and evaporated free fatty acids. Half of the reconstituted free fatty acids were added to a scintillation vial, scintillant added and the mixture counted, and 10 ml were injected into a gas chromatograph.

3.5.(iii) *Glycerol*

Plasma glycerol specific activity was determined by firstly deproteinizing the plasma by addition of $Ba(OH)_2$ and $ZnSO_4$ solutions. The glycerol concentration in the eluate was then determined by the spectrophotometric method of Galetti[118], and 100 µl of eluate was added to 10 ml of scintillant and counted.

3.5.(iv) *ß-OH Butyrate*

The plasma concentration of ß-OH butyrate was determined by a spectrophotometric method prepared by the Sigma Chemical Co (St Louis). The test involves the conversion ß-OH butyrate and NAD to acetoacetate and NADH by incubation with ß-OH butyrate dehydrogenase.

3.5.(iv) *Urea*

The plasma enrichment of ^{15}N urea was determined by firstly deproteinizing the plasma sample by addition of sulphosalicylic acid. The supernatant was then passed over a column of Dowex 50 W x 8 100-200 mesh hydrogen ion resin (Sigma Chemical Co, St Louis). The urea was washed from the column by rinsing with 2N NH_4OH. The eluate was then dried and the atom percent excess determined by isotope ratio mass spectrometry.

3.5.(v) *Leucine*

The specific activity of leucine in the plasma was determined by firstly passing the plasma over *Dowex 50-W* hydrogen ion exchange resin and washing the branch chain amino acids from the column with 4N NH_4OH. The amino acids were then incubated with amino acid oxidase and catalase enzymes. The solution were then back extracted and samples injected into a high pressure liquid chromatograph. One hundred microlitre specimens were counted on a scintillation counter.

The tissue specimens were freeze dried, defatted and hydrolyzed. The supernatant was then run over the columns as for the plasma specimens. The rest of the analysis was then completed in the same manner as described for the plasma samples.

3.5.(vi) *CO_2*

Samples of ^{14}C labelled CO_2 were collected in 3 litre anaesthetic bags and bubbled into scintillation vials to which had been added a mixture of 0.1% phenolphthalein, hydroxide of hyamine and absolute ethanol in quantities which trapped exactly 1 mmol of CO_2. As soon as the CO_2 was collected, a scintillant was added, and the solution counted.

3.5.(vii) *Plasma Electrolytes and Triglyceride*

These were determined by a Hitachi 704 Autoanalyser.

3.5.(vii) *Hormones*

Plasma Hormone Concentrations

a) IGF-I. Plasma IGF-I was measured by radioimmunoassay[40] using a rabbit antiserum to rh-metIGF-I (878/4) at a final titre of 1:150,000. The

antiserum has a cross-reaction with IGF-II of <0.1%, a minimal detectable dose of 0.06 ηg/tube and a half displacement dose of 0.40 ηg/tube.

Recombinant γ-rhIGF-I was iodinated by a modified chloramine-T method[50,126]. The ^{125}I labelled γ-rhIGF-I was purified by exclusion chromatography on a pre-albuminated *Sephadex G-50* (Pharmacia, Sweden) column (10 x 600 mm). Fractions of 40 drops were collected using 0.01 mol PBS/l (pH 6.2) containing 0.1% gelatin (w/v) as eluent. The specific activity of ^{125}I labelled g-rhIGF-I was 120-160 μCi/μg. Only the eluates corresponding to the IGF-I monomer were pooled and stored in aliquots at 4 °C. This material was used within 2 weeks of iodination.

Before immunoassay plasma samples were subject to acid-ethanol extraction with an additional cryo-precipitation step (AEC extraction). The correlation between values for sheep plasma obtained using this extraction technique and those of *Sephadex G75* chromatography in 1M formic acid was $r = 0.98$ ($p < 0.001$, $n = 10$) and the slope of the linear regression was 1.02. Using this extraction system, the interassay coefficient of variation was 9.8% and the within assay coefficient of variation was 5.0%.

The incubation mixture consisted of 100 μl diluted neutralized acid-ethanol solution containing the standard or test plasma and 200 μl of assay buffer containing IGF-I antiserum. After preincubation for 60 minutes at room temperature, ^{125}I-labelled γ-rhIGF-I (20,000 cpm) was added in 200 μl of assay buffer and the incubation was continued for 18-24 h at 4 °C. Bound and free IGF-I were separated by addition of 1 ml of a pre-precipitated second antibody containing 0.01 mol PBS/l, 1% (v/v) sheep antirabbit gammaglobulin, 0.1% (V/V) normal rabbit serum and 5% polyethylene glycol (PEG) 6000. The test tubes were then incubated for 1 hr at room temperature and centrifuged at 3000 g for 30 min at 4 °C. The supernatant was decanted and the radioactivity in the remaining pellet was determined.

b) Measurement of Circulating Molecular Forms of IGF-I. The circulating molecular forms of IGF-I in plasma were measured using a modification of the method of Butler and Gluckman[50] . In brief, aliquots of 500 µl were subjected to high-performance gel chromatography on a *Superose 12* column (10 x 300 mm, Pharmacia, Sweden) fitted to a Pharmacia Fast Protein Liquid Chromatography System calibrated as previously described[153]. The column was equilibrated with 0.1 mol phosphate/l buffer containing 0.15 mol NaCl/l and 0.02% NaN_3 (pH 7.2) and the samples were eluted with the same buffer at a flow rate of 0.5 ml/min. Fractions of 0.25 ml were collected after an 8 min delay into silicone coated tubes containing 20 µl of 1 % bovine serum albumin (BSA) (w/v) in water. The fractions containing free IGF-I eluted at fraction #49-60. Fractions in the range of 35-60 kDa (fraction #29-38) containing predominantly IGFBP1 and IGFBP-2 and fractions in the range of 150kDa (fraction #39-48) containing IGFBP-3 were pooled for the analysis of circulating molecular weight forms of IGF-I. Aliquots of the pooled fractions were subjected to AEC exraction before RIA. Recoveries of unlabelled γ-rhIGF-I added to the plasma before high-performance gel chromatography, extraction and RIA were in the order of 95-110%. Complete linearity was found in the range of 0-500 γ-rhIGF-I added per ml plasma.

The *Superose 12* column was calibrated before use with the following markers: dextran blue (MW = 200,000), thyroglobulin (MW = 330,000), alcohol dehydrogenase (MW = 150,000), BSA (MW = 68,000), carbonic anhydrase (MW = 29,000), ^{125}I labelled rhIGF-I (MW = 7,650), aprotonin (MW = 6,500), glucagon MW = 3 000) and ^{125}I (MW = 125).

c) Plasma Insulin Concentrations.

Plasma insulin was measured by radioimmunoassay using a guinea pig antiserum to ovine insulin at a final titre of 1:400,000. The assay has a

minimal detectable dose of 0.15 ηg/tube and a half displacement dose of 0.40 ηg/tube. The incubation mixture consisted of 100 µl of standard or test plasma and 200 µl of assay buffer containing ovine insulin antiserum. After preincubation at room temperature for 24 hours, ^{125}I-labelled insulin (15,000 cpm) were added in 200 µl assay buffer and the incubation was continued for 18-24 hr at 4 °C. Bound and free insulin were separated by addition of 1 ml of a second antibody complex containing 4% polyethylene glycol 6000 made up in 0.01 M phosphate buffered saline. The test tubes were then incubated for 1 hr at room temperature and centrifuged at 3000 g for 30 minutes at 4 °C. The supernatant was decanted and the radioactivity in the remaining pellet determined. The interassay coefficient of variation was 11.5% and the within assay coefficient of variation was 6.7%.

d) Glucagon

Plasma glucagon levels were measured using rabbit antiserum to at a final concentration of 1 in 90,000. This assay was performed in the Department of Endocrinology, The Princess Margaret Hospital, Christchurch. After preincubation at 4 °C, ^{125}I labelled glucagon tracer is added and the incubation continued for another 3 days at 4 °C. The second antibody and PEG complex are then added, vortexed and separated after 15 minutes incubation, and the pellets subsequently counted.

e) Human Growth Hormone

Plasma human growth hormone levels were determined by the in house radioimmunoassay of the Department of Endocrinology of Auckland Public Hospital. The first antibody was guinea pig anti-hGH, and the second , sheep anti-guinea pig. The assay was calibrated against WHO standard 66/217.

f). Cortisol

The plasma levels of cortisol was measured using the Amerlex RIA Kit (Amersham, Buckinghamshire, UK)

g) Tumour Necrosis Factor

Plasma TNF levels were measured by a commercially prepared radioimmunoassay (*TNF-IRMA;* Medgenix, Fleurus, Belgium). Samples and standards were added, along with ^{125}I labelled anti-TNF monoclonal antibody, to tubes pre-coated with oligoclonal antibodies against TNF. Following overnight incubation at room temperature, the solutions were decanted and the tubes washed twice prior to reading in a gamma counter. The assay was sensitive to 1 pg/ml of TNF. This RIA has previously been shown to detect biologically active TNF[132].

Chapter 4

The Metabolic Effects of rhGH in Surgical Patients:Isotopic Studies in the Postabsorptive State and During Intravenous Nutrition.

4.1 Introduction

As the survival of critically ill surgical patients is prolonged by improvements in intensive care, optimizing the nutritional management of such patients is becoming increasingly important. The parenteral provision of excess calories and nitrogen has not proved to be the panacea once thought, as the resultant increase in the rate of protein synthesis is often not of sufficient magnitude to compensate for the greatly accelerated rate of protein breakdown typical of such patients, allowing a state of net protein catabolism to persist (Chapter 1, Section 1.7(ii)). Excessive loss of protein reserves leads to impairment of cardiopulmonary performance, immune function and wound healing[283] and so improving the protein sparing efficacy of IVN in severely ill patients may represent a significant clinical advance.

The potential of human growth hormone to encourage protein synthesis in adult surgical patients was first described more than thirty years ago[268]. The early workers in this field used human growth hormone extracted from pituitary cadavers, but since1985 recombinant human growth hormone (rhGH) produced from bacterial DNA has been available, rekindling research interest in a possible therapeutic role for this hormone in surgical patients with an accelerated rate of protein loss. The effect of human growth hormone or its synthetic equivalent on protein metabolism has now been investigated in a number studies of normal volunteers and of patients on a variety of nutritional regimens (table 1.2). Wilmore, following early work performed in the early seventies in which he described

an improved nitrogen balance in burn patients given hGH and an adequate dietary intake[369], has reported achieving protein anabolism by administering rhGH to normal volunteers[220] and depleted surgical patients[396] who were receiving hypocaloric intravenous feeding and were subsequently in a negative nitrogen balance prior to rhGH treatment. Similar results have been described in normal volunteers given hypercaloric nutritional support[106], postoperative patients on hypocaloric intravenous feeding[266] and postoperative patients given no nitrogen and minimal caloric supplementation[350].

There remain several issues requiring further investigation. To date, no study has been published which examines whether the administration of rhGH is capable of reducing the rate of protein loss in patients with severe, acute illness who are receiving total parenteral nutrition. An effective anabolic agent would have considerable clinical potential in such patients who typically have a high rate of protein breakdown. Accordingly, we have studied the effects on protein metabolism of rhGH when administered to a group of severely ill septic or traumatized patients receiving total parenteral nutrition.

Although the changes in protein metabolism consequent to rhGH administration to patients with less severe clinical insults have been well documented, less attention has been paid to the associated changes in fat and glucose metabolism. The effects of rhGH on energy metabolism have important implications on its protein anabolic properties, so in the second part of this study we have made a detailed assessment of the effect of rhGH on energy substrate turnover and oxidation in septic or injured patients who were sufficiently well to be eating a normal ward diet.

4.2 Methods

4.2.(i) *Clinical Status of Patients* Isotopic studies were performed in 25 adult surgical patients admitted to the surgical wards of Auckland Hospital. Twelve of the patients were receiving intravenous nutrition and thirteen were on a normal ward diet. The patients were recovering from sepsis, trauma (musculoskeletal or major operative) or pancreatitis, conditions which have been previously demonstrated to be associated with an accelerated rate of net protein catabolism[303,307,313]. Patients with evidence of diabetes mellitus or overt congestive heart failure or those receiving corticosteroids were excluded.

The clinical details of the patients are summarized in tables 4.1 a&b. The twelve intravenously fed patients had been established on parenteral nutrition for a minimum of three days prior to a baseline study being performed, and were clinically stable over the time course of the study. The mean age, weight, sex distribution and sepsis severity scores[96] did not differ significantly between the treatment and control groups. The studies of the patients on a normal ward diet were begun at between day three and day twelve post admission. All patients completed the study protocol.

4.2.(ii) *Intravenous Nutrition* The intravenous nutrition solutions prescribed provided approximately 40 kcal/kg.day of non-protein calories. The energy substrate requirement was administered as a calorically equal mixture of 50% dextrose and 20% lipid solutions (*Intralipid*, KabiVitrum Laboratories, Stockholm). An amino acid solution (*Synthamin 17*, Travenol Laboratories, New South Wales) provided approximately 300mg/kg of nitrogen daily, and vitamins (*MVI 12*, Armour Pharmaceuticals, Illinois) and trace elements were added to the IVN bag. A weekly dose of vitamin K 10 mg i.m. was given routinely.

Table 4.1a *IVN Patient Details*

IVN Controls

Age	Sex	Weight	Sepsis Score	Diagnosis
23	M	76	19	Multiple trauma, subphrenic abscess
65	M	62	15	Duodenal perforation, biliary peritonitis
87	F	49	12	Pharyngeal perforation, mediastinitis
75	M	46	13	Oesophagectomy, pneumonia

IVN rhGH

Age	Sex	Weight	Sepsis Score	Diagnosis
58	F	73	12	Subphrenic abscess
73	M	75	15	Anastomotic leak, pelvic abscess
73	M	61	10	Oesophagectomy, pneumonia
54	M	70	14	Caecal perforation, peritonitis
62	M	65	14	Anastomotic leak, urinary peritonitis
58	M	82	21	Small bowel fistula, faecal peritonitis
66	F	57	11	Pancreatitis
46	F	91	15	Haemorrhagic pancreatitis, pneumonia

Table 4.1b *Postabsorptive Patient Details*

i) Protein Kinetics

Age	Sex	Weight	Diagnosis
51	M	122	Haemorrhagic pancreatitis
64	M	68	Haemopneumothorax, fractured ribs
67	F	49	Perforated duodenal ulcer, biliary peritonitis
72	M	67	Postoperative, aortic aneurysm repair
69	M	77	Pancreatitis

ii) Glucose Kinetics

Age	Sex	Weight	Diagnosis
60	F	72	Postoperative, splenectomy
65	F	48	Fractured femur
23	M	72	Haemopneumothorax, fractured ribs
82	M	61	Fractured femur

iii) Fat Kinetics

Age	Sex	Weight	Diagnosis
57	F	67	Fractured tibia and fibula
34	M	72	Fractured tibia and fibula
57	M	80	Dislocated knee
32	M	92	Fractured femur, fractured patella

4.2.(iii)*Study Design.*

(a)*IVN Studies.* The baseline protein and energy metabolism of the twelve IVN patients was measured on the first day of the study. This consisted of a primed constant infusion of isotopically labelled metabolic tracers over a three hour period and the performance of indirect calorimetry (fig 4.1a).

Three of the rhGH treated patients and all of the control patients received primed constant infusions of ^{14}C urea and 6 ^{3}H glucose. For studies in four of the rhGH treated patients, ^{15}N urea was substituted for ^{14}C urea. In five of the rhGH treated patients the rate of appearance of leucine into the plasma was determined by infusions of ^{14}C leucine. The patients were then administered either 20 IU subcutaneous injections of rhGH (*Genotropin*, KabiVitrum Laboratories, Stockholm) at 0800hrs for three days, or given no perturbation (control group). The patients were restudied 24 hours subsequent to the final injection of rhGH or the end of the control period with isotopic tracers and indirect calorimetry, allowing time for an IGF-1 response to occur after the final injection.

(b) Postabsorptive Studies. A similar protocol was followed in those patients on a normal ward diet (fig 4.1b). These patients underwent metabolic studies in the postabsorptive state (in which they were at least 12 hours postprandial). Following a baseline metabolic study with isotopic tracers and indirect calorimetry, the patients were administered three daily doses of rhGH in the manner described above. At 0900 hours, one hour after the final injection of rhGH, a second isotopic infusion was begun. This was completed at 1200 hours, so that the plasma specimens were collected four hours after the final injection of rhGH. Indirect calorimetry was also repeated during this study period. The peak plasma levels of

Figure 4.1a

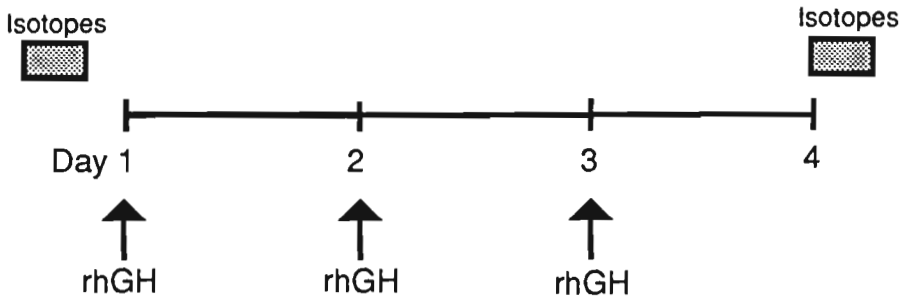
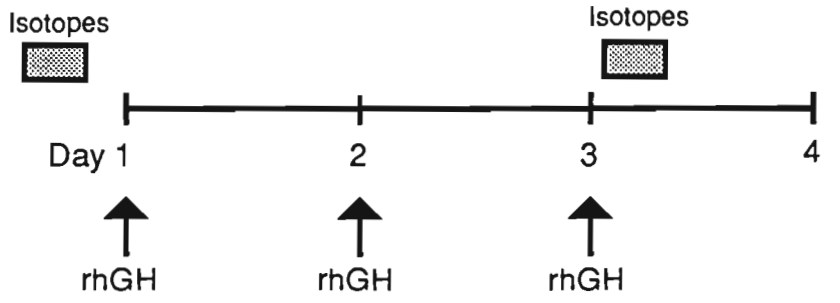


Figure 4.1b



Figures 4.1 a&b *Outline of experimental protocols. The second isotopic infusion was begun 24 hours after the final rhGH injection in the IVN patients (4.1a) and 1 hour after the final rhGH injection in the postabsorptive patients (4.1b).*

rhGH occur three to six hours after subcutaneous injection[371], and the time of isotopic infusion was chosen to coincide with this.

Glucose production and recycling were quantified in four patients with infusions of ^{14}C, 6 ^{3}H and 2 ^{3}H labelled glucose[169,270], and the rate of lipolysis and triglyceride recycling were determined by ^{14}C palmitate and ^{3}H glycerol infusions in four patients. The remaining five patients received ^{14}C leucine, and in addition ^{15}N urea was simultaneously infused in eight patients to determine the effect of rhGH on the rate of appearance of urea and hence on net protein loss.

In order to determine rates of glucose, free fatty acid and leucine oxidation, the bicarbonate pool was primed in each of the patients with an appropriate dose of $NaH^{14}CO_3$, and one mmol samples of expired $^{14}CO_2$ specimens were collected at the end of the isotopic infusions in a methylbenzonium/ethanol solution, and $^{14}CO_2$ specific activity. The rate of substrate oxidation was calculated using equation 3.2.(ii). Frayn's equations were used to derive the rate of total fat oxidation from indirect calorimetry and urea turnover (equations 3.2.(vi) a&b).

4.2.(iv) *Isotopic Infusions* The rate of appearance (Ra) of urea was determined either by a three hour infusion of ^{14}C urea (rate 1.5 ηCi/kg.min and priming dose of 450:1), or when other ^{14}C labelled tracers were used, ^{15}N urea (rate 10 μg/kg.min and 450:1 prime). Leucine kinetics were quantified by a three hour infusion of ^{14}C leucine at a rate of 3 ηCi/kg.min following an 60:1 leucine prime.

The rate of glucose production and recycling were determined with three hour infusions 2 ^{3}H, 6 ^{3}H and ^{14}C glucose at doses of 2ηCi/kg.min for the ^{3}H isotopes and 1 ηCi/kg.min for ^{14}C glucose, following 80:1 primes.

Free fatty acid kinetics were quantified with three hour infusions of 2 ^{3}H glycerol (at 2 ηCi/kg.min and 10:1 prime) and 1 ^{14}C palmitate bound to albumin(at 1 ηCi/kg.min and without prime).

4.2.(v) *Indirect Calorimetry* Indirect calorimetry was performed using a hood calorimeter with associated Sybron Taylor oxygen analyzer (Hoddeson, UK) and ADC CO_2 analyzer (Hertfordshire, UK). Following a period of accustomization to the hood, a reading was taken over a 20 minute period during the last hour of the isotopic infusion.

4.2.(vi) *Substrate Cycles* Isotopic data were used to quantify the effect of rhGH on the activity of substrate cycles. Such cycles exist when the forward and reverse reactions of a metabolic pathway, catalyzed by different enzymes, are active simultaneously[177]. The extent of glucose recycling can be determined using specifically labelled glucose tracers. If a ^{3}H attached to a specific carbon is irreversibly cleaved off by passing through one such cycle, then the activity of this cycle can quantified by measuring the rate at which counts are lost[163]. In this fashion, the activity of the glycolytic/gluconeogenic cycle is given by the difference between the rate of appearance of glucose determined by the 2 ^{3}H and 6 ^{3}H glucose tracers (Ra_2 - Ra_6)[169], and the rate of Cori cycling is given by the difference in rate of appearance between 6 ^{3}H glucose and ^{14}C glucose[270].

Intracellular triglyceride cycling occurs when the hydrolysis of triglyceride into glycerol and free fatty acids and their re-esterification into triglyceride occur simultaneously within the adipocyte[386]. The rate of intracellular triglyceride cycling is given by the equation:

$$\text{Intracellular Cycling} = 3\ \text{Ra glycerol} - \text{Ra FFA}$$

4.2.(vii) *Statistical Analysis* The Student's paired *t* test was used to determine the significance of changes between the pre and post rhHGH results, and unpaired *t* tests were used to determine intergroup differences. Results are expressed as mean +/- standard error of the mean.

4.3 Results

4.3.(i) *IVN Patients*

(a)Hormones: Post treatment plasma samples were collected from the patients receiving IVN 24 hours after the final injection of rhGH and no increase in plasma growth hormone levels from basal values was seen in these patients, reflecting the short (17 minute[329]) half life of human growth hormone (table 4.2b). The plasma level of IGF-1 was more than doubled following the rhGH treatment and there was an increase of similar magnitude in the plasma levels of insulin. No change was observed in the plasma concentration of cortisol in the treatment or control groups.

(b) Protein Metabolism There was a significant reduction in the urea Ra following rhGH administration from 9.7 +/- 1.0 μmol/kg.min to 7.5 +/- 0.8 μmol/kg.min ($p < 0.02$). When these data were converted to g protein/kg.day and the infused protein accounted for, this reduction in the urea Ra represented an almost halving of the net rate of protein loss (from 0.82 +/- 0.17 g/kg.day to 0.43 +/- 0.20 g/kg.day, $p < 0.02$) (fig 4.2). The rate of net protein loss in the control group did not change between the two study periods (0.51 +/- 0.46 g/kg.day basal and 0.66 +/- 0.43 g/kg.day post). The endogenous leucine Ra was unchanged following the rhGH course (4.4 +/- 1.1 μmol/kg.min basal and 4.4 +/- 0.8 μmol/kg.min post rhGH).

Table 4.2 *Plasma Hormone Levels*

a) IVN Controls

	Basal	Post rhGH	Significance
hGH *µg/l*	3.2 +/- 1.2	4.5 +/- 2.8	n.s.
IGF-1 *µg/l*	484 +/- 75	502 +/- 115	n.s.
Cortisol *ηmol/l*	477 +/- 65	313 +/- 49	n.s
Insulin *mIU/l*	36 +/- 22	28 +/- 15	n.s

b) IVN rhGH

	Basal	Post rhGH	Significance
hGH *µg/l*	1.4 +/- 0.0	1.6 +/- 0.3	n.s.
IGF-1 *µg/l*	434 +/- 61	1001 +/- 102	$p < 0.0003$
Cortisol *ηmol/l*	320 +/- 65	435 +/- 61	n.s.
Insulin *mIU/l*	57 +/- 11	120 +/- 30	$p < 0.03$

c) Postabsorptive Patients

	Basal	Post rhGH	Significance
hGH *µg/l*	1.4 +/- 0.3	39.9 +/- 5.6	$p < 0.0002$
IGF-1 *µg/l*	271 +/- 32	789 +/- 106	$p < 0.003$
Cortisol *ηmol/l*	422 +/- 36	365 +/- 30	n.s.
Insulin *mIU/l*	9 +/- 1	25 +/- 5	$p < 0.002$

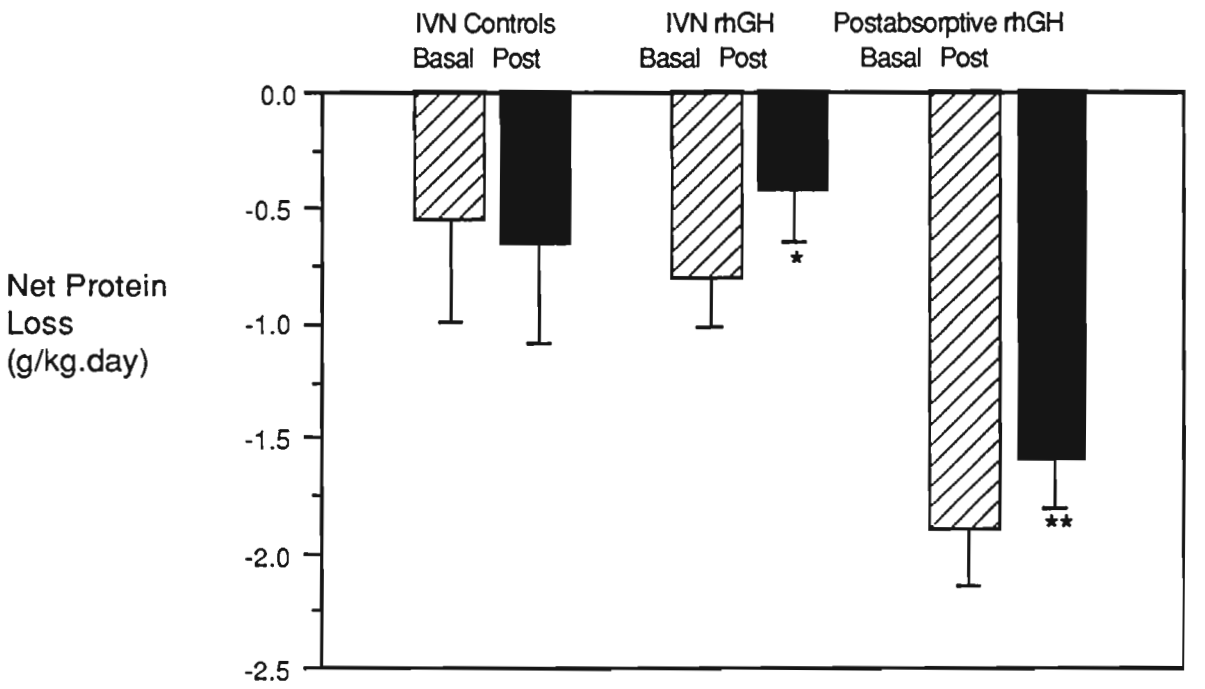


Figure 4.2 *The rate of net protein loss before and after rhGH administration in IVN and postprandial patients.*

* $p < 0.02$, ** $p < 0.05$

(c) Glucose and Fat Metabolism Following rhGH administration there was a significant increase the plasma glucose concentration (7.0 +/- 0.3 mmol/l to 8.3 +/- 0.7 mmol/l, $p < 0.03$) and an increase in the rate of endogenous glucose production (10.0 +/- 2.6 μmol/kg.min to 15.9 +/- 4.4 μmol/kg.min, $p<0.07$). No change was seen in the rate of plasma glucose clearance. There were no significant changes in measurements of glucose concentration or kinetics in the control group.

Free fatty acid and glycerol kinetics were not determined in the patients receiving IVN. However, neither the plasma concentration of free fatty acids (0.58 +/- 0.11 mmol/l basal and 0.57 +/- 0.11 mmol/l post rhGH) nor glycerol (0.14 +/- 0.02 mmol/l basal and 0.12 +/- 0.02 mmol/l) were altered in the rhGH treated group.

(d)Indirect Calorimetry There was no change in the respiratory quotient (RQ) following rhGH administration (0.94 +/- 0.01 basal and 0.93 +/- 0.02 post rhGH), nor was the rate of resting energy expenditure altered (1590 +/- 90 kcal/day basal and 1600 +/- 110 kcal/day post rhGH).

4.4.(ii) *Postabsorptive Patients*

(a) Hormones At the time of sampling during the second study, four hours after the final rhGH injection, the plasma growth hormone levels were almost thirty fold higher than basal in the postabsorptive group (table 4.2c), and both the plasma IGF-1 and insulin levels were increased nearly three fold. The plasma cortisol levels were unchanged.

(b)Protein Kinetics Following rhGH administration there was a consistent reduction in urea Ra (7.6 +/- 1.1 μmol/kg.min basal to 6.6 +/- 0.9 μmol/kg.min post rhGH, $p < 0.05$), which equated to a decrease in the rate

of net protein loss from 1.92 +/- 0.27 g/kg.day to 1.66 +/- 0.23 g/kg.day ($p < 0.05$), and there were similar decreases in both the leucine Ra (3.7 +/- 0.6 μmol/kg.min basal to 2.7 +/- 0.5 μmol/kg.min post rhGH, $p < 0.04$) and rate of leucine oxidation (0.44 +/- 0.05 μmol/kg.min basal to 0.26 +/- 0.03 μmol/kg.min post rhGH, $p < 0.005$).

(c) Glucose Metabolism There were no significant changes in the glucose concentration, rate of glucose clearance or rate of oxidation following the rhGH treatment course. The glucose Ra and recycling data are presented in table 4.3.

(d)Fat Metabolism The rate of appearance of free fatty acids into the plasma increased significantly from 7.4 +/- 2.2 μmol/kg.min to 11.1 +/- 2.6 μmol/kg.min ($p < 0.03$) in the postabsorptive patients following rhGH treatment. This was not attended by an increase in Ra glycerol of the same magnitude (3.3 +/- 0.7 μmol/kg.min vs 3.7 +/- 0.8 μmol/kg.min, NS). The disparity in these changes was reflected in a decrease in the mean percentage of intracellular triglyceride recycling from 25% to 0%. The rate of oxidation of free fatty acids increased following rhGH administration in the postabsorptive patients (from 1.3 +/- 0.4 μmol/kg.min to 1.7 +/- 0.4 μmol/kg.min, $p < 0.06$).

(e) Indirect Calorimetry The increase in free fatty acid oxidation seen in the postabsorptive patients following rhGH administration was associated with a significant reduction in respiratory quotient from 0.78 +/- 0.02 to 0.72 +/- 0.01 ($p < 0.0005$). The rhGH course was not associated with a significant change in the resting metabolic expenditure (1560 +/- 150 kcal/day basal and 1580 +/- 120 kcal/day post rhGH).

Table 4.3 *Glucose Metabolism in Postabsorptive Patients*

	Basal	Post rhGH	Significance
Ra 6* *µmol/kg.min*	15.9 +/- 1.6	16.1 +/- 1.3	n.s.
Ra 2* *µmol/kg.min*	17.9 +/- 2.1	15.9 +/- 2.3	n.s.
Ra 14* *µmol/kg.min*	9.7 +/- 1.0	9.9 +/- 0.8	n.s.
Ra 2 - Ra 6 *µmol/kg.min*	3.2 +/- 1.0	2.1 +/- 2.0	n.s
Ra 6 - Ra 14 *µmol/kg.min*	3.7 +/- 1.0	5.4 +/- 1.2	n.s

* Rate of appearance of glucose determined by simultaneous infusions of 6 and 2 ^{3}H and ^{14}C glucose tracers

4.4 Discussion

4.4.(i) *Effect on Nitrogen Balance* Despite the parenteral provision of adequate calories and protein, all but one of the IVN study patients were in a net negative nitrogen balance at the time of baseline study, reflecting the severity of the protein catabolic response to their illness. The rate of net loss of protein was nearly halved following rhGH administration, but in only one patient was a positive nitrogen balance achieved. The failure of rhGH to render the patients anabolic contrasts with the positive nitrogen balances achieved by rhGH treatment in the studies of Wilmore in normal volunteers[220] and in a group of depleted surgical patients[396], and of Sim in postoperative patients[266]. In these three studies the subjects received only about half their daily caloric requirement along with an adequate amount of nitrogen (between 100 and 200 mg/kg.day). The parenteral nutrition solution prescribed for our patients provided ample calories and 300mg/kg.day of nitrogen, and the reason why a positive nitrogen balance was not achieved despite the provision of IVN almost certainly lies in the clinical state of patients studied - all the patients receiving IVN in our study were acutely and severely ill, not simply depleted or postoperative. It would appear that the release of hormonal and inflammatory mediators accelerating the breakdown of protein was in excess of the potential of IVN and rhGH to promote nitrogen retention. Nonetheless, our results demonstrate that rhGH has a significant protein conserving effect in the septic and/or trauma patient, a group of patients whom we and others have shown to remain significantly catabolic despite optimal nutritional support[303,313,325]. Wilmore and colleagues have recently demonstrated that rhGH can continue to promote protein anabolism in surgical patients when administered for periods as long as 25 days[396]. If the protein sparing effect that we have observed in the study group receiving IVN continued at the

same rate for a period of two weeks, it would equate to a saving of nearly 1.5 kg of wet muscle mass in a 70 kg patient.

4.4.(ii) *Effect on Protein Catabolism and Synthesis* The reduction in net protein loss observed in the patients receiving IVN was not associated with a reduced rate of appearance of leucine. Assuming that leucine Ra is representative of the rate of appearance of amino acids in general, then this result suggests that the administration of rhGH did not effect a change in the rate of whole body protein catabolism. This implies that the observed improvement in nitrogen balance was a consequence of an enhanced rate of protein synthesis, a finding consistent with those of others[187].

4.4.(iii) *Effect on Energy Substrate Metabolism* In the second part of our study we examined the interaction between substrate mobilization and oxidation following rhGH administration to a group of septic and injured patients who were studied while postabsorptive. As the antagonism of the actions of insulin seen after hGH administration is believed to be a property of the hGH molecule itself[83], in this series of studies the second isotopic infusion was timed to coincide with maximal plasma hGH levels. Although neither glucose Ra or rate of oxidation were changed following rhGH treatment, free fatty acids were mobilized and oxidized at an accelerated rate. Both leucine Ra and leucine oxidation were significantly reduced. These changes are summarised in figure 4.3. We suggest that the reduction in leucine oxidation following rhGH administration was secondary to an increased reliance on fat oxidation for energy production. Presumably this protein sparing effect would last until

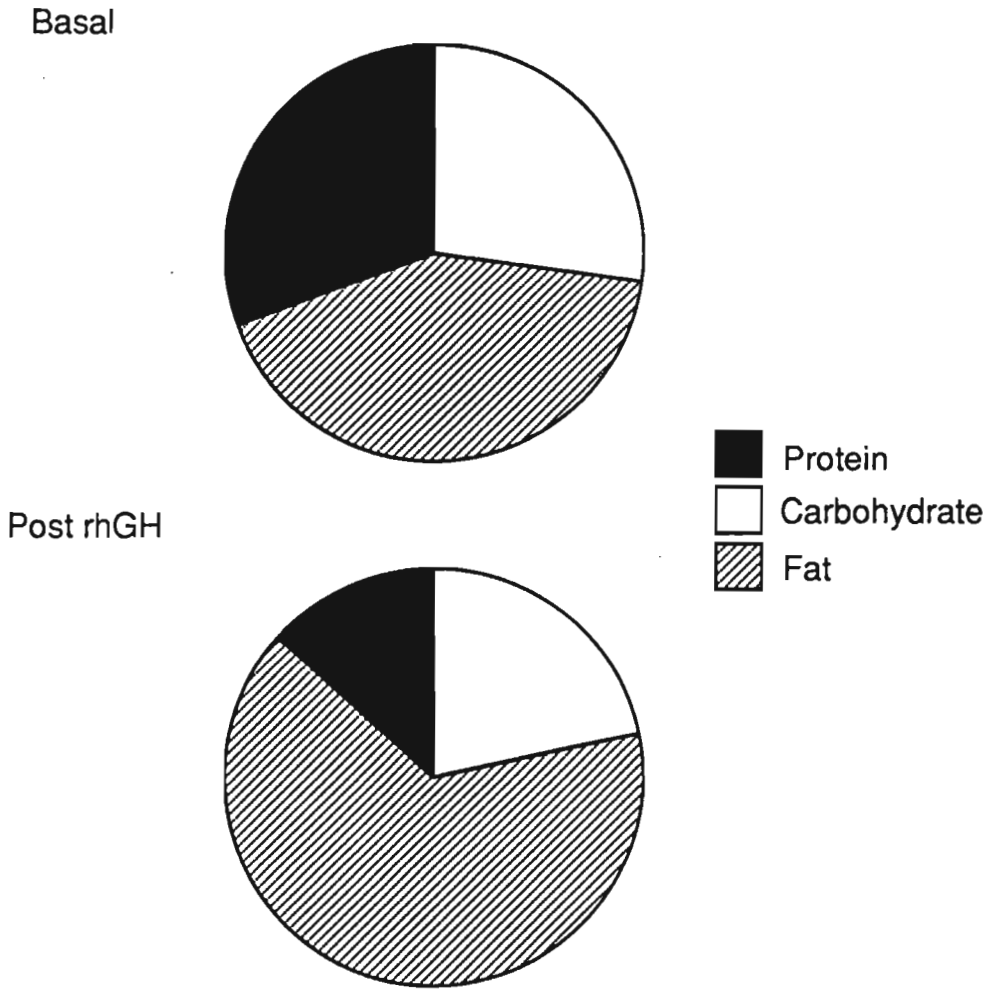


Figure 4.3 *Energy substarte oxidation in the basal state and following admininstration of rhGH. Glucose and protein oxidation were derived from the rate of oxidation of 14C labelled glucose and leucine tracers and total fat oxidation from Frayn's equation. The energy released per gram of substrate oxidized was calculated using the following data: glucose 4.1 kcal/g, fat 9.3 kcal/g and protein 5.3 kcal/g (120).*

the fat stores were exhausted, which may not be long in a depleted patient with a high energy expenditure[106].

4.4.(iv) *Effect on Substrate Recycling* An increase in glycolytic/gluconeogenic cycling of some 70% has been reported in acromegalic patients studied in the postabsorptive state[176], and we were interested to see whether rhGH would have a similar effect in surgical patients. However, no increase in the rate of glycolytic/gluconeogenic or Cori cycling was observed in the postabsorptive patients following rhGH administration in our study (table 4.3). The effect on glucose recycling observed in acromegalics may be related to their chronic exposure to in some cases very highly elevated plasma hGH levels.

However, our results suggest that rhGH administration influenced the rate of triglyceride recycling in the postabsorptive patients. Treatment with rhGH increased the rate of appearance of free fatty acids by two thirds in this group, but did not significantly alter the rate of appearance of glycerol. This suggests that the primary cause of the increase in Ra FFA was a reduction in reesterification. These data concur with those of Goodman obtained in studies of laboratory animals[129], although others have reported parallel increases in glycerol release[372]. Although results from the two parts of our study are not directly comparable because of the different timing of the second study in relationship to the final rhGH injection, no increase in FFA or glycerol concentration was seen in the IVN patients. This may simply be a reflection of the plasma hGH levels not being raised at the time of the second study in the IVN patients, as the lipolytic effects of hGH are believed to be a direct action of this hormone[83]. However, it has been reported by others that the lipolytic effect of hGH is effectively neutralized by parenteral feeding[106,396] or glucose alone[372].

4.4.(v) *Study Shortcomings* Our study may be criticised because of short term over which the isotopic measurements were made. It is very likely that the metabolic response to the rhGH injections varies over the time of absorption and catabolism of the exogenous hormone. Accordingly, the magnitude of the changes we have measured may not be consistent over a 24 hour period. Employing either a prolonged infusion of rhGH to ensure a consistent response or a prolonged infusion of isotopic tracers associated with multiple sampling over time to measure differences in response following the final rhGH injection would circumvent this interpretational difficulty, but neither of these alternatives were clinically feasible.

4.4.(vi) *Summary* We have demonstrated that a short course of rhGH to severely ill surgical patients receiving IVN is capable of almost halving their rate of net protein loss, although a mean positive nitrogen balance was not achieved in this highly protein catabolic group. In the postabsorptive setting, the protein sparing effect observed following rhGH administration appeared to result from the oxidation of fat in preference to protein, rather than from an increase in the rate of protein synthesis. The significant protein sparing effects of rhGH which we have observed in severely ill parenterally fed patients suggest that this hormone may have therapeutic potential in the nutritional management of septic or traumatized patients who require intensive support.

Chapter 5

The Effect of rhGH in Cancer Patients Measured at the Tissue Level

5.1 Introduction

The adverse influence of preoperative weight loss on the postoperative course of cancer patients undergoing surgery has been well documented[70,150,231]. Although the provision of adequate calories and nitrogen to patients whose depletion is secondary to benign disease consistently results in a positive nitrogen balance and restoration of lean body mass, this is often not the case in patients with cancer cachexia[296,311]. This may in part explain why, with the exception of the study of Muller and colleagues[247], the preoperative administration of intravenous nutrition to wasted cancer patients has been demonstrated to have had either limited or no impact on clinical outcome in prospective randomized trials[145,162,239]. On the basis of these findings, an agent which increases the efficiency of utilization of infused nutrients may have significant clinical potential.

Recombinant human growth hormone (rhGH) has been recently demonstrated to have a significant protein sparing effect in intravenously fed normal volunteers, postoperative patients with and without nutritional support, and in malnourished and severely ill surgical patients receiving intravenous nutrition (table 1.2 and Chapter 4). The provision of rHGH to patients with cancer cachexia may produce a similar improvement in nitrogen balance. As with other therapeutic manipulations attempting to replenish cachectic cancer patients, there exists the concern that rhGH will promote protein synthesis in malignant tissue. However, no evidence of tumour stimulation was found when exogenous growth hormone was given to intact cancer bearing laboratory animals[265,327]. In addition, when

provision of intravenous nutrition did not accelerate the rate of protein synthesis in malignant tissues, they concluded that it was likely that "tumour protein synthesis rates are primarily determined by the inherent nature of the tumour rather than by other environmental or remote factors"[246].

A further advantage in determining whether rhGH improves nitrogen balance without stimulating tumour growth lies in its potential use in patients with serious postoperative complications following tumour resection. Sims and colleagues have demonstrated that rhGH effectively spares protein in the first week after major abdominal operations[266,350]. It would be reassuring to know that such supportive therapy was not likely to stimulate the growth of residual tumour.

Accordingly, the present study was designed to determine whether the preoperative administration of a short course of rhGH would favourably influence host protein metabolism without accelerating tumour protein synthesis.

5.2 Methods

5.2.(i) *Patients* The study involved 10 patients who were admitted for elective resection of malignant tumours of the colon or upper rectum to the general surgical service of one surgeon. The clinical details of the patients are summarised in table 5.1. The treatment and control groups have been matched as closely as possible in regard stage of disease, age, and sex. Preoperative diagnosis was established on the basis of radiological investigation (barium enema) and confirmed by histological examination of the operative specimen.

Table 5.1 *Patient Details*

	Controls	rhGH Treated
Pair 1		
Age	80	73
Sex	M	M
Weight	45	67
Disease	Right colon, C	Splenic flexure, C
Pair 2		
Age	75	72
Sex	F	F
Weight	60	51
Disease	Ascending colon, A	Sigmoid, A
Pair 3		
Age	85	83
Sex	F	F
Weight	60	55
Disease	Sigmoid, A	Caecum, A
Pair 4		
Age	71	64
Sex	F	F
Weight	80	52
Disease	Sigmoid, B	Caecum, B
Pair 5		
Age	70	69
Sex	M	M
Weight	73	72
Disease	Upper Rectum, B	Sigmoid, B

5.2.(ii) *Study Design* To five patients a daily dose of recombinant human growth hormone (Genotropin, KabiVitrum Laboratories, Stockholm) of 20 IU was administered by subcutaneous injection at 0800 hours for two days, with the final injection being given on the morning of surgery. The patients were fasted and prepared for surgery in accordance with current surgical practice. Sixty minutes prior to the patient proceeding to the operating rooms a 20 gauge intravenous catheter was inserted into a forearm vein and a constant infusion of universally labelled ^{14}C l-leucine at a rate of 2.9 ηCi/kg.min was begun following priming of the leucine pool with 180 ηCi/kg of U ^{14}C L-leucine (60:1 prime: infusion ratio).

Following the induction of anaesthesia an18 gauge intravenous catheter was inserted into the contralateral forearm for plasma sampling. As soon as the operative specimen was removed, small samples of malignant and surrounding normal tissue were excised, blotted dry and immediately placed in liquid nitrogen. Similar specimens of skeletal muscle (rectus abdominis), skin and liver were then taken and a 40 ml blood sample withdrawn. This latter was chilled then centifuged, and the separated plasma frozen prior to analysis in our laboratory.

Preoperative radiological investigations, operative findings and histological examination of the resected specimens by Auckland Hospital pathologists were used to stage the extent of the extent of tumour invasion according to Duke's classification but including a D stage when metastatic disease was evident beyond the regional lymph nodes. The five rhGH treated patients were then matched as closely as possible in regard to stage of disease, age and sex to control patients not treated with rhGH whom we have studied previously employing identical methodology.

5.2.(iii) *Statistics* The data from control and treatment groups were compared using paired *t* tests.

5.3 Results

5.3.(i) *Hormones* The plasma levels of growth hormone, IGF-1 and insulin were significantly higher in the rhGH treated group than in the control patients (table 5.2). There was no difference in plasma cortisol levels between the two groups.

5.3.(ii) *Whole Body Protein Kinetics* The rate of appearance (Ra) of leucine in the rhGH treated patients was 1.5 +/- 0.2 μmol/kg.min, which tended to be higher but was not statistically different from the Ra leucine of the control patients (1.3 +/- 0.2 μmol/kg.min).

5.3.(iii) *Tissue Data* The malignant tissue removed from the rhGH treated patients was incorporating ^{14}C labelled leucine at the same rate as the control group (table 5.3). Similarly there were no significant differences in fractional synthetic rates of normal colon, skin or liver between treatment and control groups, although the mean FSR tended to be higher in skin and colon removed from the rhGH treated group. The FSR of skeletal muscle in the rhGH treated patients was 3.5 +/- 0.6%/dayand this was significantly higher than the mean value for the control group (2.2 +/- 0.5%/day, $p< 0.05$).

Table 5.2 *Plasma Hormone Levels*

	Controls	rhGH	Significance
HGH *μg/l*	1.6 +/- 0.3	29.7 +/- 4.8	$p < 0.01$
IGF-1 *μg/l*	310 +/- 56	489 +/- 72	$p < 0.02$
Insulin *mIU/l*	5 +/- 1	13 +/- 4	$p < 0.04$
Cortisol *ηmol/l*	751 +/- 229	741 +/- 82	n.s.

Table 5.3 *The Effect of rhGH on Tissue Protein Synthesis*

	Controls	rhGH	Significance
FSR Cancer	17.6 +/- 2.2	16.1 +/- 2.3	n.s.
FSR Colon	2.9 +/- 0.5	3.6 +/- 0.4	n.s.
FSR Skin	2.9 +/- 0.5	4.4 +/- 1.4	n.s.
FSR Muscle	2.2 +/- 0.5	3.5 +/- 0.6	$p < 0.03$
FSR Liver	23.0 +/- 4.1	24.0 +/- 3.2	n.s.
FSR Cancer/ FSR Colon	3.4 +/-0.8	3.2 +/- 0.2	n.s.

* FSR = fractional synthesis rate in %/day

5.4 Discussion

5.4.(i) *Rationale for hormonal manipulation* Cancer cachexia presents a nettlesome problem to the oncology surgeon. Although it has been clearly demonstrated that significantly wasted patients suffer from higher rates of postoperative complications than do patients without weight loss[70,150,231], efforts to reduce postoperative morbidity by preoperatively replenishing depleted patients with nutritional techniques such as intravenous hyperalimentation have not been consistently effective[145,162,239,247]. Using isotopic methodology, Shaw and Wolfe have demonstrated that although it is possible to achieve a zero nitrogen balance in cachectic cancer patients with IVN, protein anabolism is not achieved in this group as a whole[296,310]. It would appear that the changes to host protein metabolism affected by tumour bearing reduce the efficiency with which exogenous amino acid infusion improves nitrogen balance, suggesting a need for an effective form of metabolic manipulation.

The purpose of our study was to determine whether recombinant human growth hormone possesses potential as a therapeutic agent to aid in the preoperative repletion of patients with cancer cachexia. To do so, the effect of a short preoperative course of rhGH on the rate of protein synthesis was measured at the tissue level *in vivo*. This was accomplished by measurement of the rate of incorporation of a radiolabelled amino acid tracer (U ^{14}C leucine) into the protein of the tissue under study.

5.3.(ii) *Hormonal response* The dose of recombinant human growth hormone administered raised the plasma growth hormone concentrations to supraphysiologic levels at the time of sampling, which was on average four hours after the last dose. Recombinant growth hormone has a half life of approximately 15 minutes[329], and such high levels would be short-lived. Most evidence suggests that the protein synthesis stimulating effect of

growth hormone is mediated by IGF-1, the plasma IGF-1 levels were almost 60% higher in the rhGH treated group than in the control patients. The plasma insulin levels were significantly higher in the rhGH treated group (13 +/- 4 mmol/l) than in the control group (5 +/- 1 mmol/l, $p < 0.04$). This is probably a response to growth hormone's peripheral antagonism of the effect of insulin[83,242].

5.4.(iii) *Effect on protein metabolism* The fractional rate of protein synthesis of the skeletal muscle (rectus abdominis) of the rhGH treated patients was significantly higher than in the control group (3.5 +/- 0.6 %/24 hours vs 2.2 +/- 0.5 %/24 hours, $p < 0.03$), and the FSR of skin and colon tissue also tended to be more rapid in the treatment group. These data are consistent with those of other research groups who have found that the improvement in nitrogen balance following rHGH administration to normal volunteers or surgical patients resulted from an increase in the rate of protein synthesis, which may be enhanced by a reduction in the rate of protein catabolism[219,266,350].

There was no evidence of rhGH administration accelerating the rate of protein synthesis in malignant tissue, which was 16.1 +/- 2.3%/day in the rhGH treated group and 17.6 +/- 2.3%/day in the control group. The potential advantage of preoperative replenishment of wasted cancer patients would be dissipated if during the time of nutritional support the growth of malignant tissue was encouraged as much as those of the host. Recently, IGF-1 receptors have been reported to be present in breast cancer suggesting the possibility of stimulation of tumour growth by IGF-1[263]. Malignant and host tissue growth was found to be equally responsive to exogenous growth hormone in a hypophysectomized rats, but neither tumour nor host tissues were effected by growth hormone in intact mice[327]. The animal and human studies in which attempts have been made to alter

the rate of growth of malignant and host tissue by nutritional or hormonal manipulation suggest that malignant tissue is synthesizing protein at a maximal rate and that it can further enhanced only in situations were a specific deficiency is reversed (eg refeeding in starvation[282] or hormone replacement following hypothysectomy[98]).

5.4.(iv) *Study shortcomings* However, our results require caution in interpretation. The methodology employed in this study measures only the rate of protein synthesis, and so effects of rhGH on the rate of protein catabolism would pass undetected. Accordingly, it is difficult to comment conclusively on the effect of this hormone on the net protein content of the tissues studied. However, several studies addressing the manner in which growth hormone affects protein kinetics conclude that it does so primarily by accelerating protein synthesis.

Another cause for reservation is the relationship between the effects of rhGH on protein kinetics and the nutritional status of the individual being studied. There is now substantial evidence to suggest that the anabolic properties of hGH are most clearly manifest when the hormone is given in conjunction with adequate protein intake, and that these effects tend to be attenuated by starvation[106]. The patients whom we studied had fasted overnight as part of their routine operative preparation, and it is possible that the effect of rhGH on the rate of protein synthesis in the fed state may be different. Also to be considered is the histological uniformity of our patients, all of whom had tumours arising from the epithelium of the colon or upper rectum. It may well be that tumours originating from other tissues may be responsive to growth hormone administration.

5.4.(v) *Summary* Our data suggest that rhGH may have therapeutic potential for the cachectic cancer patient and that its role in the preoperative

replenishment and postoperative support of such patients is worthy of further evaluation.

Chapter 6

The Effects of Infusion of IGF-I, IGF-II and Insulin on Glucose and Protein Metabolism in Fasted Lambs

6.1 Introduction

As insulin-like growth factors I and II share considerable structural homology with insulin[35], it is not surprising that the three hormones have qualitative similarities in their biological actions[16,394]. IGF-I and II have been observed *in vitro* to increase glucose uptake and to possess anti-lipolytic activity in cell cultures[395], but with a molar potency which is considerably less than that of insulin[36,264]. These findings have been reproduced *in vivo* during IGF I and II and insulin infusions in rats[393] and following the administration of IGF-I and insulin bolus injections to human subjects[134]. However, the IGFs have been reported to possess considerably more mitogenic and growth promoting activity than insulin in *in vitro* preparations[116], and to be more effective in accelerating protein synthesis and reducing protein breakdown in L6 myotube cultures[279]. IGF-I has the ability to restore growth in hypophysectomized[135,290] and diabetic rats[292]. Recently it has been reported that acute IGF-1 infusions reduce the rate of whole body protein catabolism in normal adult rats [170]. It has been suggested that while growth may be primarily mediated by the paracrine production of IGF in tissues, circulating IGF may play a role in coordinating the anabolic state of the organism[259]. IGF-II appears to have some growth promoting potential in hypophysectomized rats, but to be a less potent anabolic agent than IGF-I[224,291]. The ability of the three hormones to interact with each other's receptors and tissue differences in receptor distribution may account for many of the similarities in their biological

effects. It has been suggested that whereas the actions of IGF-I on glucose metabolism are mediated by insulin receptors, stimulation of protein synthesis by IGF-I is mediated by IGF receptors[184].

In contrast to insulin, virtually all IGF is non-covalently bound to several distinct forms of specific carrier proteins[81,222]. The IGF binding proteins greatly extend the plasma half-life of these hormones[153] and may serve as reservoir function, ensuring that small amounts of IGFs are constantly available to the tissues[34]. It has been suggested that only free IGF-I and II have hypoglycaemic activity[393]. While the binding proteins appear to inhibit some IGF-I actions[232], there is evidence from *in vitro* studies that the association of IGF-I with plasma binding protein enhances its mitogenic activity[34,97].

As have been summarized in chapters 1 and 4, there are several studies which report improvement in the nitrogen balance of severely septic or injured patients following the administration of human growth hormone. As IGF-I may mediate the anabolic effects of growth hormone, IGF-I may possess some clinical potential in the nutritional support of such critically ill patients who typically have an accelerated rate of protein catabolism and who may have some degree of growth hormone resistance[80]. We have therefore undertaken a study of the effects on protein and glucose metabolism of a short term infusion of pharmacological doses of rhIGF-I. The effects of this infusion were compared to a molar equivalent dose of rhIGF-II and to an insulin infusion of equivalent hypoglycaemic potential. A lower dose of rhIGF-I which did not induce hypoglycaemia was infused in order to attempt to determine whether there was a difference in the sensitivity of carbohydrate and protein metabolism to IGF-I.

6.2 Materials and Methods

6.2.(i) *Animals and Preparation* The studies were performed using cryptorchid crossbred lambs which were fasted for 48 hours and prepared in the manner described in section 3.4.(i).

6.2.(ii) *Protocol* The experimental protocol is illustrated in fig 6.1. The priming dose for the isotopic infusions was administered at 0900 hrs, and during the first 180 minutes normal saline and isotopes were infused simultaneously while isotopic equilibrium was being reached. At the end of this period blood specimens were collected to enable a baseline measurement of metabolic parameters. Following this, the test infusions were begun and continued over a 300 minute period during which time plasma specimens were taken at 60 minute intervals. At the end of the test infusions, the animals were sacrificed by intravenous injection of 5g phenobarbitone and tissue samples of liver, heart, diaphragm and psoas and hind limb adductor muscle were taken as rapidly as possible, washed in cold normal saline, blotted dry and rapidly frozen in liquid nitrogen. All blood specimens were collected in chilled, heparinized tubes and were immediately centrifuged and the separated plasma was frozen prior to being analysed.

6.2.(iii) *Hormone Infusions* The animals were infused during the test period with one of five solutions: normal saline at a rate of 10 ml/hr (n = 6), rhIGF-I at a dose of 50 μg/kg.hr (n = 6) or 15 μg/kg.hr(n = 4), rhIGF-II at a dose of 50 μg/kg.hr (n = 4), or recombinant human insulin at a dose of 1 μg/kg.hr (n = 4). The insulin dose was empirically chosen following preliminary experiments to result in the same percentage reduction in the plasma glucose concentration as the high dose (50 μg/kg.hr) IGF-I infusion. This represents an rhIGF-I: insulin molar ratio of 50:1.

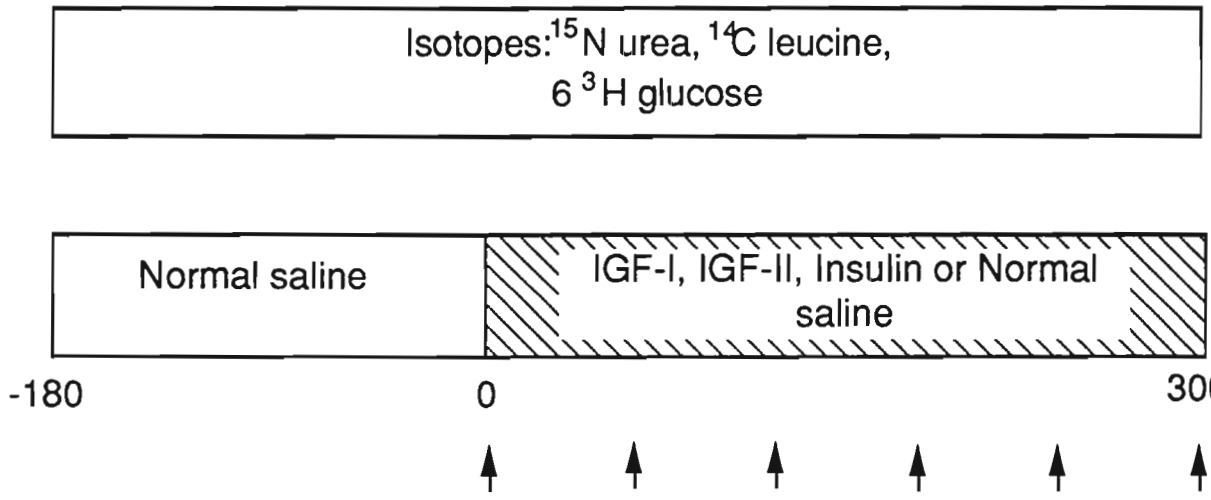


Figure 6.1 *The experimental protocol. Plasma specimens were taken at 60 minute intervals between 0 and 300 minutes.*

The low (15 μg/kg.hr) dose rhIGF-I was selected because although it resulted in a doubling of the baseline plasma IGF-1 concentration, it did not significantly alter glucose kinetics. The rhIGF-I was donated by Kabi Peptide Hormones (Stockholm, Sweden) and the rhIGF-II by Elanco (Greenfield, Illinois). The recombinant human insulin was obtained from Eli Lilly (Fegersheim, France). Immediately prior to infusion, all of the hormones were dissolved in 55 ml normal saline and 0.5 ml of the plasma from the animal to be infused was added to reduce hormone binding to the surface of the syringe and plastic tubing. The hormone/normal saline solutions were infused at a rate of 10 ml/hr.

6.2.(iv) *Isotopic Infusions* The rates of turnover of metabolic substrates were measured by the technique of primed constant infusion of stable and radioactively labelled tracers. The rate of appearance (Ra) of glucose was measured by 6 ^{3}H glucose infusion of 7.5ηCi/kg.min following an 80:1 prime. The rate of appearance (Ra) of urea was determined by a primed constant infusion of ^{15}N urea (rate 10 μg/kg.min and 450:1 prime) and whole body leucine kinetics were quantified by a primed constant infusion of ^{14}C leucine at a rate of 7.5 ηCi/kg.min following an 60:1 leucine prime.

6.2.(v) *Statistics* Dunnett's test was applied to repeated measures taken over the duration of the infusions. Similarly, Dunnett's variation of one way factorial ANOVA was used to compare the tissue rates of fractional synthesis of the hormone treated with those of normal saline infused controls. The data are expressed as means +/- SEM.

6.3 Results

6.3.(i) *Plasma Hormone Concentrations* The low and high dose rhIGF-I infusions increased the plasma IGF-I concentration above baseline by two and five fold respectively, whereas infusion of rhIGF-II reduced the plasma IGF-1 concentration by almost half (fig 6.2). The plasma concentration of IGF-I did not change significantly during the insulin infusion.

The distribution of IGF-1 between binding-protein bound (35-60 kDa and 150 kDa) and free (7kDa) forms was determined at 120 minutes and 300 minutes during the IGF-1 infusions and at 300 min during the saline infusions (figs 6.3 a-c). The level of free IGF-1 in the normal saline controls was below the limit of sensitivity of the column chromatographic extraction and radioimmunoassay (40 μg/l); however measurable amounts were present following both the rhIGF-1 infusions (67 +/- 4 μg/l and 240 +/- 54 μg/l for the low and high dose infusions respectively). The pattern of distribution of the protein bound and free forms of IGF-1 were almost identical between the low and high dose IGF-1 infusions, and the infused rhIGF-1 appeared to associate preferentially with the 35-60 kDa rather than the 150 kDa binding proteins.

The plasma insulin levels were below the limit of sensitivity of the RIA employed (0.15 μg/l) both before and during normal saline, IGF-I and IGF-II infusions. Insulin infusion significantly increased the plasma insulin level from <0.15 μg/l to 0.73 +/- 0.13 μg/l at 300 minutes .

6.3.(ii) *Glucose Metabolism*

i) Normal saline. The plasma glucose concentration, glucose rate of appearance and the rate of glucose clearance did not alter significantly over the duration of the normal saline infusion (fig 6.4 a-c)

ii) rhIGF-I 15 μg/kg.hr. Despite a doubling of the plasma IGF-I level by the end of this infusion and the presence of detectable quantities of free IGF-I in the plasma, no changes in the three parameters of glucose metabolism were observed.

iii) rh IGF-I 50 μg/kg.hr. There was a 41 % reduction ($p < 0.01$) in the plasma glucose concentration by the end of the high dose IGF-I infusion, which reflected a 55 % increase ($p < 0.01$) in the rate of glucose clearance (from 3.8 +/- 0.6 ml/kg.min to 5.9 ml/kg.min). The rate of glucose appearance was unaltered.

iv) rhIGF-II 50 μg/kg.hr. Glucose clearance increased by 15 % over the course of infusion of this hormone ($p < 0.05$). However, as this effect was associated with a tendency for the Ra glucose to increase, the plasma glucose concentration was not significantly changed.

v) Insulin 1 μg/kg.hr. The plasma glucose concentration decreased by 44% over the duration of this infusion, from 3.6 +/- 0.3 mmol/l to 2.0 +/- 0.5 mmol/l ($p < 0.05$). There was a transient reduction in the Ra glucose which was maximal at 180 minutes, but a progressive increase in the rate of glucose clearance.

6.3.(iii) *Plasma Electrolytes*

None of the test infusions altered the plasma concentrations of Na^+, Cl^- or urea. Over the course of both the high dose rhIGF-I and insulin infusions, the plasma K^+ concentration was reduced from 3.8 +/- 0.1 mmol/l to 3.1 +/- 0.1 mmol/l ($p < 0.01$) and from 3.9 +/- 0.1 mmol/l to 3.3 +/- 0.1 mmol/l ($p < 0.01$) respectively.

6.3.(iv) *Protein Metabolism*

i) Normal saline. The Ra urea in one of the animals decreased markedly, and the data from this animal was disregarded, being more than 5 standard deviations beyond the mean. There was a small but consistent reduction in the mean rate of net protein loss of 3% ($p < 0.05$) over the 300 minute normal saline infusion (fig 6.5). The Ra leucine was not altered significantly by the normal saline infusion (fig 6.6), and the protein fractional synthetic rates of the tissues samples are illustrated in figs 6.7 a-e.

ii) IGF-I 15 μg/kg.hr. The rate of net protein loss was reduced from 4.1 +/- 0.4 g/kg.day to 3.5 +/- 0.3 g/kg.day during the low dose IGF-I infusion ($p < 0.05$). This equates to an 11 % reduction in the rate of net protein loss relative to the normal saline infused controls. The Ra leucine was not significantly altered by this dose of IGF-I (fig 6.6), and in none of the tissues sampled was the FSR significantly different from those of the normal saline infused controls (figs 6.7 a-e).

iii) IGF-I 50 μg/kg.hr. The rate of net protein loss decreased significantly over the time of the high dose rhIGF-I infusion from 3.5 +/- 0.2 g/kg.day to 2.9 +/- 0.2 g/kg.day ($p < 0.005$), which represents a 15% reduction in NPL relative to the saline infused controls (fig 6.5). The rate of leucine appearance fell sequentially over the time of the infusion from 5.0 +/- 0.4 μmol/kg.min to 3.4 μmol/kg.min ($p < 0.01$) (fig 6.6). The FSR in adductor and psoas muscle were increased ($p < 0.05$) The FSR in cardiac and diaphragmatic and cardiac muscle were approximately twice that observed in the saline controls ($p < 0.01$) (figs 6.7a-d). Similarly, the FSR of liver tissue was significantly higher in the high dose IGF-I infused animals ($p < 0.05$) (fig 6.7e).

iv) IGF-II 50 μg/kg.hr. The rate of NPL was not significantly altered by the infusion of this hormone. There was a tendency for the Ra leucine

to decline over the early part of the infusion (fig 6.6). In none of the tissues sampled was the FSR significantly different from those measured in the saline infused controls.

v) Insulin 1 μg/kg.hr. A reduction in NPL from 4.1 +/- 0.4 g/kg.day to 3.5 +/- 0.3 g/kg.day ($p < 0.05$) was observed during the insulin infusion, representing a 12% reduction relative to the normal saline infused controls. The leucine Ra was not significantly altered (4.6 +/- 0.2 μmol/kg.min to 4.0 +/- 0.5 μmol/kg.min), and in none of the tissues sampled was the FSR different from the control values.

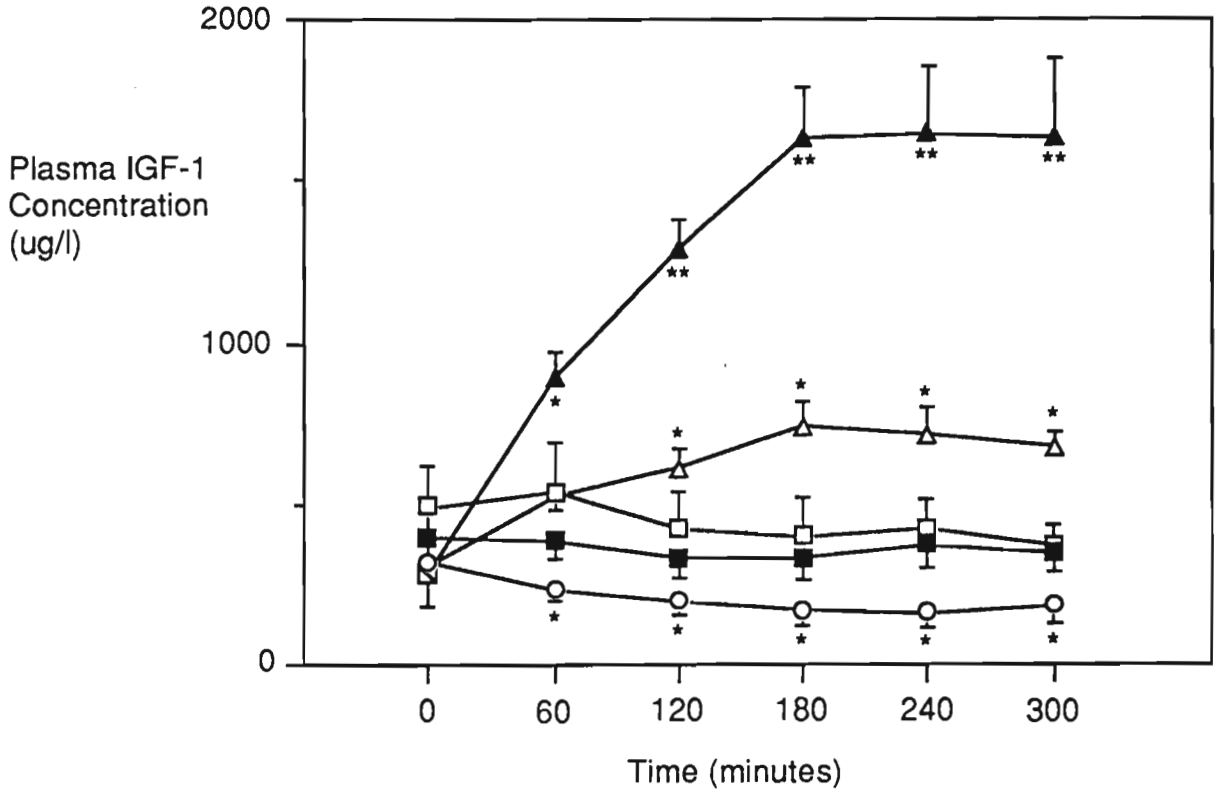
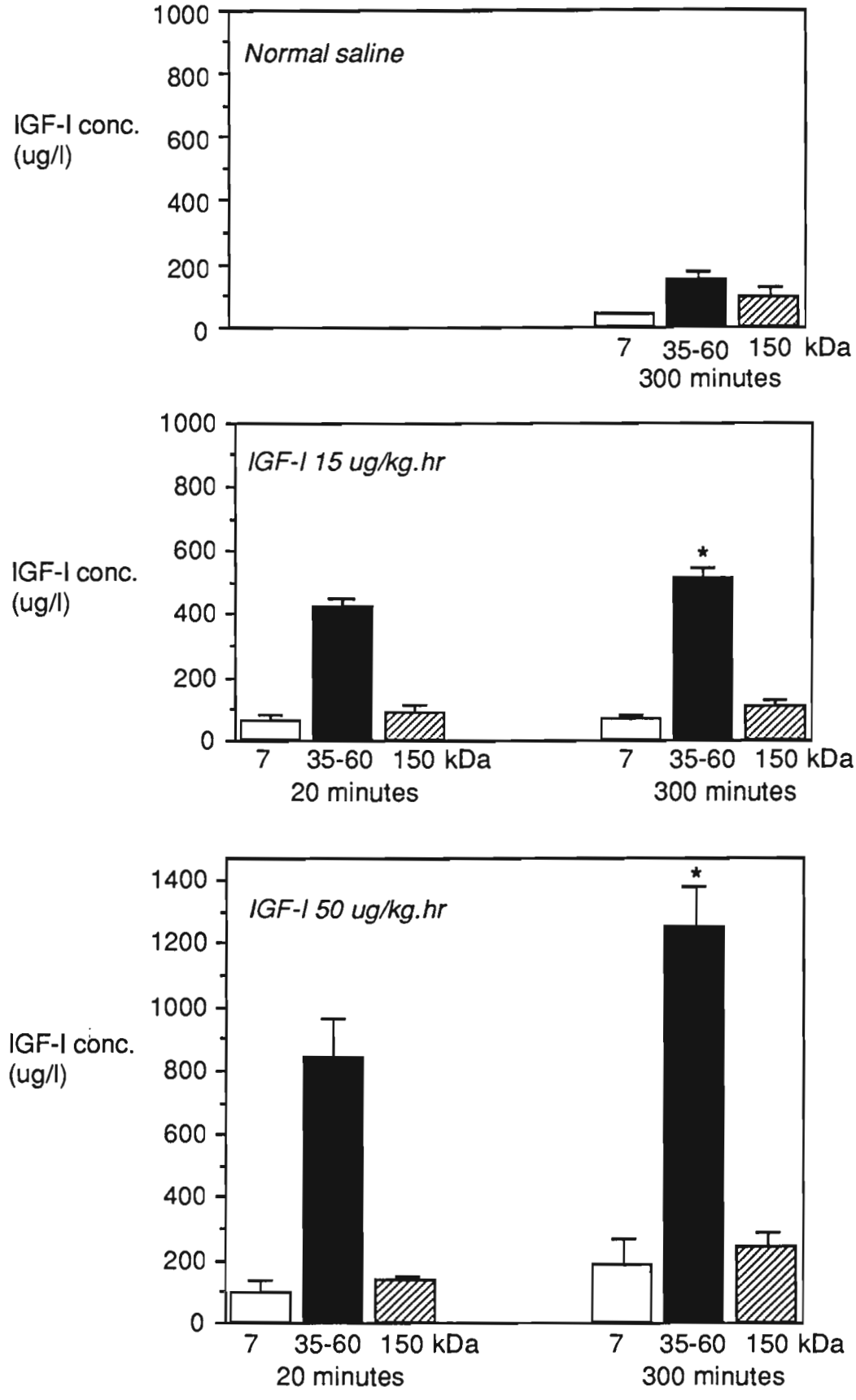


Figure 6.2 *Plasma IGF-I concentrations during hormone infusions. rhIGF-I caused a dose related increase in plasma IGF-I levels, whereas IGF-II infusion halved the plasma IGF-I concentration. rhIGF-I 15 ug/kg.hr (—△—), rhIGF-I 50 ug/kg.hr (—▲—), rhIGF-II 50 ug/kg.hr (—○—), insulin 1 ug/kg.hr (—■—) and normal saline (—□—) infusion.*

* $p < 0.05$, ** $p < 0.005$ *compared to 0 min.*

Figures 6.3 a-c. *(Following page) The distribution of IGF-I across the IGF binding proteins. The 7kDa fraction represents free IGF-I and the 35-60 and 150kDa forms are IGF associated with low and high molecular weight binding proteins. The concentration of free IGF-I in the normal saline infused animals was below the minimum detectable dose of the RIA (< 40 ug/l).*

* *$p < 0.05$ compared to normal saline infused controls.*


Normal saline
IGF-I conc. (ug/l)
1000
800
600
400
200
0
7
35-60
150 kDa
300 minutes
IGF-I 15 ug/kg.hr
IGF-I conc. (ug/l)
*
7
35-60
150 kDa
20 minutes
300 minutes
IGF-I 50 ug/kg.hr
IGF-I conc. (ug/l)
1400
1200
1000
800
600
400
200
0
*
20 minutes
300 minutes

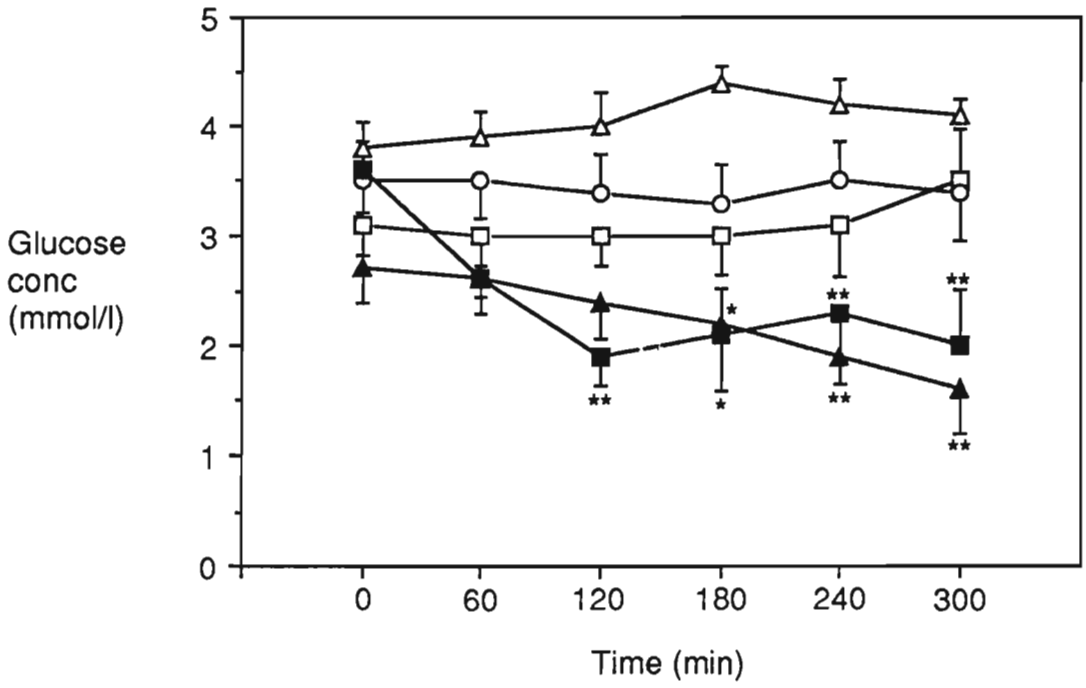


Figure 6.4a *Effect of rhIGF-I 15 ug/kg.hr* (—△—), *rhIGF-I 50 ug/kg.hr* (—▲—), *rhIGF-II 50 ug/kg.hr* (—○—), *insulin 1 ug/kg.hr* (—■—) *and normal saline* (—□—) *infusion on glucose concentration.*

* $p < 0.05$, ** $p < 0.01$ *compared to 0 min.*

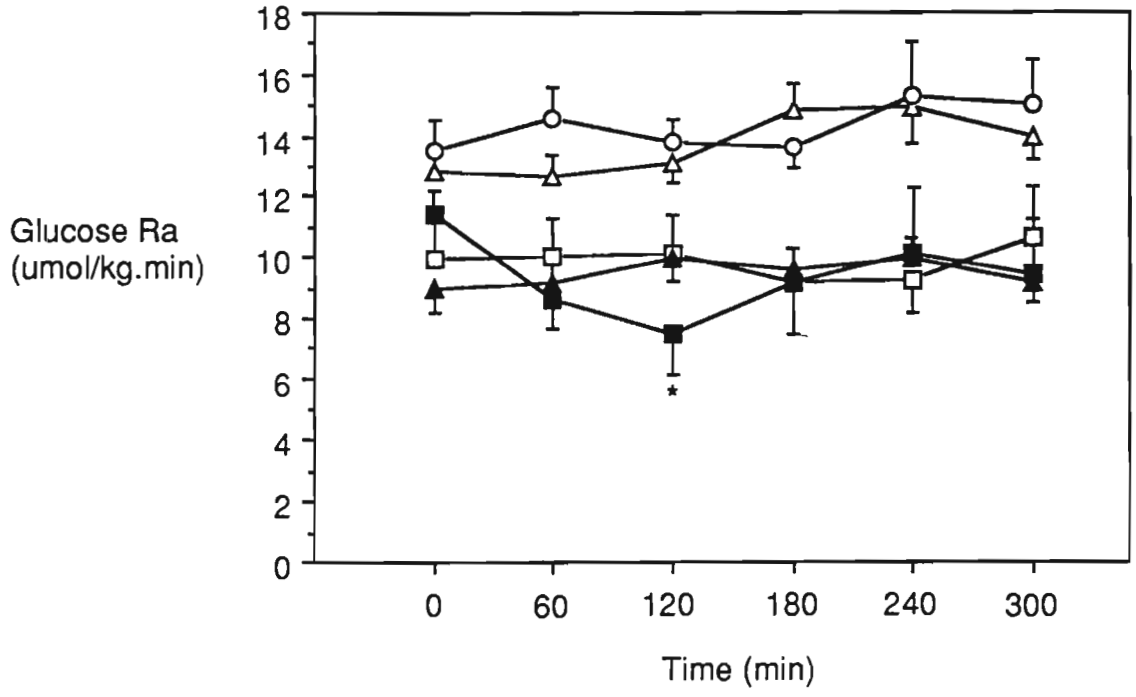


Figure 6.4b *Effect of rhIGF-I 15 ug/kg.hr* (—△—), *rhIGF-I 50 ug/kg.hr* (—▲—) *rhIGF-II 50 ug/kg.hr* (—○—), *insulin 1 ug/kg.hr* (—■—) *and normal saline* (—□—) *infusion on glucose rate of appearance.*

* $p < 0.05$ *compared to 0 min.*

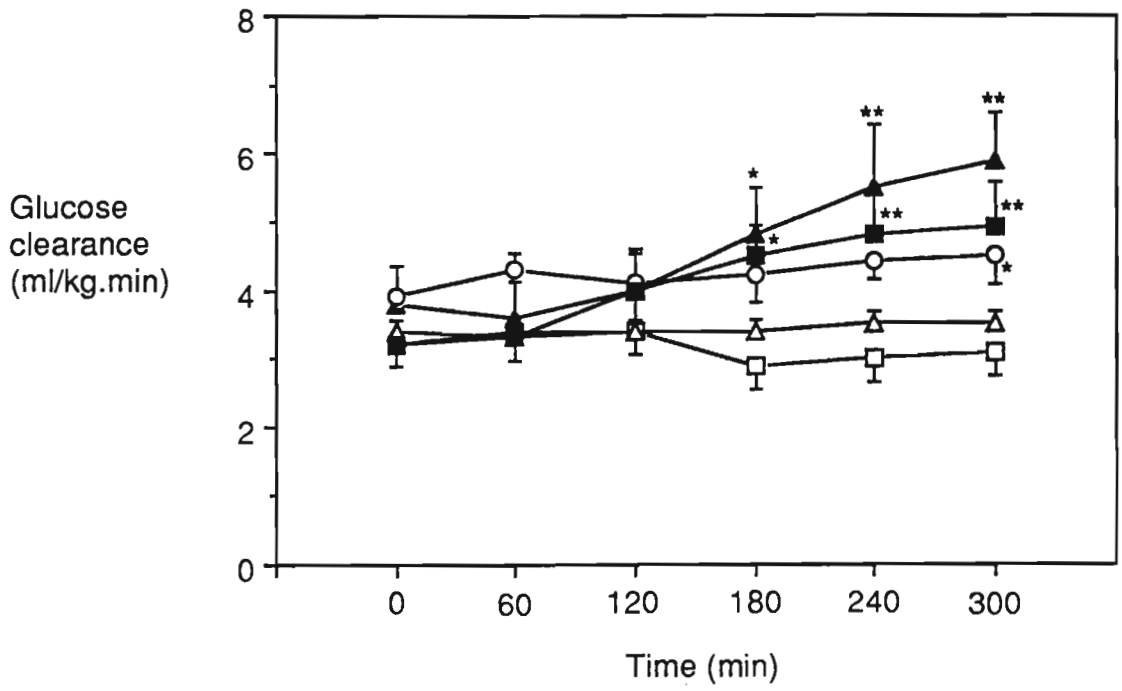


Figure 6.4c *Effect of rhIGF-I 15 ug/kg.hr (—△—), rhIGF-I 50 ug/kg.hr (—▲—) rhIGF-II 50 ug/kg.hr (—○—), insulin 1 ug/kg.hr (—■—) and normal saline (—□—) infusion on rate of glucose clearance.*

* $p < 0.05$, ** $p < 0.01$ *compared to 0 min.*

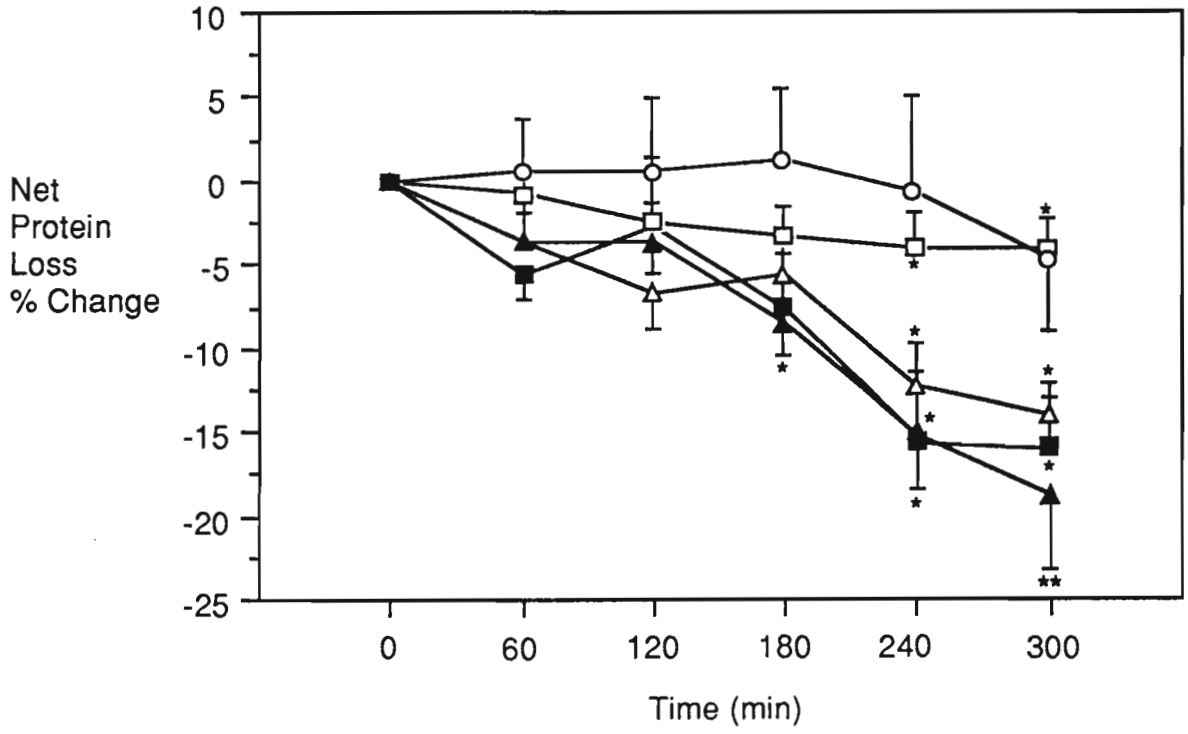


Figure 6.5 *Percentage change of net protein loss during hormone infusion: rhIGF-I 15 ug/kg.hr (—△—), rhIGF-I 50 ug/kg.hr (—▲—), rhIGF-II 50 ug/kg.hr (—○—), insulin 1 ug/kg.hr (—■—) and normal saline (—□—).*

** $p < 0.05$, ** $p < 0.01$ compared to 0 min.*

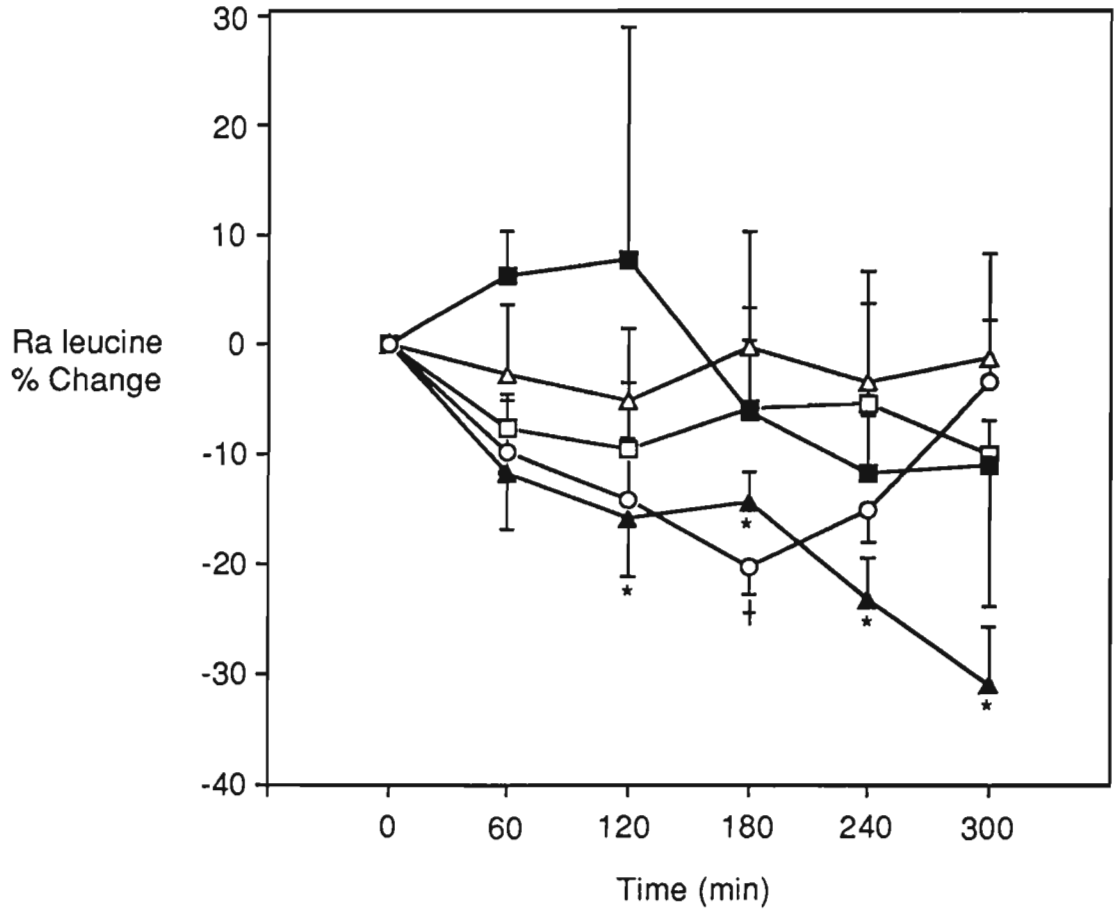
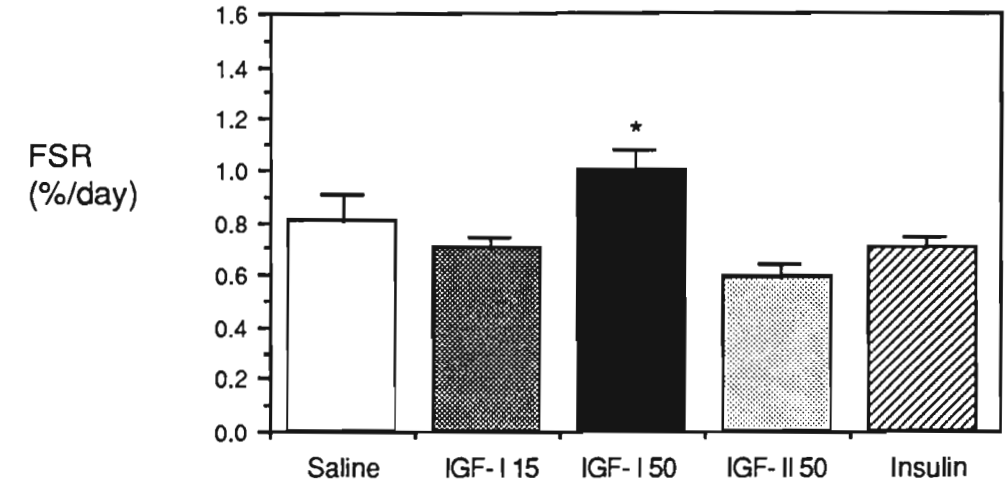


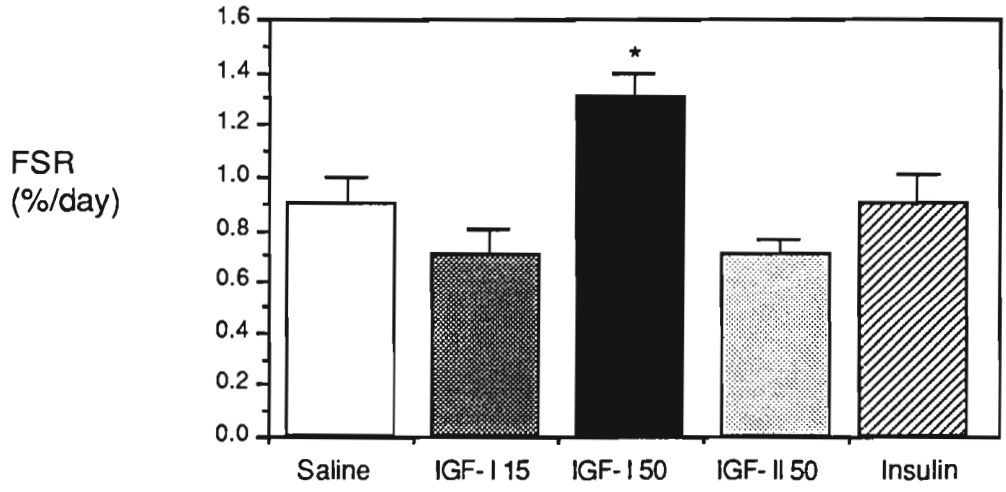
Figure 6.6 *Percentage change of leucine Ra during hormone infusion: rhIGF-I 15 ug/kg.hr (—△—), rhIGF-I 50 ug/kg.hr (—▲—), rhIGF-II 50 ug/kg.hr (—○—), insulin 1 ug/kg.hr (—■—) and normal saline (—□—).*

* $p < 0.05$, † $p < 0.10$ *compared to 0 min.*

(a) Adductor



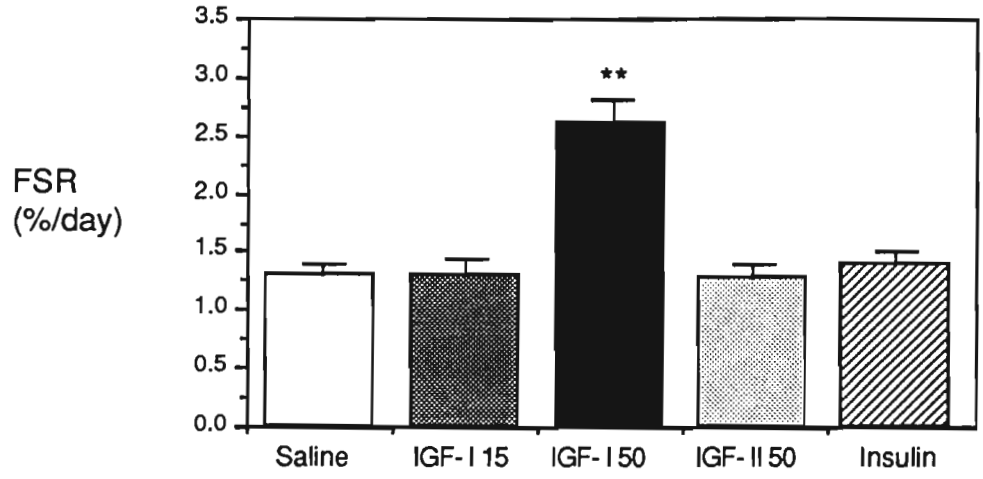
(b) Psoas



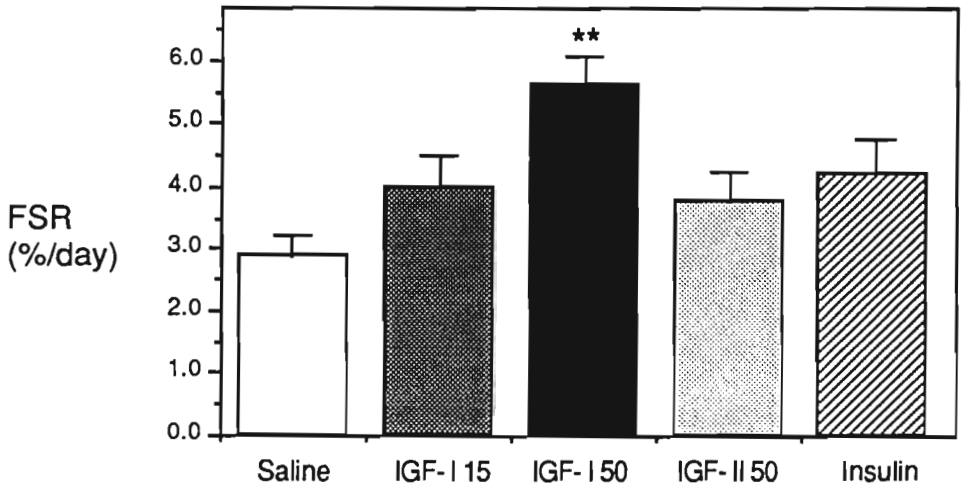
Figures 6.7 a - e *(Above and following page) Fractional synthetic rate of protein synthesis in skeletal and cardiac muscle and liver tissue.*

** $p < 0.05$, ** $p < 0.01$ compared to normal saline infused controls.*

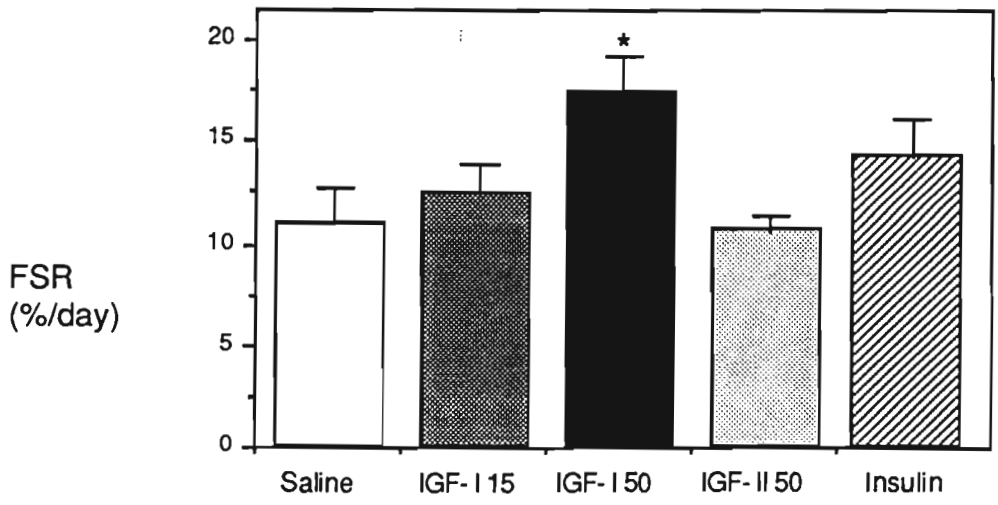
(c) Diaphragm



(d) Heart



(f) Liver



6.4 Discussion

6.4.(1) *Introduction* In this study, isotopic tracer methodology was used to investigate the metabolic effects of short term infusions of rhIGF-I and II in fasted lambs. These effects were compared to those of an insulin infusion of the same hypoglycaemic potential as the rhIGF-I infusion. The metabolic response to a lower dose of rhIGF-I which did not induce hypoglycaemia was also investigated. We observed that rhIGF-I is more potent than rhIGF-II in increasing the rate of peripheral glucose clearance and less potent than insulin (with an rhIGF-I: insulin dose ratio of 50:1 having the same hypoglycaemic effect). The rhIGF-I and insulin infusions reduced the rate of net protein loss to a similar extent, although only the high dose rhIGF-I infusion clearly stimulated protein synthesis in all of the tissues sampled. At lower doses of rhIGF-I infusion, protein metabolism was altered in the absence of any effect on carbohydrate metabolism. The rhIGF-II infusion did not reduce the rate of net protein loss or stimulate protein synthesis.

6.4.(ii) *Effect of Non-Binding Protein Bound Hormone* In the physiological state less than 1% of the total plasma IGF-I is not associated with binding proteins[81,222] and although the amount of free IGF-II has not been measured, it is likely that only negligible amounts are present under normal conditions given the relatively greater affinity of human[395], ovine[153] and rat[34] binding proteins for IGF-II relative to IGF-I. The hypoglycaemic action of the IGFs has been attributed to the presence of free hormone in the plasma[393] as protein bound IGF-I may be prevented from interacting with the insulin receptor. When large amounts of exogenous IGF are infused rapidly, the capacity of the binding proteins may be exceeded allowing unbound IGF in the plasma. At the end of both the low

and high dose rhIGF-I infusions 10% of the total plasma IGF-I was in the free form, but in absolute quantities there was almost four times more free IGF-I following the high dose infusion (figs 6.3 b and c). Only the high dose infusion effected an increase in the rate of peripheral glucose clearance (fig 6.4). It would appear that a threshold level of free IGF-I in the plasma must be exceeded prior a measurable increase in glucose clearance occurring. rhIGF-II also significantly accelerated the rate of glucose clearance, but the magnitude of this increase was less than one third as great as that of the same dose of rhIGF-I. Although not measured directly, we infer from this result that there was a significant amount of free IGF-II present in the plasma and that this was responsible for the increase in glucose clearance. The apparently less potent hypoglycaemic action of the IGF-II may reflect the greater affinity of IGF-II for the plasma binding proteins and consequently reduced amount of free IGF-II present. Alternatively, free IGF-II may be cleared more rapidly than free IGF-I.

6.4.(iii) *Effects on Glucose Metabolism* The actions of the high dose rhIGF-I and rhIGF-II infusions on glucose metabolism could be distinguished from those of insulin. Whereas the hypoglycaemic action of insulin resulted from both an increase in the rate of glucose clearance and a reduction in the rate of glucose release, both IGF-I and IGF-II failed to suppress the rate of glucose production. Jacob and colleagues[170] have described a similar disparity in response between insulin and IGF-I in 24 hour fasted adult rats. These observations suggest that not all the actions of IGF-I on carbohydrate metabolism can be ascribed to actions on the insulin receptor. The issue of whether the effects of IGF-I on carbohydrate metabolism are mediated via the insulin receptor has been controversial[184]. Our data favour at least some independent effects. The differential effect of the hormones on carbohydrate metabolism may reflect a greater sensitivity

of hepatic tissues to the hypoglycaemic actions of insulin and would be consistent with the relative paucity of IGF receptors on liver cells except during times of growth[57].

6.4.(iv) *Effects on Protein Metabolism* The high and low dose rhIGF-I and insulin infusion resulted respectively in a 15%, 11% and 12% reduction in the rate of net protein loss relative to the normal saline infused controls (fig 6.5). The ability of IGF-I to promote growth after chronic administration or conserve protein over the course of short term infusions has been observed in hypophysectomized[290] and diabetic rats[292], but there is little *in vivo* data clarifying whether the anabolic actions of this hormone represent a reduction in catabolism, a stimulation of protein synthesis, or a combination of both. IGF-I administration to starved mice reduces weight loss significantly[259], suggesting a role of circulating IGF-I as a regulator of anabolic state. In our animals which received the high dose rhIGF-I infusion the rate of leucine flux was significantly lowered, implying that the rate of whole body protein catabolism is reduced by IGF-I. As the rate of ^{14}C labelled leucine incorporation into the five tissues sampled was elevated, we conclude that the protein conserving effects of IGF-I result from both a reduction of catabolism and an acceleration of protein synthesis.

Our observations contrast with those of Jacob and coworkers[170] who have reported that in normal adult rats a high dose IGF-I infusion of short duration reduced the rate of protein degradation without accelerating the rate of synthesis. Rather, infusion of IGF-I reduced the rate of leucine uptake by muscle and liver tissue and the effects of IGF-I on protein kinetics observed in this study were almost indistinguishable from those of insulin. There exist several differences between our study and that of Jacob and coworkers which may account for the disparity in the observed effects on protein synthesis. In the latter study, IGF-I was infused at a rate five times

faster than in our own and a substantial loading dose was administered. It is probable that the amount of free IGF-I present in the plasma was such that there was considerable crossreaction with the insulin receptors, and hence a masking of differences of action of IGF-I and insulin. There exists also the possibility of differences in response between adult rats used in their study and growing lambs used in the present study; for example, hepatocytes appear to lose their IGF-I receptors with maturity[57].

The mechanism of the protein conservation seen in the low dose rhIGF-I and insulin infused animals is less clear than in the high dose IGF-I group. Although in both groups the mean Ra leucine tended to decrease over the duration of the infusion, in neither was this change consistent nor statistically significant. Similarly the rate of leucine incorporation into skeletal muscle, which because of their bulk have the greatest influence on whole body kinetics, were not significantly increased. However, our methodology would underestimate changes to the FSR of tissues as a result of the hormone infusion. This is because during the initial 180 minutes of ^{14}C leucine infusion (prior to the beginning of hormone infusion), the tissues incorporated labelled leucine at the basal rate, which would then act to dilute the effect of any change in the rate of tracer uptake over the subsequent 300 minute hormonal infusion. Accordingly, the methodology employed may have failed to detect a small increase in fractional synthesis rate following the low dose rhIGF-I or insulin infusions. Nevertheless, given the magnitude of the effect on FSR and Ra leucine of the high dose rhIHF-I infusion, it is clear that there are important differences in the effect of rhIGF-I and insulin infusion on protein metabolism.

No protein anabolic effects were attributable to the rhIGF-II infusions: this result is consistent with the limited *in vivo* data on the effects of this hormone on growth. Schoenle and coworkers[291] administered a six day subcutaneous infusion of IGF-I and IGF-II to hypophysectomized rats and

found that IGF-I but not IGF-II led to an increase in body weight and that although both hormones stimulated widening of the costal cartilage, IGF-II was clearly less potent than IGF-I. Both hormones have been reported to increase collagen synthesis and reduce collagen degradation in rat calvarial cultures, but IGF-I was approximately three times more potent than IGF-II [224]. The protein anabolic potential, if any, of infused rhIGF-II is likely to be counteracted by the resultant lowering plasma of IGF-I levels which has been observed by ourselves (fig 6.2) and by others in rats transplanted with IGF-II secreting tumours[370].

6.4.(v) *Role of Circulating Binding Protein Form* It is of interest that the effects of rhIGF-I on carbohydrate metabolism required a marked increase in free IGF-I. During the infusion of rhIGF-I, most associated with the 35-60 kDa binding protein fractions and there was no significant increase in the 150 kDa bound form. In contrast, following growth hormone administration the increase in circulating IGF-I is associated with both fractions and particularly with the 150 kDa form[84]. While the function of the differeing circulating forms of IGF-I has been a subject of speculation[125], no firm conclusions can be drawn from the available data. The present observations suggest that IGF-I does not have to be incorporated into the 150 kDa complex to have anabolic and/or anti-catabolic effects. As free IGF-I does not not rise substantially after growth hormone administration in sheep (but remains < 2 μg/l, Hodgkinson, Bass and Gluckman, personal communication), it would appear most likely that IGF-I in the 35-60 kDa fraction is responsible for the anabolic actions we observed. This size range includes at least three potential forms of IGF-I: that associated with BP -1, BP-2 and BP-3 not complexed into the 150 kDa form. Whether IGF-I leaves the circulation in association with any of these proteins or must first

dissociate is is unresolved[29]. Whether the effects we observed relate to one specific form of IGF-I is also unkown.

6.4.(v) *Summary and Speculation* We have observed that infused rhIGF-I can reduce plasma glucose by increasing glucose clearance and conserve protein by both encouraging protein synthesis in various tissue and by reducing protein metabolism. These observations support the postulation of O'Sullivan and colleagues[259] that circulating IGF-I plays a role as a regulator of protein metabolism in the fasted state. The metabolic effects of rhIGF-I could be distinguished from those of an insulin infusion of equal hypoglycaemic activity: the insulin infusion reduced the rate of glucose production whereas rhIGF-I did not, and insulin did not accelerate tissue protein synthesis. These observations suggest that at least some of the metabolic effects of circulating IGF-I are mediated by receptors other than those for insulin. As a low dose rhIGF-I infusion was able to reduce net protein loss without influencing glucose kinetics, we infer that protein metabolism is more sensitive than carbohydrate metabolism to circulating rhIGF-I. The protein sparing effect of rhIGF-I did not appear to be shared by rhIGF-II infusion of the dose investigated.

The anabolic effects of rhIGF-I may have therapeutic potential for severely septic or multiply injured surgical patients with a high rate of protein catabolism. In particular, stimulation of protein synthesis in cardiac and diaphragmatic muscle by exogenous rhIGF-I, combined with a putative positive inotropic action of this hormone[342], may be useful in the treatment of sepsis related cardiopulmonary failure.

Chapter 7

The Effects of Recombinant hIGF-I on Protein and Glucose Metabolism in rhTNF Infused Lambs

7.1 Introduction

The continued nitrogen loss which occurs in severely septic or multiply injured patients despite their being provided with optimal parenteral nutrition (IVN) has been discussed in chapter 1.. The difficulty in achieving a positive nitrogen balance in such patients has encouraged the investigation of various methods of pharmacological and hormonal manipulation designed to either limit protein breakdown or encourage its synthesis. However, few of these methods have yielded encouraging results. Even insulin, whose protein conserving properties have been well established in normal volunteers[117], has not consistently reduced nitrogen loss in severely ill surgical patients[217].

In the study reported in chapter 4 it was demonstrated that recombinant human growth hormone (rhGH) is an effective anabolic agent in surgical patients receiving IVN. The protein sparing effects of hGH are believed to be largely mediated via production of insulin-like growth factor I (IGF-I), and administration of recombinant human IGF-I has been reported to restore growth in hypophysectomized rats[135], to have a protein sparing effect in fasted intact rats[170] and to reduce weight loss in fasted mice[259]. Infusion of rhIGF-I was observed in the study described in chapter 6 to increase the *in vivo* fractional synthetic rate of protein in skeletal, diaphragmatic and cardiac muscle in lamb. These studies suggest that IGF-I may have properties useful in the nutritional support of severely ill surgical patients in whom anabolism is difficult to achieve. Indeed it has been suggested that

circulating IGF-I may be a physiological regulator of protein metabolism[259]. In the present study we wished to investigate whether the anti-catabolic effects of rhIGF-I are preserved in the septic state.

A septic/catabolic state was achieved experimentally by infusing fasted lambs with recombinant tumour necrosis factor (rhTNF). TNF, a peptide released by macrophages in response to endotoxin, has been increasingly implicated as one of the most important effectors of the metabolic response to sepsis[233]. Infusion of rhTNF replicates many of the clinical and metabolic features of sepsis, including fever, hypotension, anorexia, hyperglycaemia and a negative nitrogen balance[105,236,335], and passive immunization of baboons against TNF protects them from an otherwise lethal innoculum of *E.coli* [336]. The aim of this study was to determine whether the protein sparing action of rhIGF-I infusion is preserved during rhTNF infusion.

7.2 Materials and Methods

7.2.(i) *Animal Preparation* The studies were performed using cryptorchid crossbred lambs of average weight 19 kg and age range 3 to 5 months which were fasted for 48 hours and prepared in the manner described in section 3.4.(i).

7.2.(ii) *rhTNF and rhIGF-I Infusions* The experimental protocol is illustrated in figure 7.1. The animals were divided into four groups, all of which received a 480 minute infusion of isotopically labelled tracers. The 480 minute infusion was divided into two periods; a baseline period of 180 minutes during which isotopic equilibrium was reached, followed by a test period of 300 minutes during which the effects of rhIGF-I infusion were

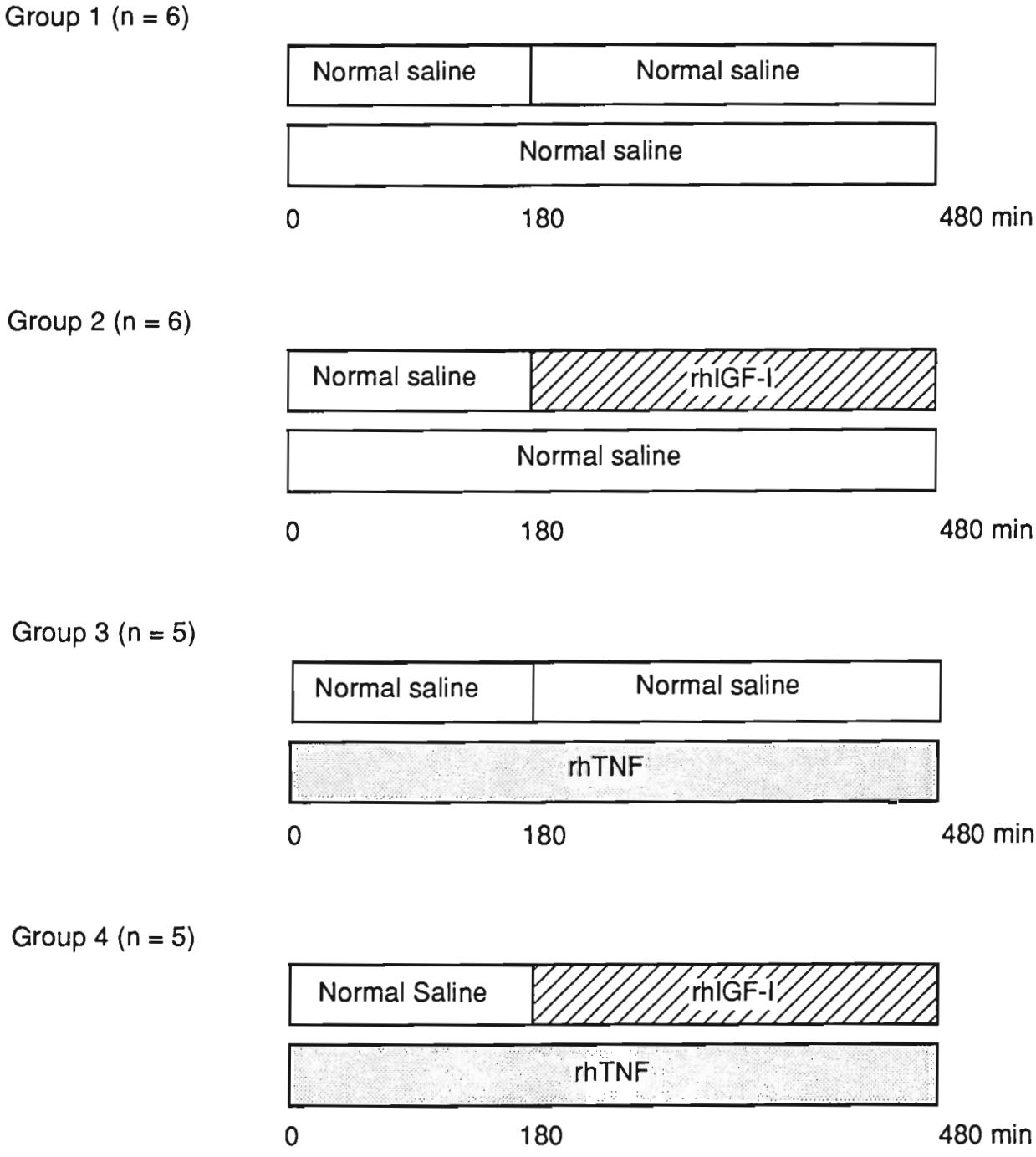


Figure 7.1 *The experimental protocol. In groups 2 and 4, rhIGF-I was infused during the second period at a dose of 50 ug/kg.hr. Groups 3 and 4 received a 480 minute infusion of rhTNF at 1 ug/kg.hr. All groups received a primed constant infusion of isotopic tracers for 480 minutes.*

determined. The protocol was designed to enable measurement of the effects of rhIGF-I in the basal state and during rhTNF infusion.

Groups 3 and 4 received a 480 minute infusion of rhTNF at a dose of 1 μg/kg.hr, while groups 1 and 2 were infused with normal saline at a rate of 6 ml/hr. The rhTNF was donated by Genentech (San Francisco, California) and was dissolved in a 2 mg/ml solution of bovine serum albumin immediately prior to infusion, and this solution was infused at a rate of 6 ml/hr.

Groups 1 and 3 were infused during the test period (from 180 min to 480 min) with a second normal saline infusion at a rate of 10 ml/hr while groups 2 and 4 received rhIGF-I at a dose of 50 μg/kg.min. Immediately prior to infusion, the rhIGF-I was dissolved in 55 ml normal saline and 0.5 ml of the plasma from the animal to be infused was added to reduce hormone binding to the surface of the syringe and plastic tubing. The hormone/normal saline solutions were infused at a rate of 10 ml/hr.

7.2.(iii) *Sampling* Blood specimens were removed at the beginning and end of the baseline period and at 60 minute intervals during the test period. These specimens were collected in chilled, sterile heparinized tubes and were immediately centrifuged and the separated plasma frozen prior to being analysed.

7.2.(iv) *Isotopic Infusions* The rate of appearance (Ra) of urea was determined by a primed constant infusion of ^{15}N urea (rate 10 μg/kg.min and 450:1 prime) and the glucose Ra was measured by 6 ^{3}H glucose infusion of 7.5 ηCi/kg.min following an 80:1 prime.

7.2.(v) *Statistics* Dunnett's test was applied to repeated measures taken over the duration of each test infusion. Unpaired *t* tests were used to make

comparisons between different groups at a particular time of sampling. Whether rhTNF infusion influenced the actions of rhIGF-I infusion over time was determined by using a three way ANOVA. All data expressed graphically are represented as the mean +/- standard error.

7.3 Results

7.3.(i) *Clinical Response to rhTNF Infusion* The lambs did not appear distressed over the time of the rhTNF infusion, and their behaviour could not be discerned from that of the saline infused animals. The mean rectal temperature increased 1.4 +/- 0.3 °C in the rhTNF/saline infused animals (group 3, $p < 0.05$) and 1.1 +/- 0.1 °C in the rhTNF/rhIGF-I infused animals (group 4, $p < 0.05$); these rises did not differ significantly from each other (fig 7.2).

7.3.(ii) *Hormonal Responses*

Plasma TNF. There was a significant increase in plasma TNF level at both 180 min and 480 min in both rhTNF infused groups of animals (3 and 4, table 7.1). Plasma TNF levels were lower at the end of the infusions than at 180 min in both groups, despite the constant infusion of rhTNF over this period of time. There was no significant difference in the plasma TNF level at the end of the infusion between the rhTNF infused animals who did and did not receive rhIGF-I (groups 3 and 4).

Plasma IGF-I. rhIGF-I infusions in both control (group 2) and rhTNF (group 4) infused animals increased the plasma IGF-I level approximately five fold above the baseline values (fig 7.3).

Plasma Cortisol. rhTNF infusion increased plasma cortisol from 17 +/- 6 µg/l to 102 +/- 13 µg/l in group 3 and from 17 +/- 8 µg/l to 76 +/- 10 µg/l in group 4 ($p < 0.05$, table 7.1). Plasma cortisol levels were

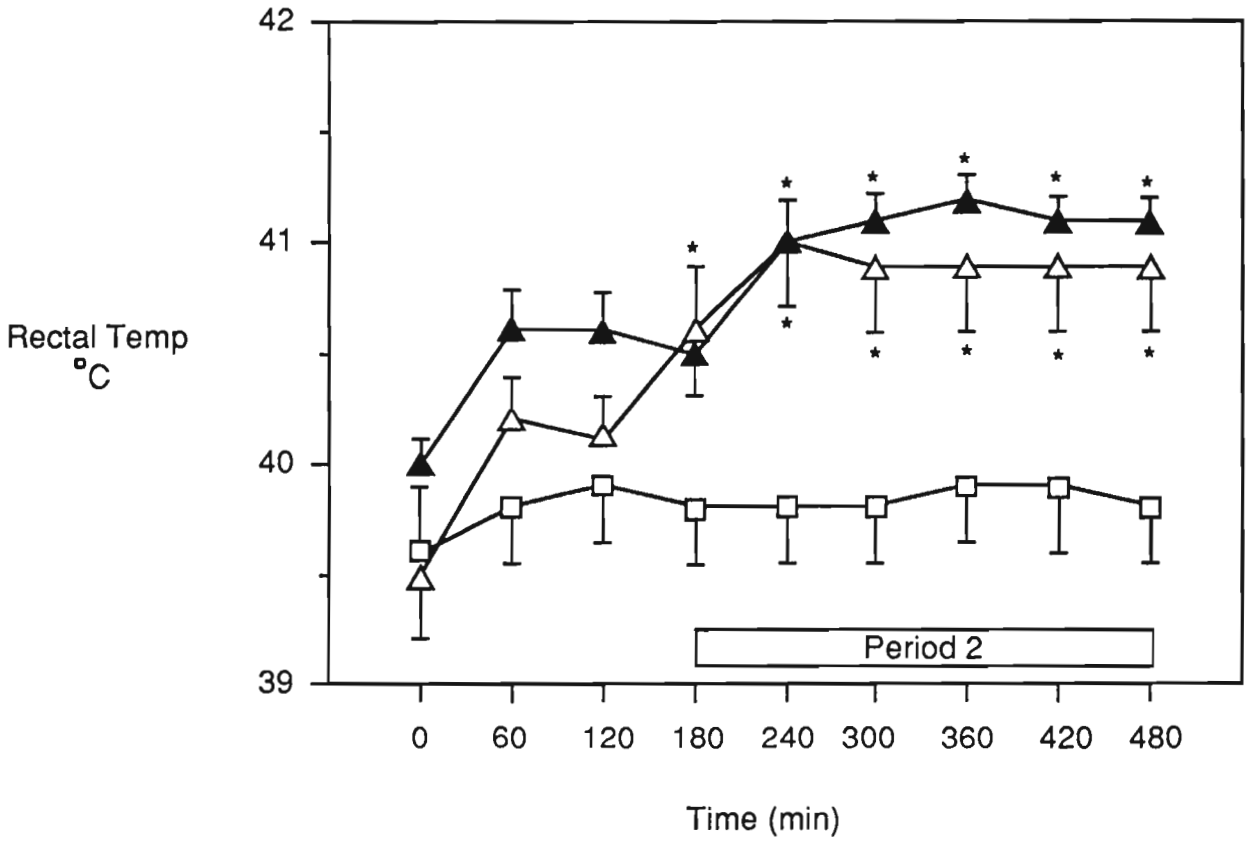


Figure 7.2 *Rectal temperature during rhTNF infusion. Values are means +/- SEM. Saline/saline (group 1,), rhTNF/saline (group 3,), rhTNF/rhIGF-I (group 3,). * $p < 0.05$ compared to 0 min.*

Table 7.1 *Effect of rTNF and rhIGF-I Infusions on Plasma TNF and Hormone Concentrations*

Plasma TNF (ng/l)

Time (min)	0	180	480
TNF/Saline	1 +/- 1	9,544 +/- 656*	8091 +/- 629*
TNF/IGF-I	7 +/- 6	9,245 +/- 529*	8559 +/- 398*

Plasma Cortisol (ug/l)

Time (min)	0	180	480
TNF/Saline	17 +/- 6	102 +/- 13*	52 +/- 11*
TNF/IGF-I	17 +/- 8	76 +/- 10*	61 +/- 8*

Plasma Glucagon (ng/l)†

Time (min)	0	180	480
TNF/Saline	80 +/- 9	133 +/- 28	152 +/- 20
TNF/IGF-I	70 +/- 9	166 +/- 6	117 +/- 17

Plasma Insulin (ug/l)

Time (min)	0	180	480
TNF/Saline	< 0.15	0.37 +/- 0.07*	0.51 +/- 0.17*
TNF/IGF-I	< 0.15	0.60 +/- 0.12*	< 0.15

* $p < 0.05$ compared to 0 min.

† Mean data for groups 3 and 4: 0 min, 75 +/- 6 ng/l; 180 min, 125 +/- 14 ng/l*; 480 min, 134 +/- 14 ng/l*.

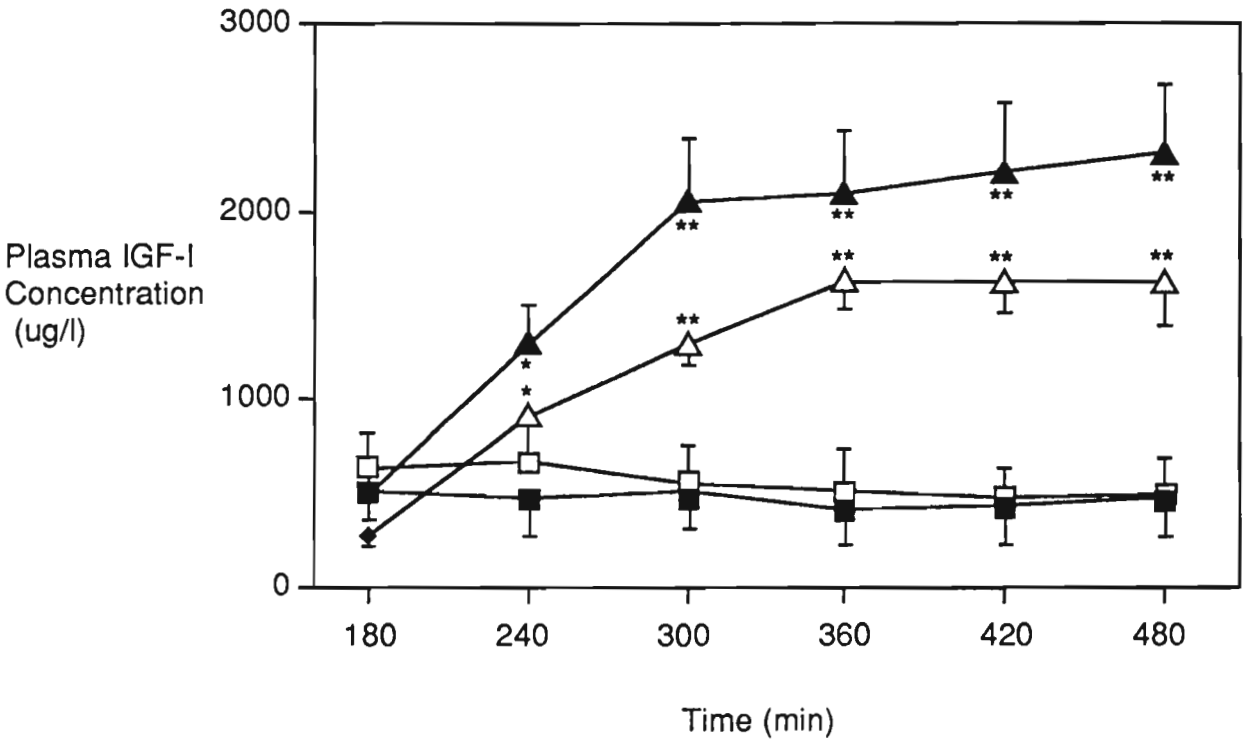


Figure 7.3 *Plasma IGF-I concentration during rhIGF-I and rhTNF infusions compared to normal saline infused controls. rhIGF-I infusions began at 180 min and ended at 480 min. Values are means +/- SEM. Saline/saline(group 1,-□-), saline/rhIGF-I (group 2, -△-), rhTNF/saline (group 3, -■-), rhTNF/rhIGF-I (group 4,-▲-).*

* $p < 0.05$, ** $p < 0.01$ *compared to 180 min*

maintained significantly above baseline levels throughout the duration of the second period.

Plasma Glucagon. The mean glucagon level of groups 3 and 4 increased from 75 +/- 6 ηg/l at 0 min to 125 +/- 14 ηg/l at 180 min ($p< 0.05$) and 134 +/- 14 ηg/l at 480 min ($p < 0.01$).

Plasma Insulin. rhTNF infusion resulted in an increase in plasma insulin concentration by 180 min (table 7.1). Plasma insulin levels remained elevated in group 3, but were reduced to below the minimal detectable levels of the RIA following rhIGF-I infusion (group 4).

7.3.(iii) *Glucose Metabolism*

Response to rhTNF Infusion. By the end of the baseline period (180 min) the plasma glucose concentration was significantly higher in the rhTNF infused animals (groups 3 and 4) than in the saline controls (groups 1 and 2, table 7.2). The higher mean plasma glucose concentration in the rhTNF infused animals resulted from an accelerated Ra glucose and a tendency for the rate of peripheral glucose clearance to be reduced.

Response to rhIGF-I Infusion. rhIGF-I infusion was associated with 41% reduction in plasma glucose concentration in the saline controls (group 2, $p < 0.01$) and a 43% reduction in the rhTNF/rhIGF-I infused animals (group 4, $p < 0.005$) (table 7.3). Over the same time span (180 min to 480 min) there was a 21% reduction in the plasma glucose concentration in the rhTNF/saline control animals ($p < 0.01$), but no significant change in the normal saline infused controls. The reduction in glucose concentration was in all cases due to an increase in the rate of peripheral glucose clearance (fig 7.4), as the glucose Ra consistently remained unchanged over the duration of the second period (table 7.3).

Table 7.2 *Effect of rhTNF Infusion on Glucose Metabolism Determined at 180 minutes.*

	Normal Saline (Groups 1&2)	rhTNF (Groups 3&4)	Significance §
Glucose Conc *mmol/l*	2.9 +/- 0.2	4.0 +/- 0.1	$p < 0.001$
Glucose Ra *µmol/kg.min*	9.5 +/- 0.6	11.8 +/- 1.1	$p < 0.07$
Glucose Clearance *ml/kg.min*	3.5 +/- 0.3	3.0 +/- 0.3	n.s.

§ Two-tailed unpaired *t* test

Table 7.3 *Effect of rhIGF-I and rhTNF Infusion on Glucose Metabolism*

	Glucose Concentration *mmol/l*			Glucose Ra *μmol/kg.min*		
	180 min	480 min	Signif.*	180 min	480 min	Signif.*
Saline/Saline (group 1)	3.1 +/- 0.2	3.3 +/- 0.4	n.s.	9.9 +/- 1.0	10.5 +/- 1.9	n.s.
Saline/rhIGF-I (group 2)	2.7 +/- 0.3	1.6 +/- 0.4	$p < 0.01$	9.0 +/- 0.7	9.1 +/- 0.5	n.s.
rhTNF/Saline (group 3)	3.9 +/- 0.2	3.1 +/- 0.1	$p < 0.01$	11.7 +/- 1.6	13.1 +/- 1.4	n.s.
rhTNF/rhIGF-I (group 4)	4.0 +/- 0.2	2.3 +/- 0.2	$p < 0.005$	12.0 +/- 1.7	12.1 +/- 1.5	n.s.

* Paired *t* test.

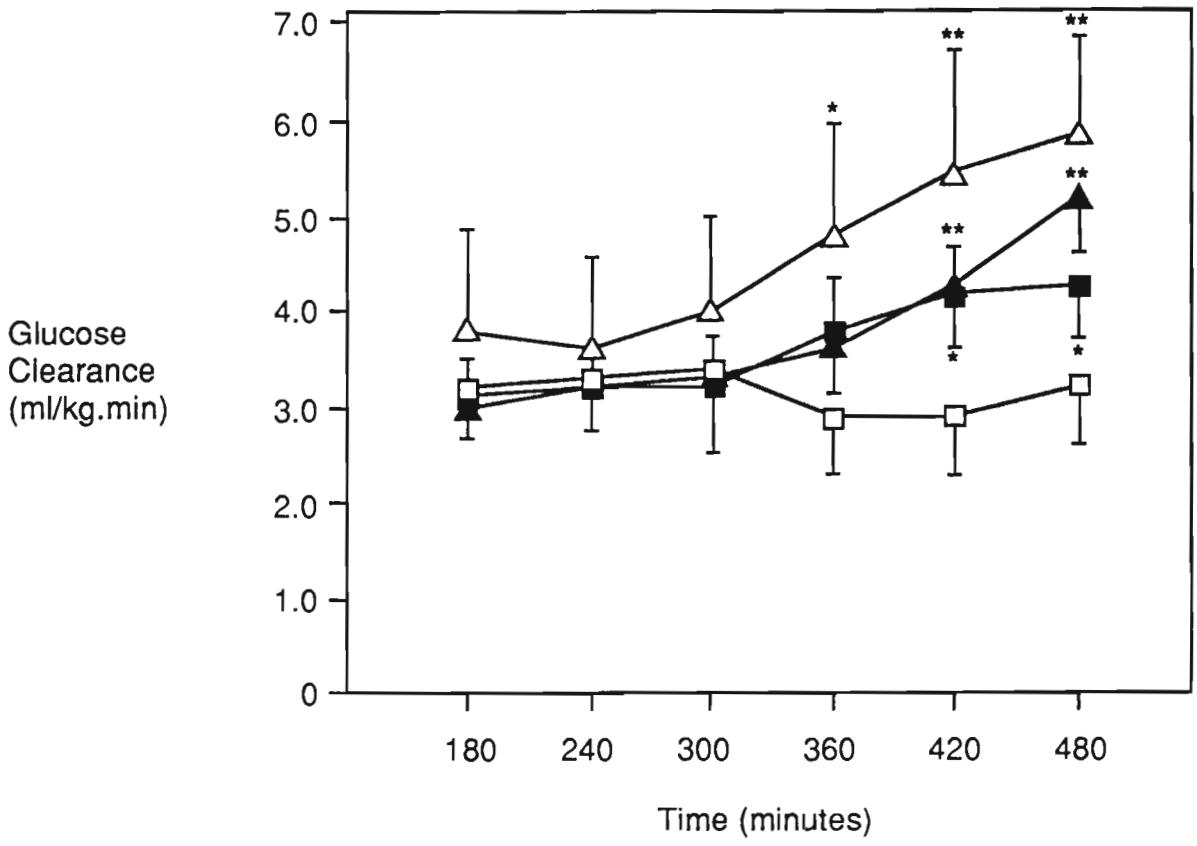


Figure 7.4 *Effects of rhTNF and rhIGF-I infusion on glucose clearance.*

Values are means +/- SEM. Saline/saline (group1, -□-), saline/rhIGF-I (group 2, -△-), rhTNF/saline (group 3, -■-), rhTNF/rhIGF-I (group 4, -▲-).

** $p < 0.05$, ** $p < 0.01$ compared to 180 min.*

7.3.(iv) *Protein Metabolism*

Response to rhTNF Infusion. By the end of the baseline period the plasma urea concentration was significantly higher in the rhTNF infused animals (groups 3 and 4, 7.7 +/- 0.7 mmol/l) than in the normal saline infused controls (groups 1 and 2, 6.1 +/- 0.3 mmol/l) ($p < 0.05$), although the rates of appearance of urea were not significantly different between the two groups (16.3 +/- 1.0 μmol/kg.min vs 15.2 +/- 0.6 μmol/kg.min respectively), suggesting that the increased plasma urea concentration was a reflection of a reduction in the rate of urea clearance.

Response to rhIGF-I Infusion. rhIGF-I infusion reduced significantly the rate of net protein loss (NPL) from 3.5 +/- 0.2 g/kg.day to 2.9 g/kg.day ($p < 0.005$) in the saline/rhIGF-I group and from 4.3 +/- 0.3 g/kg.day to 3.3 +/- 0.3 g/kg.day ($p < 0.005$) in the rhTNF/rhIGF-I treated animals. These data are illustrated in fig 7.5, expressed as percentage reduction in the rate of net protein loss. Relative to the saline infused controls (groups 1 and 3), rhIGF-I infusion resulted in a 15% reduction of NPL in both the control animals (group 2) and the rhTNF infused animals (group 4).

7.3.(v) *Plasma Glycerol and Triglyceride Concentrations.* There were no differences in the plasma concentrations of glycerol and triglyceride between the saline (1 and 3) and rhTNF (2 and 4) infused groups and the end of the baseline period (180 min), nor were these concentrations altered by rhIGF-I infusion.

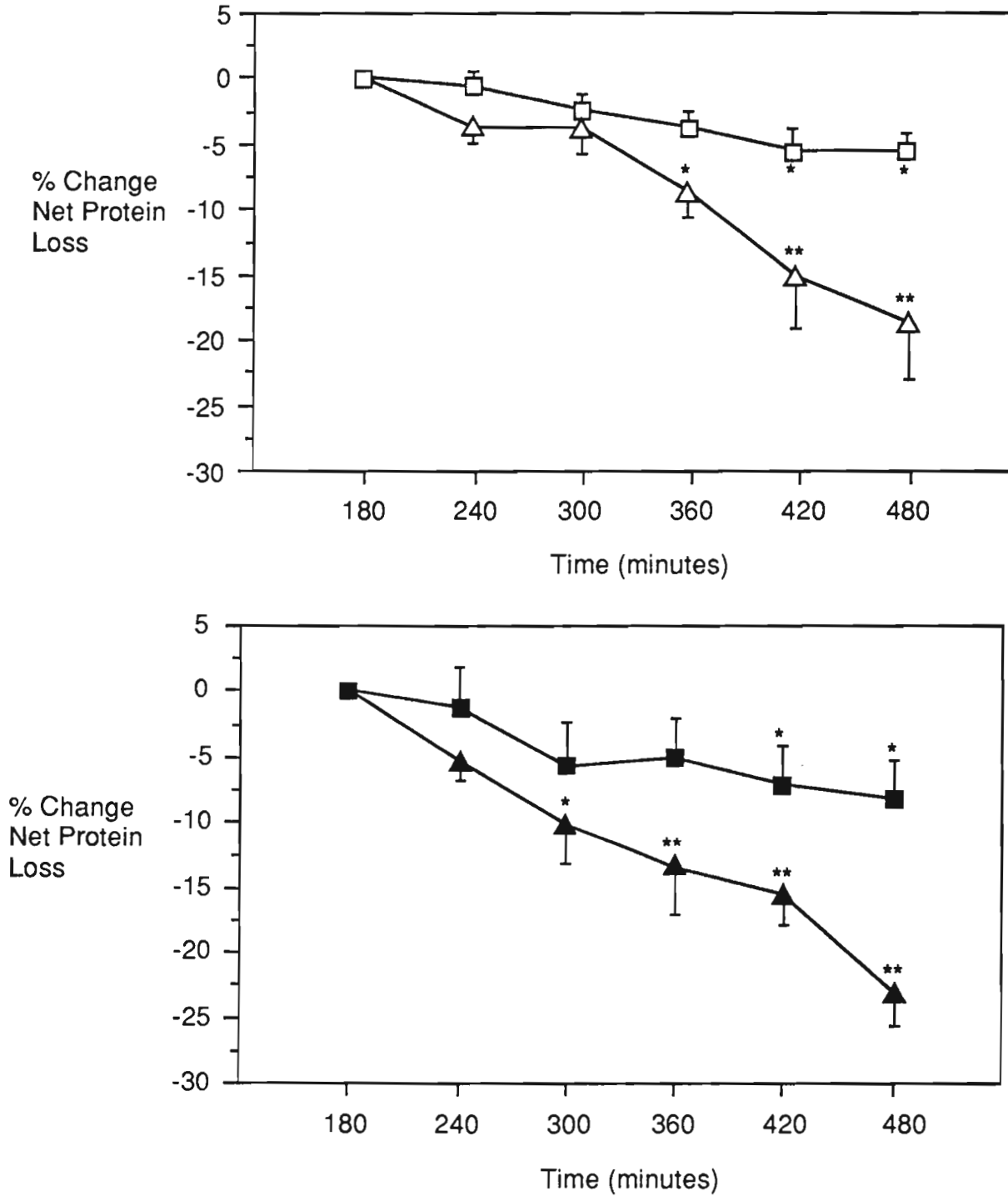


Figure 7.5 *Percentage change of net protein loss during rhIGF-I infusion. Values are means +/- SEM. Saline/saline (group 1,-□-) , saline/rhIGF-I (group 2, -△-), rhTNF/saline (group 3,-■-), rhTNF/rhIGF-I (group 4, -▲-).*

** $p < 0.05$, ** $p < 0.01$ compared to 180 min.*

7.4 Discussion

7.4.(i) *Introduction* The primary aim of this study was to investigate whether rhIGF-I preserves its protein sparing effect when administered to animals with a catabolic state analogous to that of sepsis. It was found that the extent to which rhIGF-I inhibited the rate of net protein loss was not influenced by pretreatment and simultaneous infusion of rhTNF, suggesting that rIGF-I warrants further investigation as an anabolic agent in the metabolic support of septic patients.

7.4.(ii) *Effects of rhTNF on Temperature and Plasma Hormone Levels* The animals were infused with rhTNF because this provided a reproducible method of provoking many of the clinical, hormonal and metabolic responses associated with sepsis. All of the animals infused with rhTNF became febrile within 60 minutes, and a significant temperature elevation was maintained throughout the 480 minute duration of the infusion. The plasma concentrations of cortisol, glucagon and insulin were elevated after 180 minutes of rhTNF infusion, mimicking the counter-regulatory hormone release and hyperinsulinaemia which are commonly observed in septic patients. Although we did not measure the plasma concentration of catecholamines, others have reported an increase in plasma catecholamine levels following TNF bolus administration to dogs[337].

7.4.(iii) *Effects of rhTNF on Glucose Metabolism* Some aspects of the metabolic response to sepsis were seen after 180 minutes of rhTNF infusion, such as an elevation in the plasma glucose concentration and rate of appearance. The ability of TNF to encourage glycogenolysis has been observed *in vitro* [195], and increases in glucose Ra have been previously

reported *in vivo* in rats following bolus doses of TNF[12]. Although the mean rate of peripheral uptake of glucose was not significantly different from that of the controls at 180 minutes in the rhTNF infused animals, in those animals infused with rhTNF and normal saline (group 3) during the second period there was a steady increase in the rate of peripheral glucose clearance which was not seen in the saline control group (group 1, fig 7.4). This was associated with gradual, but not significant, increase in the glucose production so that the resultant plasma glucose concentration was not altered. Explanation for this observation may lie in the reduced plasma cortisol and increased plasma insulin measured at 480 min compared with 180 min in this group. However, rhTNF may have direct effcte on glucose uptake: *in vitro* data suggests that TNF itself is able to increase the uptake of glucose into muscle cells[195].

7.4.(iv) *Effect of rhTNF on Fat Metabolism* Infusion of rhTNF did not appear to influence the plasma concentrations of glycerol or triglyceride in our study. Beutler and Cerami[24] have suggested that TNF is responsible for the hyperlipidaemia associated with infectious diseases and others have described an increase in the serum triglyceride levels shortly after the administration of TNF to rats[101]. However, there is evidence which suggests that TNF stimulates the hepatic synthesis of triglycerides only in fed animals[102] which may explain why no hypertriglyceridaemia was seen in the present study in which fasted animals were infused. Starnes and coworkers have reported an increase in glycerol turnover in patients administered TNF[317], but Kettelhut and Goldberg failed to observe a change in the rate of lipolysis in rats despite injecting very high doses for five days[179].

7.4.(v) *Effect of rhTNF on Protein Metabolism* We observed no significant change in the rate of net protein loss in the TNF infused animals to that of fasting alone. This is consistent with the results of Michie and coworkers[235], who found no accelerated net protein breakdown following the infusion of TNF to both human subjects and rats. However, others have reported that adminstration of TNF results both in increased muscle proteolysis[105,317] and increased hepatic protein synthesis, so that in the short term there may conceivably be no change in the net protein loss measured at the level of the whole body while these two effects remain in balance.

7.4.(vi) *Plasma TNF Levels* TNF levels are variably raised or normal in septic patients[294]. It is thought that this peptide is released from macrophages relatively early in the clinical course and that this initiates many of the clinical and metabolic events seen in established cases of sepsis[234]. It is therefore difficult to relate plasma TNF concentrations to sepsis severity, or to describe a septic range of TNF levels. The TNF levels which we measured during the infusion of rhTNF were considerably higher than those seen following endotoxin injection to human subjects[236] and approximately half the peak TNF level measured in rapidly lethal *E. coli* induced bacteraemia in baboons[336]. Interestingly, the plasma TNF concentration fell significantly between180 and 480 minutes in both the rhIGF-I and normal saline infused animals, despite the TNF being infused at a constant rate over this time. A similar fall in plasma TNF concentration during a constant infusion was noted by Michie and coworkers who determined that this was not not caused by deterioration of the TNF infusate and suggested that it was a result of as yet unknown changes in the pharmacokinetics of the molecule over the duration of the infusion[236].

7.4.(vii) *IGF-I Plasma Levels* The infusion of IGF-I increased the mean plasma IGF-I level more than five fold in both those animals who were simultaneously infused with normal saline and those with rhTNF. In the study described in Chapter 6, it was observed that at the end of 300 minute IGF-I infusions of 50 μg/kg.hr that approximately10% of the IGF-I present in the plasma is in the free form, which implies that the capacity of the plasma IGF-I binding proteins has been saturated. The hypoglycaemic effect of IGF-I is brought about only by the free form, this action being prevented by association with the IGF-I binding proteins[232,393]. rhTNF infusion did not appear to inhibit the insulin-like action of free IGF-I as infusion of rhIGF-I reduced plasma glucose concentration by a similar percentage in the control and rhTNF treated animals (table 7.3). The individual potentials of rhIGF-I and rhTNF to accelerate the peripheral clearance of glucose were partly additive, as when infused together the clearance of glucose increased 73%, as opposed to 55% and 39% respectively for rhIGF-I and rhTNF infused alone.

7.4.(viii) *Effect of rhIGF-I on Protein Metabolism* The ability of rhIGF-I to reduce the rate of net protein loss was not affected by the simultaneous infusion of rhTNF (fig 7.5). This result is consistent with our recent report of rhGH administration halving the rate of protein loss in a group of septic patients who were continuing to lose protein despite receiving parenteral nutrition (Chapter 5). Plasma IGF-I levels increased more than two fold in these patients, and it is likely that IGF-I mediated the protein anabolic effects of the growth hormone. IGF-I has been demonstrated to reduce protein breakdown and accelerate protein synthesis in several *in vitro* preparations[128,224], and to possess protein sparing activity *in vivo* in fasted adult rats[170]. Increases in the *in vivo* rate of protein synthesis of skeletal, diaphragmatic and cardiac muscle in fasted lambs during rhIGF-I infusion

were observed in the study described in Chapter 6 . No such increase in the rate of protein synthesis was observed in lambs infused with an insulin dose of equivalent hypoglycaemic potential, which suggests that the effect is mediated by interaction with the IGF-I receptors.

7.4.(ix) *Summary* The protein anabolic properties of rhIGF-I, and their preservation despite high plasma levels of TNF, suggest possible therapeutic potential in the support of severely septic, protein catabolic patients. Recombinant hIGF-I may offer some advantages over rhGH as an anabolic agent in critically ill patients as there appears to a degree of growth hormone resistance in such patients, so that the IGF-I response to hGH administration is attenuated[80]. Similarly, neonates undergoing major surgery, whose GH receptors have yet to develop, may benefit from rhIGF-I support .

Chapter 8

The Circulating Molecular Weight Forms of Infused Recombinant Insulin-like Growth Factor-I and Effects on Glucose and Fat Metabolism in Lambs.

8.1 Introduction

As the major constituent of the non-suppressible insulin-like activity of plasma, circulating IGF-I has been attributed with considerable hypoglycaemic potential[257]. The normal animal is protected from IGF-I induced hypoglycaemia because of its association with specific plasma binding proteins which prevent IGF-I from exerting insulin-like actions[393]. Whether there are significant amounts of free IGF-I present in the plasma under physiological conditions has been debated[222,389]. However, if the plasma binding capacity is saturated by administration of a sufficient dose of IGF-I, the resultant free IGF-I can lower the blood glucose concentration[393].

We examined the effects of infusion of several different doses of IGF-I on glucose metabolism in order to clarify how the distribution of IGF-I across the IGF-I binding proteins influences its actions on glucose kinetics. Although the hypoglycaemic action of IGF-I administration has been reported in both animal[393] and human studies[134], this has been simply ascribed to its affinity for the insulin receptor as a result of the considerable homology between the peptides and their receptors[116]. Few *in vivo* studies have addressed the mechanism of IGF-I induced hypoglycaemia. We have used isotopic tracer methodology to determine the effects of IGF-I on glucose production and peripheral glucose clearance, and have measured the effects of IGF-I infusion on glycerol and free fatty acid concentrations.

8.2 Materials and Methods

8.2.(i) *Animals and Preparation* The animals involved in these studies were cryptorchid crossbred lambs of an age range between 3 and 5 months which were fasted for 24 hours prior to being studied, and prepared in the manner described in Section 3.4.(i).

8.2.(ii) *Protocol* The priming dose for the isotopic infusions was administered at 0900 hrs, and during the first three hours normal saline and glucose tracer were infused simultaneously while isotopic equilibrium was being reached (fig 8.1). Blood specimens were collected at 10 minute intervals over the last 20 minutes of this period to enable baseline measurements to be made. Following this, the test infusions were begun and continued over a 90 minute period during which time plasma specimens were taken at times 70, 80 and 90 minutes. All blood specimens were collected in chilled, heparinized tubes and were immediately centrifuged and the separated plasma was frozen prior to being analysed.

8.2.(iii) *Hormone Infusions* The animals were infused during the test period with rhIGF-I at doses of 2.5, 20, 40 and 120 μg/kg.hr (n = 3 for each dose). Immediately prior to infusion, all of the hormones were diluted in 35 ml normal saline and 0.5 ml of the plasma from the animal to be infused was added to reduce hormone binding to the surface of the syringe and plastic tubing. The infusates were infused at a rate of 20 ml/hr.

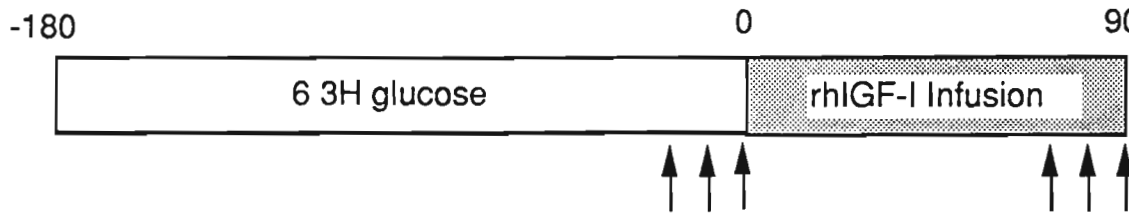


Figure 8.1 *The experimental protocol. A primed constant infusion of 6 3H glucose was begun 180 minutes prior to the rhIGF-1 infusions to ensure adequate time for isotopic equilibrium to be reached. Plasma specimens were removed at the times indicated by the arrows.*

8.2.(iv) *Isotopic Infusions* The rate of appearance of glucose was determined by a primed constant infusion of 6 ^{3}H glucose of 5.0 ηCi/kg.min (prime ratio 80:1).

8.2.(v) *Statistics* Dunnett's test was applied to repeated measures taken over the duration of the infusions, and Dunnett's variation of one way factorial ANOVA was used to compare the concentrations of IGF-I binding forms at the end of the 90 minute infusions of variable doses. All data are expressed as the mean +/- SEM.

8.3 Results

8.3.(i) *Plasma IGF-I Concentrations* The total plasma IGF-1 concentration was not altered by infusion of rhIGF-I at 2.5 μg/kg.hr (fig 8.2). The mean plasma IGF-I level increased from 687 +/- 129 μg/l to 851 +/- 65 μg/l by the end of the 20 μg/kg.hr infusion, but this change did not reach statistical significance. Infusion of rhIGF-I at 40 μg/kg.hr increased the plasma IGF-I concentration from 537 +/- 40 μg/l to 1281 +/- 118 μg/l ($p < 0.05$), and the 120 μg/kg.hr dose increased the plasma IGF-I concentration from 524 +/- 7 μg/l to 2763 +/- 114 μg/l ($p < 0.005$). The plasma concentration of free (7kDa form) IGF-1 remained below the limit of the sensitivity of the radioimmunoassay (<40 μg/l) at the end of the two low dose infusions (figs 8.3 a&b). There were detectable amounts of free IGF-I present by the end of the 40 and 120 μg/kg.hr infusions (205 +/- 41 μg/l and 510 +/- 150 μg/l respectively) (figs 8.3 c&d). The mean plasma concentration of 35-60kDa binding forms of IGF-I was almost twofold higher by the end of the 20 μg/kg.hr infusions than at the end of the 2.5 μg/kg.hr infusions. The 35-60 kDa form concentration was significantly higher at the end of the 40

μg/kg.hr infusion (801 +/- 95 mg/l, $p < 0.05$) and of the 120 μg/kg.hr (1873 +/- 174 μg/l, $p < 0.01$), when compared to plasma concentration of this binding form at the end of the 2.5 μg/kg.hr infusion. None of the higher doses of rhIGF-I infusion increased the 150 kDa form significantly above the concentration measured at the end of the 2.5 μg/kg.hr infusion.

8.3.(ii) *Plasma Glucose Kinetics* The 2.5, 20 and 40 μg/kg.hr infusions of rhIGF-I did not alter the plasma glucose concentration, whereas the 120 μg/kg.hr infusion reduced the plasma glucose concentration from 3.5 +/- 0.2 mmol/l to 1.9 +/- 0.2 mmol/l($p < 0.05$) (fig 8.4a). None of the infusions significantly changed the rate of appearance of glucose into the plasma compartment, although there was a tendency for the Ra glucose to be lower by the end of the 120 μg/kg.hr infusion (fig 8.4b). The rate of glucose clearance, unchanged by the lower dose infusions of rhIGF-I, was significantly increased by the 120 μg/kg.hr infusion from 3.8 +/- 0.4 ml/kg.min to 5.6 +/- 0.4 ml/kg.min ($p < 0.05$) (fig 8.4c).

8.3.(iii) *Plasma Free Fatty Acid and Glycerol Concentrations* The plasma concentrations of free fatty acid and glycerol were not significantly changed by any dose of rhIGF-I infusion..

8.3.(iv) *Plasma Electrolytes* Plasma potassium concentration fell over the course of the 120 μg/kg.hr rhIGF-1 infusion from 3.7 +/- 0.1 mmol/l to 2.6 +/- 0.5 mmol/l ($p < 0.05$), but was not altered by infusions of a lower dose. Plasma urea concentrations were not changed by any of the doses infused.

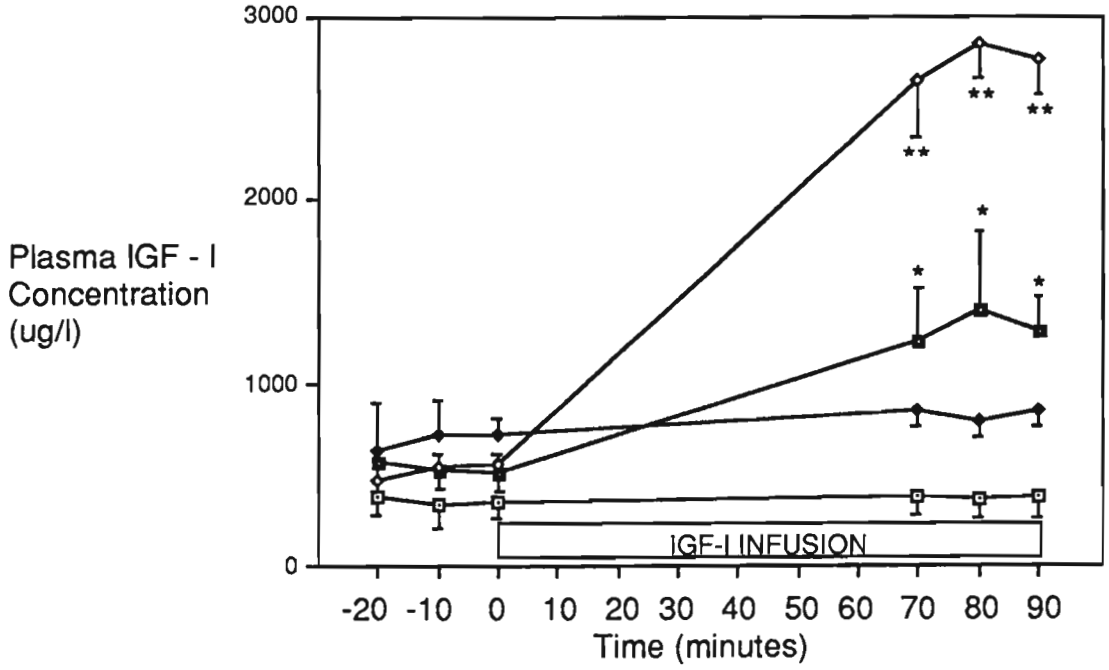
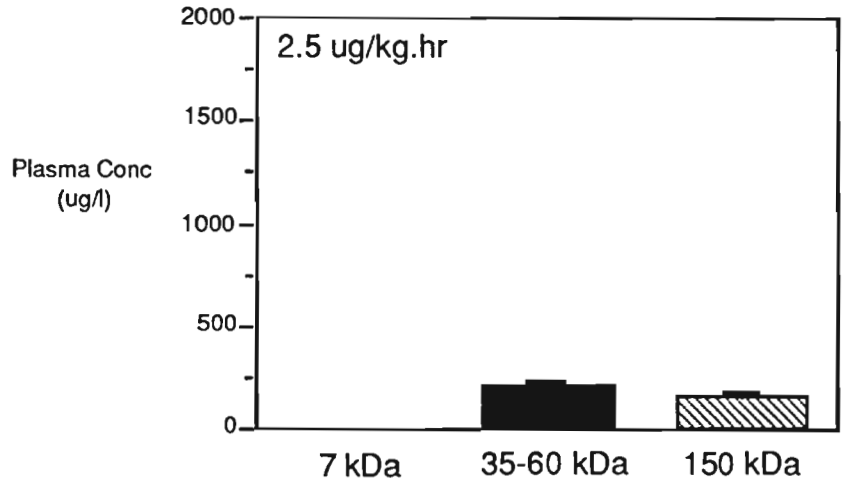


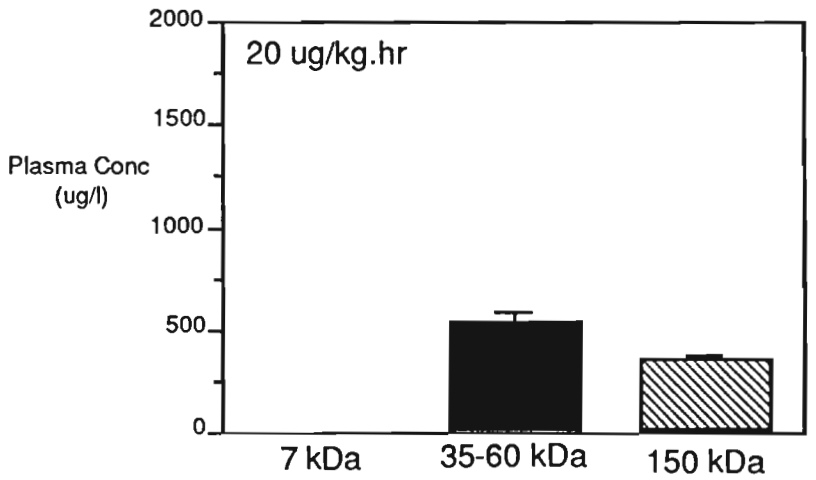
Figure 8.2. *Total plasma IGF-I concentrations over the duration of rhIGF-I infusion at doses: 2.5 ug/kg.hr (—▫—), 20 ug/kg.hr (—◆—), 40 ug/kg.hr (—■—) and 120 ug/kg.hr (—◇—).*

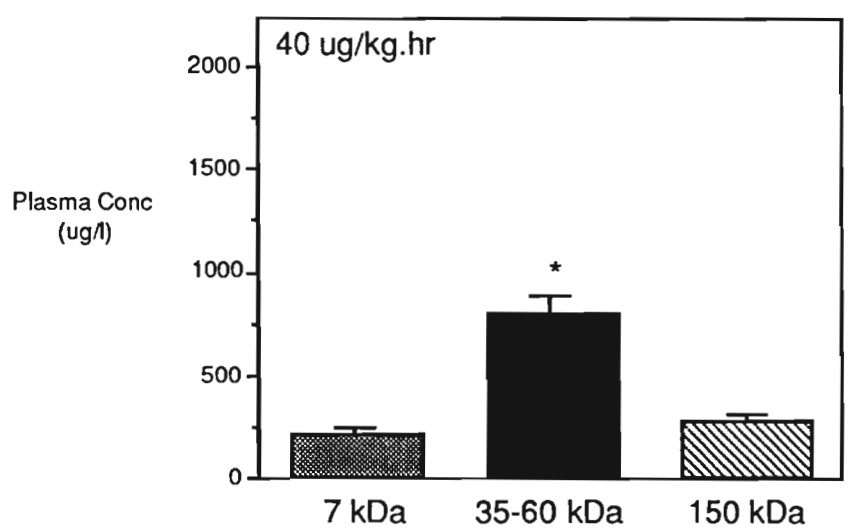
** $p < 0.05$, ** $p < 0.005$*

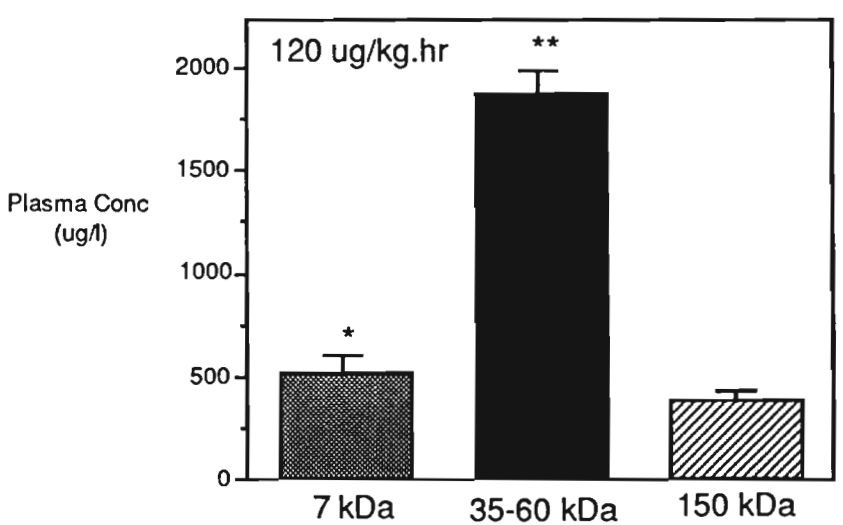
Figures 8.3 a-d.(over page) *IGF-I binding forms at t = 90 minutes (ug/l). The 7 kDa fraction represents free IGF-I and the 35-60 and 150 kDa forms are IGF associated with low and high molecular weight binding proteins.*

$^{*}p < 0.05$, $^{**}p < 0.01$ compared to saline infused controls.


2.5 ug/kg.hr
Plasma Conc (ug/l)
2000
1500
1000
500
0
7 kDa
35-60 kDa
150 kDa

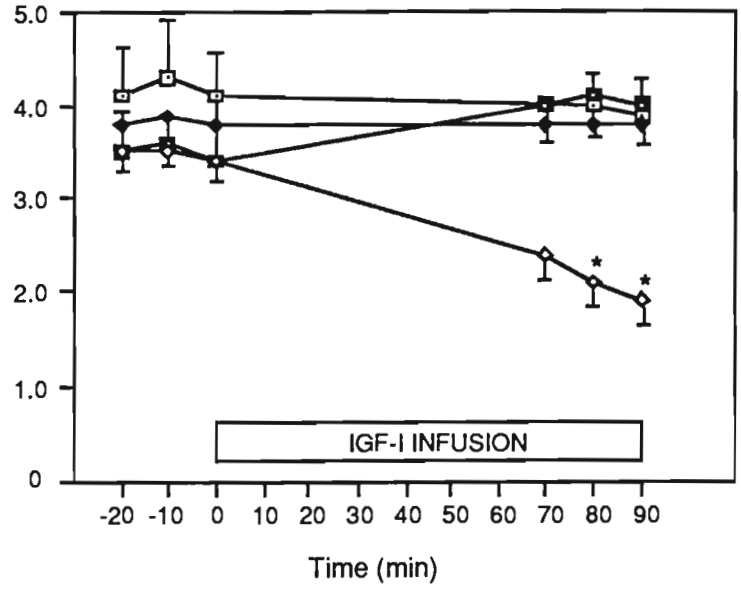

20 ug/kg.hr
Plasma Conc (ug/l)
2000
1500
1000
500
0
7 kDa
35-60 kDa
150 kDa


40 ug/kg.hr
Plasma Conc (ug/l)
2000
1500
1000
500
0
*
7 kDa
35-60 kDa
150 kDa

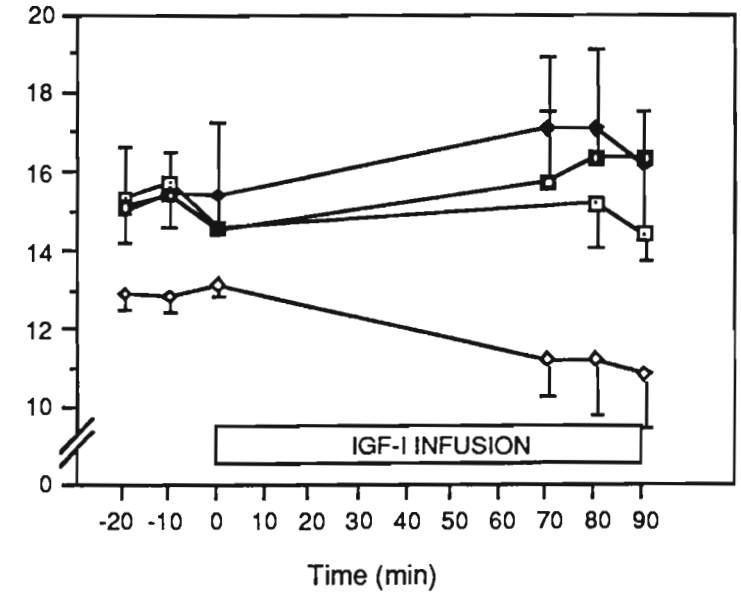

120 ug/kg.hr
Plasma Conc (ug/l)
2000
1500
1000
500
0
*
**
7 kDa
35-60 kDa
150 kDa

Figures 8.4 a-c (over page) *Effects of rhIGF-I infusion on glucose concentration (a), glucose rate of appearance (b) and glucose clearance (c). Rates of infusion; 2.5 ug/kg.hr (-□-), 20 ug/kg.hr (-◆-), 40 ug/kg.hr (-■-) and 120 ug/kg.hr (-◇-)* $* p < 0.05$

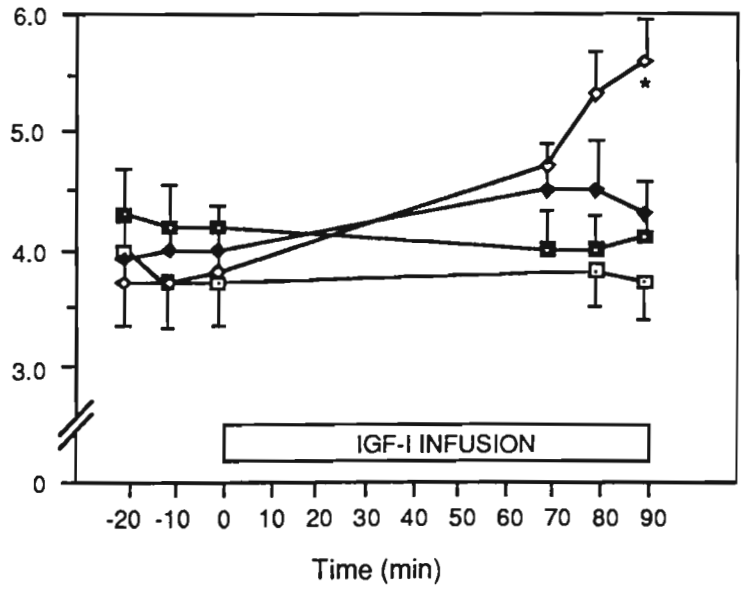
Plasma Glucose Concentration (mmol/l)



Glucose Ra (umol/kg.min)



Glucose Clearance (ml/kg.min)



8.4. Discussion

8.4.(i) *Hypoglycaemic Action of Free IGF-I* Our data support the assertion that the hypoglycaemic action of circulating IGF-I is attributable to the free form. The highest dose of rhIGF-I infusion (120 μg/kg.hr) was associated with a high plasma concentration of free IGF-I and significantly lowered the plasma glucose concentration, but when the infused dose was insufficient to saturate the plasma IGF-I binding capacity, plasma glucose levels remained unchanged. However, an intermediate dose of 40 μg/kg.hr saturated the plasma binding capacity but did not lower the plasma glucose concentration. This may reflect the relatively weaker hypoglycaemic potency of free IGF-I compared to insulin[116], and subsequent requirement for a threshold concentration to be passed before hypoglycaemic effects are seen.

8.4.(ii) *Role of Plasma Binding Proteins* The role of the plasma binding proteins has been a source of considerable speculation [156]. They were initially viewed as having primarily a reservoir function, as there is no tissue or organ which specifically stores IGF in concentrations larger than those observed in the plasma[16] However, *in vitro* studies have suggested that the mitogenic potential of rhIGF-I is potentiated by association with binding proteins[34]. Combined with other reports of the ability of IGF-I associated to IGF-binding proteins to pass freely from the plasma to the tissues[152], the possibility is raised of IGF-I/binding protein complexes being the form which promotes growth. In our study, the majority of infused IGF-I was found in the 35-60 kDa fraction (fig 8.3a-d). The relatively lower concentration of free IGF-I probably reflects the more rapid rate of clearance of free IGF-I relative to the 35-60 kDa and 150 kDa forms[156]. A similar

pattern of distribution of infused rhIGF-I was observed in the study described in Chapter 6. It was found that acute rhIGF-I infusions in two doses, the higher of which lowered the plasma glucose concentration and the lower of which did not despite doubling the plasma IGF-I concentration, both reduced the rate of protein loss in fasted lambs. It is tempting to speculate that the anabolic action of rhIGF-I observed in these lambs was attributable to the marked increase of the 35-60kDa molecular weight form, which consists of IGF-I associated with IGFBP-1, IGFBP-2 and the b subunit of IGFBP-3. However, the *in vivo* physiological roles of the IGFBPs will probably not be definitively elucidated until sufficient quantities are synthesized to enable infusion into animal models.

8.4.(iii) *Effect on Glucose Kinetics* The 120 μg/kg.hr infusion lowered plasma concentration of glucose primarily by increasing the rate of glucose clearance by 51% ($p < 0.05$). This effect was associated with a fall in the mean rate of glucose production by 16%, which did not reach statistical significance. The predominance of the glucose clearing effect of IGF-I has been previously reported in fasted rats[170]. The kinetics of the hypoglycaemic action of IGF-I contrast with those of insulin, to which glucose production is more sensitive than glucose uptake[277]. We speculate that this disparity may be a result of the hypoglycaemic actions of IGF-I being mediated by IGF type 1 receptors which are numerous in many tissues including muscle[208] but sparse in hepatic tissue[57]. However, attributing an observed response to a particular hormone-receptor interaction is difficult in the case of insulin and IGF-I, because of the strutural homology shared by both the hormones and their respective receptors and subsequent overlapping of receptor affinities. The concept of insulin acting primarily on glucose and amino acid transport and of IGF-I having a predominantly mitogenic role now appears to be an oversimplification, as it becomes increasingly clear that both hormones can

exhibit metabolic and mitogenic actions[123,128,184,264]. The physiological function performed by these hormones probably relates to the number of receptors and their relative affinities

8.4.(iv) *Effect on Fat Metabolism* Although insulin is antilipolytic when infused at doses below that required to alter glucose kinetics[397], we have observed that glucose metabolism is more sensitive than fat metabolism to infused IGF-I. This observation is consistent with those of others who have observed relatively much smaller decreases in plasma free fatty acid levels than plasma glucose levels following bolus injections of IGF-I in both normal rats[393] and human subjects[134]. The absence of anti-lipolytic effect of IGF-I in our study provides further evidence for the supposition that the metabolic actions of IGF-I are not simply mediated by cross-reaction with insulin receptors.

8.4.(v) *Summary* We have observed that IGF-I infusion is hypoglycaemic if the dose infused is sufficient to saturate the plasma binding protein capacity, although it appears that a threshold plasma concentration of free IGF-I concentration must be exceeded. The predominant effect of IGF-I on glucose clearance and lack of anti-lipolytic action contrast with the previously reported properties of insulin, and suggest the IGF-I may be acting via IGF rather than insulin receptors.

Chapter 9

Summary and Conclusions

The studies described in this thesis have sought to define the metabolic actions of anabolic peptide hormones which may be therapeutically useful in slowing the accelerated rate of protein loss which occurs in critically ill surgical patients.

The effects of a short course of recombinant human growth hormone in septic or injured surgical patients were determined using primed constant infusions of isotopically labelled tracers. Some patients were studied while receiving parenteral nutrition as part of their clinical management, and others were studied in the postabsorptive state. It was found in both groups of patients that the plasma IGF-I and insulin concentrations were significantly elevated after rhGH treatment. In those patients who were receiving parenteral nutrition, the rate of net protein loss was reduced from 0.82 +/- 0.17 g/kg.day to 0.43 +/- 0.20 g/kg.day ($p < 0.02$). The rate of protein catabolism was unchanged, which suggests that the protein conservation resulted from an increase in the rate of protein synthesis. If this rate of protein conservation were to be maintained for a period of two weeks, it would equate to a saving of almost 1.5 kg of wet muscle in a 70 kg patient. This is the first report describing the protein conserving effects of rhGH in acutely ill patients receiving full parenteral nutritional support. There was a rise in the plasma glucose concentration and rate of glucose production in parenterally fed patients and these changes probably reflect the insulin-antagonistic properties of rhGH. The reduction in the rate of protein loss in postabsorptive patients after rhGH treatment was more modest (from 1.92 +/- 0.27 g/kg.day to 1.66 +/- 0.23 g/kg.day, $p < 0.05$), and this was a result of a

reduction in the rate of protein oxidation, probably secondary to an increase in the rate of free fatty acid mobilization and oxidation.

A small study was performed in which the effect of a short course of rhGH on the rate of uptake of isotopically labelled leucine into various tissues was determined in patients undergoing resection for colonic tumours. It was found that there was an increase in the rate of leucine uptake by skeletal muscle ($p < 0.03$), but none by the malignant tissue. These data suggest that rhGH may have potential in the treatment of cancer cachexia, but any wider investigation need be addressed with caution because of the possibility of tumour stimulation.

It is widely assumed that most of the protein anabolic actions of hGH are a result of its stimulation of the production of IGF-I in the liver and in various other target organs. Only recently has sufficient rhIGF-I been available to enable the effects of infused rhIGF-I to be measured in a large animal model. In fasted lambs, rhIGF-I was infused at two doses, one which induced hypoglycaemia (50 μg/kg.hr) and the other which did not despite doubling plasma IGF-I levels (15 μg/kg.hr). Both rhIGF-I infusions resulted in significant reductions in the net rate of loss of protein over the 300 minutes of the infusion (of 15% and 11% respectively). Only the higher rate of rhIGF-I infusion increased the rate of tissue protein synthesis relative to normal saline infused controls. The increased rate of protein synthesis was most marked in cardiac and diaphragmatic tissue, which may be of clinical benefit in preserving cardiopulmonary performance in critically ill surgical patients requiring nutritional support. The metabolic actions of rhIGF-I were compared to an insulin dose of the same hypoglycaemic potential as the high dose rhIGF-I infusion, and also to an rhIGF-II infusion of 50 μg/kg.hr. It was found that the insulin infusion decreased the rate of net protein loss by12% ($p < 0.05$) which was a fall of similar magnitude to the reduction seen during the rhIGF-I infusion, however there was no

stimulation of protein synthesis in any of the tissues. This suggests that the reduction in NPL following insulin administration was a result of a decrease in the rate of protein catabolism. Whereas insulin infusion both reduced the rate of glucose production and increased the rate of glucose clearance, rhIGF-I had a significant effect only on glucose clearance. Recombinant IGF-I did not alter free fatty acid or glycerol concentrations in the doses infused. These differences suggest that the actions of rhIGF-I are not mediated simply by cross-reaction with the insulin receptor. Infusion of rhIGF-II did not alter the rate of NPL or protein synthesis, but brought about a small increase in the rate of glucose clearance.

Having established that circulating IGF-I was capable of reducing the rate of net protein loss, the next question addressed was whether this effect would be preserved in the face of the catabolic hormonal environment seen in the septic or injured patient. In order to answer this question, an animal model was developed in which an infusion of recombinant human tumour necrosis factor was used to induce many of the features of sepsis: the rhTNF infused animals were pyrexic and had an elevated rate of glucose production and increased plasma concentrations of cortisol, glucagon and insulin. Despite this hormonal and metabolic background, rhIGF-I infusion had exactly the same protein conserving effect as was observed in normal fasted animals. This result suggests that rhIGF-I may preserve its protein sparing effects when infused in the clinical setting of severe surgical illness.

The major question which remains to be answered is to what extent will the favourable biochemical changes observed following the administration of these anabolic peptide hormones equate to improvements in physiological function and, ultimately, into speeding the recovery of severely septic or injured patients.

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Appendix 1

Methods of Sample Analysis

Contents

1. Expired $^{14}CO_2$ Analysis

1.1 REAGENTS

0.1% Phenolphthalein solution (w/v)
Hydroxide of hyamine
Absolute ethanol
0.15 N HCl

1.2 METHOD

(Note: This solution should be made up fresh and standardized as soon as possible)

1) In a 125 ml Erlenmeyer flask add:

4.0 ml 0.1 % phenolphthalein
20.0 ml hydroxide of hyamine
36.0 ml absolute ethanol

Cover with parafilm and mix.

2) Transfer 3.0 ml to a scintillation vial and titrate with 0.15 N HCl. The titration should take 6.67 ml of HCl to turn the hyamine clear.

3) The rest of the hyamine solution is then quickly transferred in 3.0 ml portions to glass scintillation vials and tightly capped.

4) Each vial should contain 1.00 mEq of hyamine hydroxide and be capable of trapping 1.00 mmol of CO_2.

5) After the vial has been used to collect the expired air, 10 ml of the toluene/0.4% PPO* solution is added as the scintillation fluid and then counted.

* toluene 2l, ethylene glycolmonomethyl ether 1l, PPO 16.5 g

2. Preparation and Balancing of $Ba(OH)_2$ and $ZnSO_4$ Solutions

2.1 REAGENTS

$Ba(OH)_2$
$ZnSO_4$
Ascarite
2% phenolphthalein

2.2 METHODS

A. Preparation of 4.73% $Ba(OH)_2$

1) Heat 1800 ml water, add 94.6 g $Ba(OH)_2$ slowly while stirring.

2) Stir the solution overnight, loosely covered with foil to prevent the absorption of CO_2 from the air.

3) Filter the solution through a double layer of #1 filter paper into a bottle which has the flow hose connected at the bottom. Keep the bottle and the funnel covered as much as possible.

4) Stopper the bottle with a drying tube filled with Ascarite.

5) Place a similar drying tube on the top of the burette. Keep the drying tubes stoppered until actually in use and make sure the flow hose is tightly clamped.

B. Preparation of 5.5% $ZnSO_4$

1) In a bottle with a flow hose connected at the bottom dissolve 110 g $ZnSO_4$ in 2l water.

2) Stopper with a glass stopper.

C. Balancing

1) Add 50 ml distilled H_2O, 2-4 drops 2% phenolphthalein, and 10ml $ZnSO_4$ into a 125 ml Erhlenmeyer flask.

2) Titrate with $Ba(OH)_2$ until the solution becomes slightly pink and the colour remains for 2-3 min. This should take 10 ml of $Ba(OH)_2$. If less is required , add water to the $Ba(OH)_2$, and if more is required add water to the $ZnSO_4$.

3) When precipitating proteins, add the $Ba(OH)_2$ to the sample first, then add an equal amount of $ZnSO_4$.

3. Analysis of ^{14}C Glucose Specific Activity

3.1 REAGENTS

4.73% $Ba(OH)_2$
5.50% $ZnSO_4$

3.2 METHODS

1) Centifuge blood 10 minutes to separate plasma then place plasma into a new tube.

2) Precipitate proteins from plasma by adding equal volumes of the $Ba(OH)_2$ and $ZnSO_4$ stock solutions. For 1ml plasma use 1.5 ml of each solution. Vortex immediately then centrifuge at 15,000 rpm for 15 minutes.

3) Transfer supernatant to another test tube.

4) Run the supernatant through anion and cation exchange columns*. Columns are prepared by placing a cotton plug in a 14 cm pasteur pipette then adding 5 cm resin to the pipette and rinsing with water. Collect the eluate in test tubes.

5) Place samples into vortex evaporator and dry.

6) Add 1ml of distilled H_2O and shake to dissolove contents of vial. Transfer 0.5 ml of this solution to another scintillation vial then add 0.5 ml of water and 10 ml hydrofluor. Cap and count in scintillation counter. Determine the glucose concentration in the remaining 0.5 ml**.

* Dowex -50W (cation) 200 - 400 mesh (Sigma Chemical Co, St Louis)

Dowex 1x8 (anion) 200-400 mesh (Serva Feinbiochemica, Heidelberg)

** Performed by an Hitachi 704 Autoanalyser

4. Analysis of 6 ^{3}H Glucose Specific Activity

4.1 REAGENTS

4.73% $Ba(OH)_2$
5.50% $ZnSO_4$

4.2 METHODS

1) Deproteinize 1 ml plasma by method described in Appendix 3.2, and place supernatant in a test tube.

2) Place samples into vortex evaporator and dry.

3) Add 1ml of distilled H_2O and shake to dissolove contents of vial. Transfer 0.5 ml of this solution to another scintillation vial then add 0.5 ml of water and 10 ml hydrofluor. Cap and count in scintillation counter. Determine the glucose concentration in the remaining 0.5 ml.

5. Analysis of ^{14}C Palmitate Specific Activity

5.1 REAGENTS

Heptadecanoic acid standard - prepared fresh
1:10:40 solution of 1N HCl: n-heptane: isopropanolol
Chloroform
Boron trifluoride methanol
Petroleum ether

5.2 METHOD

A. Extraction for Gas Chromatography

1) Into 16 x 125 mm pyrex screw top tubes add
   a) 1ml plasma
   b) 75 ml heptadecanoic acid standard
   c) 5 ml of HCl: *n* -heptane: isopropanolol solution to each tube.

2) Cap and shake by hand for 10 minutes.

3) Add to each tube: 2ml distilled H20 and 3ml *n* - heptane.

4) Cap and shake by hand for 5 min.

5) Remove upper phase into 13mm x 100 mm screw top tubes.

6) Evaporate under nitrogen at 40 °C.

B. Measurement of Total Free Fatty Acid Concentration and Specific Activity

1) To evaporated extract add boiling chips and 1 ml boron trifluoride methanol. Mix.

2) Boil for 2 minutes. Cool under tap water.

3) Add 1 ml petroleum ether (40-50 °C). Mix well on vortex then pipette upper phase into a glass tube.

4) Repeat step 3 on lower phase.

5) Evaporate at 40 °C, add 200 µl chloroform and mix well.

6) Add 100 ul of this solution to a scintillation vial, evaporate, add 1 ml H_2O and 10 ml scintillation fluid and count. Inject 10 µl of solution into the chromatograph.

6. Analysis of 2 and 6 ^3H Glucose Specific Activity

6.1 REAGENTS

$Ba(OH)_2$ and $ZnSO_4$
Hexokinase
Phosphoglucose isomerase
$MgCl_2$
ATP
Sorenson's Buffer pH 7.4

6.2 METHODS

1) Deproteinize 1 ml plasma with $Ba(OH)_2$ and $ZnSO_4$ and pour supernatant over Dowex columns as described in Appendix 3.2.

2) A 1 ml aliquot is evaporated to dryness in a vortex and reconstituted with 1 ml H_2O (sample A).

3) Another 1 ml aliquot is incubated at room temperature with 2 units hexokinase and 9 units phosphoglucose isomerase in the presence of $MgCl_2$ abd ATP (both at a concentration of 1.8 mmol) at a pH of 7.4 (Sorenson's buffer) for 1 hour then evaporated to dryness and redissolved in 1 ml H_2O (sample B).

4) 0.5 ml aliquots of samples A and B are then placed in scintillation vials, 0.5 ml of H_2O and 10 ml of scintillation fluid added and they are counted on the dual ^3H and ^{14}C channel of a beta counter. The counts from sample A on the ^3H channel represent the combined activities of the 2 and 6 ^3H glucose tracers. The ^3H counts in sample B are from the 6 ^3H glucose tracer only, the 2 ^3H having been cleaved by the enzymes and then evaporated. The difference in the number of counts between samples A and B represents the activity of the 2 ^3H glucose

tracer. The glucose concentrations of the remaining 0.5 ml aliquots are then determined.

5) A further sample was processed in the same manner as the other samples, but 50 µl of 2 ^{3}H infusate was added to one half and 50 µl of 6 ^{3}H infusate was added to the other half. The samples were then incubated as described above. The ratio of counts incubated/not incubated should give values of ~ 5% for 2 ^{3}H glucose and ~95% for 6 ^{3}H glucose. This provides a correction factor for the small percentage of ^{3}H in the 2 ^{3}H infusate on positions other than the 2 carbon.

7. Analysis of Plasma Glycerol Concentration

7.2 REAGENTS

Phosphoric acid, 4N
Potassium metaperiodate, 0.015 N
Phenyl hydrazine hydrochloride
Potassium ferricyanide, 1% w/v in H_20
Sulphuric-Acetic Acid reagent
(Place 80 ml H_20 in a 1l Ehrlenmeyer flask sitting in a sink of cold water)
Isopropanol

7.2 METHOD

1) Deproteinize 0.5 ml specimens of plasma with $Ba(OH)_2$ and $ZnSO_4$ solutions as described in Appendix 3.2.

2) Remove 0.5 ml of the supernatant solution and place this in glass test tube.

3) Prepare a series of glycerol standards of concentration 0, 10, 20, 30, 40 and 50 mg/ml and add 0.5 ml of each standard to a glass test tube.

4) To each tube in order add 50 ul of 4N phhosphoric acid and 100µl of potassium metaperiodate. After 15 minutes add 200 µl of freshly prepared and filtered phenyl hydrazine solution. After 10 minutes follow with 200 µl of potassium ferricyanide. Place immediately in an ice-bath for exactly 5 minutes, adding 1 ml of sulphuric-acetic acid solution and 1 ml isopropanol upon removal. Vortex after each addition.

5) Read absorbance at 520 ηm 20 minutes after final addition.

8. Analysis of ^{15}N Urea Enrichment

8.1 REAGENTS

Sulphosalicylic acid, 15% w/v
Hydrochloric acid, 1N
Ammonium hydroxide, 2N
Dowex 50 W x 8 100-200 mesh hydrogen ion resin

8.2 METHODS

A. Column Preparation and Pre-Treatment

1) Make columns by pushing a small quantity of glass wool down into the stem of 14 cm Pasteur pipette. Approximately 3.5 cm of resin should be placed into the pipette.

2) Wash the column with 1 ml of 1N HCl.

3) Wash the column with deionized H_2O until pH = 7.

B. Sample Preparation

1) Mix 1 ml plasma with 1 ml sulphosalicylic acid, centrifuge and transfer the supernatant to the prepared column.

2) After the sample has run through the column completely, rinse the tube which held the sample with 0.5 ml H_2O, and run this through the column. Then rinse the column with deionized H_20 until pH = 7.

3) Discard all of the wash.

4) Run 2N NH_4OH through the column. Collect the eluate in small (13 x 100 mm) pyrex screw top tubes.

5) Dry under N_2 as follows: warm water bath, gentle N_2 flow, and cap while warm having flushed with nitrogen.

6) Determine atom percent excess by isotope ratio mass spectrometry.

9. Analysis of Plasma ß-Hydroxybutyrate Concentration

9.1 REAGENTS

ß-HBD Reagent (Sigma Chemical Co)
ß-HBD Dehydrogenase 50 units/ml (Sigma Chemical Co)
ß-HBD Calibrator Solution, 4.8 mmol/l

9.2 METHODS

1. Pipette 3.0 ml of ß-HBD reagent and 0.05 ml of ß-HBD dehydrogenase solution into each tube and warm to 37 °C.

2. Add 0.05 ml of either deionized water, calibrator solution or samples to appropriately labelled test tubes. Mix by gentle inversion.

3. Incubate each tube for 10 minutes at 37 °C.

4. Read and record absorbance of all tubes at 340 nm within 30 minutes.

5. Subtract absorbance of blank from absorbance of samples and calibrator solution to obtain the change in absorbance (Δ A) due to ß-HBD. The ß-HBD concentration is calculated from the equation (Δ A sample/Δ A calibrator) x concentration of calibrator.

10. Analysis of ^{14}C Leucine Specific Activity in Plasma and Tissues.

10.1 REAGENTS

$Ba(OH)_2$
$ZnSO_4$
Nor-leucine standard (250 µmol/l)
Leucine standard (100 µmol/l)
Dowex 50-W hydrogen ion exchange resin
Hydrochloric Acid, 6N, 1N and 0.01 N
Ammonium hydroxide, 4N
Oxidase/ Catalase Reagent
(for each sample: amino acid oxidase 4.5 ul, catalase 0.05 mg, 0.5 N Tris buffer pH = 7.6)
Dichlormethane
Phosphate buffer pH = 7.0
Defatting mixture
(chloroform: heptane: methanol - 45:45:10)

10.2 METHODS

A. Plasma Specimens

1) Into plastic tubes measure for:
test: 1 ml plasma and 1ml nor-leucine standard
standard 1: 1 ml leucine standard
standard 2: 1 ml leucine standard and 1 ml nor-leucine standard
blank: 1 ml H2O

2) In all tubes adjust the pH with 1 N HCl until pH = 7.

3) Centrifuge to clear fibrin clots.

4) Prepare columns by placing 4 cm of Dowex 50-W hydrogen ion exchange resin in a 14 cm Pasteur pipette and wash with H_2O.

5) Run the plasma samples through the columns and discard the eluate.

6) Wash each column four times with 1 ml 0.01 ml HCl.

7) Wash each column four times with 1 ml NH_4OH, collecting the eluate in scintillation vials. Freeze dry the samples.

8) Prepare fresh Oxidase/Catalase reagent and add 1 ml to each sample. Mix well, flush with O_2 and incubate at 37 °C in shaking water bath for 90 minutes. Add 1 N HCl until pH < 1.

9) Add 20 ml dichloromethane, vortex for one minute and aspirate off the upper phase and transfer this to 16 x 125 mm tubes. Back extract with 0.35 ml 0.1 N phosphate buffer (pH = 7.0), shaking the mixture for one minute then centrifuging it. Transfer the upper phase into small cups. Inject samples into a high pressure liquid chromatograph, and add 0.1 ml samples to scintillation vials, adding 0.9 ml H_2O and 10 ml scintillation fluid.

B. Tissue Specimens

1) Cut tissues specimen into approximately 30 mg pieces and freeze dry them. Add 5 ml of the defatting solution and leave for 48 hours. Pour off the chloroform mixture and absorb the excess

with tissue paper, then dry the specimens in a drying oven.

2) Place the specimens in a hydrolysing tube and add 2 ml 6 N HCl. Hydrolyse for 12 hours, centrifuge the specimens and run the supernatant through the columns in the same manner described in step 4 above for the plasma specimens.

Appendix 2

Experimental Data: Patient Studies

Table of Contents

The following tables present the raw data of the studies which have been described in Chapters 4 & 5

2.2.(iv) Protein metabolism

2.3.(v) Indirect calorimetry and substrate oxidation

2.4 *Intraoperative Study*

2.4.(i) Plasma hormone levels

2.4.(ii) Protein metabolism

Table 2.1.(i) *Plasma Hormone Levels in IVN Controls*

	AA1		AA2		AA3		AA4		Mean +/- SEM		Signif.
	Basal	**Post**	**Basal**	**Post**	**Basal**	**Post**	**Basal**	**Post**	**Basal**	**Post**	
hGH *μg/l*	5.4	10.0	1.5	1.1	2.7	2.7			3.2 +/- 1.2	4.5 +/- 2.8	n.s.
IGF-I *μg/l*	424	315	634	712	395	478			484 +/- 75	502 +/- 115	n.s.
Insulin *mIU/l*			42	14	40	57	13	13	36 +/- 22	28 +/- 15	n.s.
Cortisol *ηmol/l*	455	315	641	324	325	425	485	186	477 +/- 65	313 +/- 49	n.s.

Table 2.1.(ii) *Glucose Metabolism in IVN Controls*

	AA1		AA2		AA3		AA4		Mean +/- SEM		Signif
	Basal	Post	Basal	Post	Basal	Post	Basal	Post	Basal	Post	
Glucose concentration *mmol/l*	6.0	6.0	7.6	6.3	3.3	8.9	6.1	5.4	5.8 +/- 0.9	6.7 +/- 0.8	n.s.
Ra Glucose total *µmol/kg.min*	24.0	27.7	56.4	34.1	27.1	26.6	32.2	30.0	34.9 +/- 7.4	29.6 +/- 1.7	n.s.
Ra Glucose endogenous *µmol/kg.min*	11.2	14.9	34.7	12.4	19.9	19.7	13.3	11.1	19.8 +/- 5.3	14.5 +/- 1.9	n.s.
Glucose Clearance *ml/kg.min*	4.0	4.6	7.4	5.4	8.2	3.0	5.3	5.6	6.3 +/- 1.0	4.7 +/- 0.6	n.s.

Table 2.1.(iii) *Protein and Fat Metabolism in IVN Controls*

	AA1		AA2		AA3		AA4		Mean +/- SEM		Signif
	Basal	Post	Basal	Post	Basal	Post	Basal	Post	Basal	Post	
Ra Urea *µmol/kg.min*	3.1	4.0	9.1	10.5	14.0	13.8	8.7	8.9	8.7 +/- 2.2	9.3 +/- 2.0	n.s.
Net Protein Loss *g/kg.day*	0.54	0.32	-0.63	-1.03	-1.67	-1.62	-0.23	-0.29	-0.51 +/-0.46	-0.66 +/-0.43	n.s.
Glycerol Conc. *mmol/l*	0.15	0.16	0.15	0.15	0.16	0.28			0.15 +/- 0.01	0.20 +/- 0.04	n.s.
FFA Conc. *mmol/l*	0.72	0.79	0.67	0.52	0.96	0.91			0.78 +/- 0.04	0.74 +/- 0.12	n.s.

Table 2.2.(i) *Plasma Hormone Levels in rhGH Treated IVN Patients*

	A1		A2		A3		A5		A6		A7		A8		A9	
	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post
HGH $\mu g/l$	1.3	2.2	1.6	1.6			1.9	1.8	0.7		1.8		1.2	0.8		
IGF-I $\mu g/l$	231	999	416	889	192	512	532	1100	372	1175	449	1169	709	1434	574	726
Cortisol $\eta mol/l$	447	592	121	192	43	542	470	334	373	302	462	467	321	615		
Insulin mIU/l	95	263	57	121	28	43	85	135	21	29	44	90	68	156		

	Mean +/- SEM		Signif.
	Basal	Post	
HGH $\mu g/l$	1.4 +/- 0.2	1.5 +/- 0.3	n.s.
IGF-I $\mu g/l$	434 +/- 61	1001+/-102	$p<0.001$
Cortisol $\eta mol/l$	320 +/- 65	435 +/- 61	n.s.
Insulin mIU/l	57 +/- 11	120 +/- 30	$p<0.03$

Table 2.2.(ii) *Glucose Metabolism in rhGH Treated IVN Patients*

	A1		A2		A3		A5		A6		A7		A8		A9	
	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post
Conc. *mmol/l*	8.4	9.2	6.8	7.3	6.5	6.7	7.4	8.0	5.9	6.3	6.3	7.3	6.3	9.7	8.3	11.8
Ra total *µmol/kg.min*	25.5	26.3	31.2	32.6	40.3	65.0	19.2	31.0	21.2	25.3	22.0	20.6	19.3		20.3	24.8
Ra endog *µmol/kg.min*	12.3	13.1	16.2	17.6	24.5	40.2	5.5	17.3	3.3	7.4	7.9	6.5	5.8		4.4	8.9
Clearance *ml/kg.min*	3.0	2.9	4.6	4.5	6.2	9.7	2.6	3.9	3.6	4.0	3.5	2.8	3.1		2.4	2.1

	Mean +/- SEM		Signif.
	Basal	Post	
Conc. *mmol/l*	7.0 +/- 0.3	8.3 +/- 0.7	$p < 0.03$
Ra total *µmol/kg.min*	24.9 +/- 2.6	32.3 +/- 5.7	n.s.
Ra endog *µmol/kg.min*	10.0 +/- 2.6	15.9 +/- 4.4	$p < 0.07$
Clearance *ml/kg.min*	3.6 +/- 0.4	4.3 +/- 1.0	n.s.

Table 2.2.(iii) *Fat Metabolism in rhGH Treated IVN Patients*

	A1		A2		A3		A5		A6		A7		A8		A9	
	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post
Free Fatty Acid Conc. *mmol/l*	1.13	0.94	0.68	0.55	0.61	0.92	0.53	0.41	0.18	0.12	0.28	0.24	0.40	0.55	0.82	0.80
Glycerol Conc. *mmol/l*	0.16	0.24	0.23	0.12	0.12	0.07	0.15	0.08	0.11	0.12	0.08	0.05	0.14	0.16	0.13	0.13

	Mean +/- SEM		Signif.
	Basal	Post	
Free Fatty Acid Conc. *mmol/l*	0.58 +/- 0.11	0.57 +/- 0.11	n.s.
Glycerol Conc. *mmol/l*	0.14 +/- 0.02	0.12 +/- 0.02	n.s.

Table 2.2.(iv) *Protein Metabolism in rhGH Treated IVN Patients*

	A1		A2		A3		A5		A6		A7		A8		A9	
	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post
Ra Urea *µmol/kg.min*	6.8	6.1	6.7	6.3	9.1	7.2	13.4	10.2			11.7	10.7	8.8	5.6	11.1	6.6
NPL *g/kg.day*	-.30	-.13	-.49	-.42	-.65	-.16	-1.14	-.81			-1.30	-1.05	-.47	0.44	-1.41	-.89
Ra leucine *µmol/kg.min*							2.0	2.2	3.6	3.6	8.4	7.3	3.4	4.2	4.6	4.8
Leucine Ox. *µmol/kg.min*							0.60	0.43	0.85	0.63	1.10	0.87	0.53	0.50	0.58	0.36

	Mean +/- SEM		Signif.
	Basal	Post	
Ra Urea *µmol/kg.min*	9.7 +/- 1.0	7.5 +/- 0.8	$p < 0.02$
NPL *g/kg.day*	0.82 +/- 0.17	0.43 +/- 0.20	$p < 0.02$
Ra leucine *µmol/kg.min*	4.4 +/- 1.1	4.4 +/- 0.8	n.s.
Leucine Ox. *µmol/kg.min*	0.73 +/- 0.11	0.56 +/- 0.09	$p<0.01$

Table 2.2.(v) *Calorimetry and Substrate Oxidation in rhGH Treated IVN Patients*

	A1		A5		A6		A7		A8		A9	
	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post
RQ	0.92	1.01	0.91	0.94	0.94	0.89	0.92	0.91	0.95	0.93	0.97	0.92
REE *kcal/day*	1680	1430	1450	1610	1340	1440	1420	1890	1750	1280	1890	1950
CHO Ox.* *µmol/kg.min*	14.3	18.0	20.6	15.0	12.9	28.3	8.3	11.9	13.6	15.5	14.5	14.3
Fat Ox.** *µmol/kg.min*	0.2	0	0	0	0	0	0	0	1.8	0	0	0

	Mean +/- SEM		Signif.
	Basal	Post	
RQ	0.94 +/- 0.01	0.93 +/- 0.02	n.s.
REE *kcal/day*	1590 +/- 90	1600 +/- 110	n.s.
CHO Ox.* *µmol/kg.min*	14.0 +/- 1.6	17.2 +/- 2.3	n.s.
Fat Ox.** *µmol/kg.min*	0.3 +/- 0.3	0.0 +/- 0.0	n.s.

* Carbohydrate and total fat oxidation rates derived from Frayn's Equation (Eq 3.2.(vi))

** Assumes averge molecular weight of 250 Daltons for FFAs.

Table 2.3.(i) *Plasma Hormone Levels in rhGH Treated Postabsorptive Patients*

	B1		B2		B3		B4		B5		E1		E2		E3	
	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post
HGH $\mu g/l$	1.1	30.3	0.5	30.4	0.6	48.8	0.5	32.4	2.2	26.7	2.0	21.4	2.1	57.0	3.2	23.0
IGF-I $\mu g/l$	151	319	264	1028	232	923	257	858	336	1470	119	338	284	623	402	1100
Cortisol $\eta mol/l$	628	234	491	403	345	274	599	513	491	521	357	371			195	339
Insulin mIU/l	22	54	7	26	6	22	11	35	13	58	7	23	4	6	8	16

	E4		C1		C2		C3		C4		Mean +/- SEM		Signif.
	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Basal	Post	
HGH $\mu g/l$	0.9	29.0	0.8	79.0			1.2	61.0			1.4 +/- 0.3	39.9 +/- 0.3	p<0.0002
IGF-I $\mu g/l$	119	355	295	1100			293	874	497	482	434 +/- 61	1001+/-102	p<0.0003
Cortisol $\eta mol/l$	357	371	321	225	470	329	337	494	472	299	320 +/- 65	435 +/- 61	n.s.
Insulin mIU/l	9	11	8	21	5	7	13	24			57 +/- 11	120 +/- 30	p<0.002

Table 2.3.(ii) *Glucose Metabolism in rhGH Treated Postabsortive Patients*

	E1		E2		E3		E4		Mean +/- SEM		Signif.
	Basal	Post	Basal	Post	Basal	Post	Basal	Post	Basal	Post	
Glucose Conc. *mmol/l*	5.6	6.5	6.0	6.4	5.7	5.3	5.7	5.6	5.8 +/- 0.1	6.0 +/- 0.3	n.s.
Glucose Ra 6 *µmol/kg.min*	17.3	14.3	19.7	19.9	14.7	15.2	12.1	15.0	15.9 +/- 1.6	16.1 +/- 1.3	n.s.
Glucose Ra 2 *µmol/kg.min*	22.0	20.3			16.0	12.1	15.6	15.2	17.9 +/- 2.1	15.9 +/- 2.3	n.s.
Glucose Ra 14. *umol/kg.min*	9.8	9.2	12.6	12.0	8.0	8.2	8.5	10.3	9.7 +/- 1.0	9.9 +/- 0.8	n.s.
Ra 2 - Ra 6 *µmol/kg.min*	4.7	6.0			1.3	0	3.5	0.2	3.2 +/- 1.0	2.1 +/- 2.0	n.s.
Ra 6 - Ra 14 *µmol/kg.min*	7.5	3.2	7.1	7.9	0	6.7	0.2	3.6	3.7 +/- 2.1	5.4 +/- 1.2	n.s.
Gluc. Clearance *ml/kg.min*	3.1	2.2	3.3	3.1	2.6	2.9	2.1	2.7	2.7 +/- 0.2	2.8 +/- 0.3	n.s.

Table2.3.(iii) *Fat Metabolism in rhGH Treated Postabsorptive Patients*

	C1		C2		C3		C4		Mean +/- SEM		Signif.
	Basal	Post	Basal	Post	Basal	Post	Basal	Post	Basal	Post	
Ra Glycerol *μmol/kg.min*	2.9	3.5	2.5	3.0	5.3	6.0	2.5	2.3	3.3 +/- 0.7	3.7 +/- 0.8	n.s.
Ra Palmitate *μmol/kg.min*	1.6	3.1	2.1	2.7	0.9	1.7	3.8	4.5	2.1 +/- 0.6	3.0 +/- 0.6	$p < 0.03$
Ra FFA *μmol/kg.min*	5.7	11.5	7.0	9.4	3.3	5.6	13.6	18.0	7.4 +/- 2.2	11.1 +/- 2.6	$p < 0.03$
FFA Oxidation *μmol/kg.min*	1.0	1.5	2.0	2.4	0.9	1.1			1.3 +/- 0.4	1.7 +/- 0.4	$p < 0.06$

Table 2.3.(iv) *Protein Metabolism in rhGH Treated Postabsorptive Patients*

	B1		B2		B3		B4		B5		E1		E2		E3	
	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post
Ra Urea *µmol/kg.min*	13.6	10.5	9.6	8.3	6.2	4.5	8.3	8.9	5.8	4.0	3.4	3.1	7.4	7.3	6.5	6.1
NPL* *g/kg.day*	3.4	2.6	2.4	2.1	1.6	1.1	2.1	2.2	1.5	1.0	0.9	0.8	1.9	1.8	1.6	1.5
Ra Leucine *µmol/kg.min*	2.4	2.0	3.5	1.7	4.7	3.2	5.3	4.5	2.4	2.3						
Leucine Ox. *µmol/kg.min*	0.58	0.31	0.40	0.23	0.33	0.16	0.53	0.36	0.35	0.25						

	Mean +/- SEM		Signif.
	Basal	Post	
Ra Urea *µmol/kg.min*	7.6 +/- 1.1	6.6 +/- 0.9	$p < 0.05$
NPL* *g/kg.day*	1.9 +/- 0.3	1.6 +/- 0.2	$p < 0.05$
Ra Leucine *µmol/kg.min*	3.7 +/- 0.6	2.7 +/- 0.5	$p < 0.04$
Leucine Ox. *µmol/kg.min*	0.44 +/- 0.05	0.26 +/- 0.03	$p < 0.005$

* NPL = total protein loss

Table 2.3.(v) *Calorimetry and Substrate Oxidation in rhGH Treated Postabsorptive Patients*

	B1		B2		B3		B4		B5		E1		E2		E3	
	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post
RQ	0.77	0.72	0.78	0.67	0.79	0.76	0.67	0.67	0.80	0.72	0.77	0.74	0.71	0.70	0.84	0.75
RME *kcal/day*	2714	2452	1540	1620	960	1080	1280	1360	1170	1180	1510	1700	1180	1210	1660	1740
CHO Ox. *μmol/kg.min*	3.0	0	2.0	0	3.5	2.4	0	0	2.5	0	3.6	0.9	0	0	8.6	1.4
Fat Ox. *μmol/kg.min*	3.7	4.9	2.8	6.1	2.6	3.9	4.4	4.8	1.7	3.3	4.0	5.7	6.1	7.8	2.1	4.6

	E4		C1		C2		C3		Mean +/- SEM		Signif.
	Bas.	Post	Bas.	Post	Bas.	Post	Bas.	Post	Basal	Post	
RQ	0.82	0.77	0.76	0.69	0.85	0.77	0.80	0.68	0.78 +/- 0.02	0.72 +/- 0.01	$p < 0.0002$
RME *kcal/day*	1320	1350	1340	1480	1670	1730	2390	2110	1560 +/- 150	1584 +/- 120	n.s.
CHO Ox. *μmol/kg.min*							16.6	0	4.4 +/- 1.7	0.5 +/- 0.3	$p < 0.06$
Fat Ox. *μmol/kg.min*							0	13.6	3.0 +/- 0.6	6.1 +/- 1.0	$p < 0.001$

Table 2.4.(i) *Plasma Hormone Concentrations in Control and rhGH Treated Operative Patients*

	D6	D4	D7	D3	D8	D2	D9	D1	D10	D5
	Basal	rhGH	Basal	rhGH	Basal	rhGH	Basal	rhGH	Basal	rhGH
GH $\mu g/l$		25.3	1.3	46.5	0.9	27.4	2.2	32.4	1.9	17.1
IGF-I $\mu g/l$	196	561	343	356	502	706	312	513	199	310
Insulin mIU/l	0	5	5	11	3	16	7	6	8	27
Cortisol $\eta mol/l$	1500	708	152	883	616	850	514		975	524

	Mean +/- SEM		
	Basal	rhGH	Significance
GH $\mu g/l$	1.6 +/- 0.3	29.7 +/- 4.8	$p < 0.02$
IGF-I $\mu g/l$	310 +/- 56	489 +/- 72	$p < 0.04$
Insulin mIU/l	5 +/- 1	13 +/- 4	$p < 0.08$
Cortisol $\eta mol/l$	751 +/- 229	741 +/- 82	n.s.

Table 2.4.(ii) *Protein Metabolism in Control and rhGH Treated Operative Patients*

	D6	D4	D7	D3	D8	D2	D9	D1	D10	D5
	Basal	rhGH	Basal	rhGH	Basal	rhGH	Basal	rhGH	Basal	rhGH
Ra Leucine *µmol/kg.min*	1.6	1.9	0.7	1.6	0.9	0.9	1.6	1.5	1.6	1.8
Skin FSR *%/day*	1.7	6.3	3.0	1.2	2.3	2.8	4.5		2.9	7.1
Muscle FSR *%/day*	3.5	4.9	1.4	2.3	2.9	2.7	0.9	2.7	2.3	4.7
Liver FSR *%/day*	35.0	36.0	20.0	23.0	16.0	19.0		24.0	21.0	18.0
Colon FSR *%/day*	5.3	6.1	6.8		5.4	6.3	2.1	7.0	11.5	
Cancer FSR *%/day*	21.0	21.0	19.4	14.3		12.0	11.0	22.0	19.0	11.0

	Mean +/- SEM		
	Basal	rhGH	Significance
Ra Leucine *µmol/kg.min*	1.3 +/- 0.2	1.5 +/- 0.2	n.s.
Skin FSR *%/day*	2.9 +/- 0.5	4.4 +/- 1.4	n.s.
Muscle FSR *%/day*	2.2 +/- 0.5	3.5 +/- 0.6	$p < 0.05$
Liver FSR *%/day*	23.0 +/- 4.1	24.0 +/- 3.2	n.s.
Colon FSR *%/day*	2.9 +/- 0.5	3.6 +/- 0.4	n.s.
Cancer FSR *%/day*	17.6 +/- 2.2	16.1 +/- 2.3	n.s.

Appendix 3

Animal Studies: Raw Data

Table of Contents

The following tables present the raw data of the studies which have been described in Chapters 6, 7 and 8.

3.2.(iii) Plasma glucose concentration

3.2.(iv) Plasma potassium concentration

3.2.(v) Glucose Ra

3.2.(vi) Glucose clearance

3.2.(vii) Plasma glycerol concentration

3.2.(viii) Plasma free fatty acid concentration

3.2.(ix) Plasma ketone concentration

3.2.(x) Plasma triglyceride concentration

3.2.(xi) Urea Ra

3.2.(xii) Net protein loss

3.2.(xiii) Leucine Ra

3.2.(xiv) Plasma urea concentration

3.3 rhIGF-I 50 μg/kg.hr

3.3.(i) Plasma IGF-I concentration

3.3.(ii) IGF-I binding forms

3.3.(iii) Plasma glucose concentration

3.3.(iv) Plasma potassium concentration

3.3.(v) Glucose Ra

3.3.(vi) Glucose clearance

3.3.(vii) Plasma glycerol concentration

3.3.(viii) Plasma free fatty acid concentration

3.3.(ix) Plasma ketone concentration

3.3.(x) Plasma triglyceride concentration

3.3.(xi) Urea Ra

3.3.(xii) Net protein loss

3.3.(xiii) Leucine Ra

3.3.(xiv) Plasma urea concentration

3.4 rhIGF-II 50 μg/kg.hr

3.4.(i) Plasma IGF-I concentration

3.4.(ii) Plasma glucose concentration

3.4.(iii) Plasma potassium concentration

3.4.(iv) Glucose Ra

3.4.(v) Glucose clearance

3.4.(vi) Plasma glycerol concentration

3.4.(vii) Plasma free fatty acid concentration

3.4.(viii) Plasma ketone concentration

3.4.(ix) Plasma triglyceride concentration

3.4.(x) Urea Ra

3.4.(xi) Net protein loss

3.4.(xii) Leucine Ra

3.4.(xiii) Plasma urea concentration

3.5 rhInsulin 1 μg/kg.hr

3.5.(i) Plasma IGF-I concentration

3.5.(ii) Plasma glucose concentration

3.5.(iii) Plasma potassium concentration

3.5.(iv) Glucose Ra

3.5.(v) Glucose clearance

3.5.(vi) Plasma glycerol concentration

3.5.(vii) Plasma free fatty acid concentration

3.5.(viii) Plasma ketone concentration

3.5.(ix) Plasma triglyceride concentration

3.5.(x) Urea Ra

3.5.(xi) Net protein loss

3.5.(xii) Leucine Ra

3.5.(xiii) Plasma urea concentration

3.6 Protein Fractional Synthetic Rates

3.6.(i) Normal saline

3.6.(ii) rhIGF-I 15 μg/kg.hr

3.6.(iii) rhIGF-I 50 μg/kg.hr

3.6.(iv) rhIGF-II 50 μg/kg.hr

3.6.(v) Insulin 1 μg/kg.hr

3.7 rhTNF Infusions

3.7.(i) Plasma total IGF-I concentration

3.7.(ii) Plasma TNF and insulin concentrations

3.7.(iii) Plasma cortisol and glucagon concentrations

3.7.(iv) Plasma glucose concentration

3.7.(v) Glucose Ra

3.7.(vi) Glucose clearance

3.7.(vii) Plasma glycerol concentration

3.7.(viii) Plasma triglyceride concentration

3.7.(ix) Plasma potassium concentration

3.7.(x) Plasma urea concentration

3.7.(xi) Urea Ra

3.7.(xii) Net protein loss

3.8 90 Minute rhIGF-I Infusions

3.8.(i) Plasma total IGF-I concentration

3.8.(ii) Plasma IGF-I binding forms

3.8.(iii) Plasma glucose concentration

3.8.(iv) Glucose Ra

3.8.(v) Glucose clearance

3.8.(vi) Plasma glycerol and free fatty acid concentrations

Table 3.1.(i) *Normal Saline Infusion: Plasma Total IGF-I Concentration (μg/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
116	510	543	477	669	706	478
133	746	730	668		501	458
135	555	452	398	350	330	411
138	360	317	338	390	367	334
146	236	217	197	191	181	194
Mean +/- SEM	481 +/- 87	451 +/- 89	416 +/- 78	514 +/- 138	468 +/- 88	485 +/- 117

Table 3.1.(ii) *Normal Saline Infusion: Plasma IGF-I Binding Form Concentration at t = 300 min (μg/l)*

	7 kDa		35-60 kDa		150 kDa	
Lamb	Concentration	% Total	Concentration	% Total	Concentration	% Total
135	<40	0	155	67	148	33
138	<40	0	132	57	75	43
146	<40	0	154	71	63	29
Mean +/- SEM	<40	0	147 +/- 8	65 +/- 4	95 +/- 27	35 +/- 4

Table 3.1.(iii) *Normal Saline Infusion: Glucose Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
112	2.5	2.6	2.6	2.5	2.4	2.3
116	3.1	2.8	3.2	4.1	4.5	4.4
133	3.6	3.8	3.6			4.7
135	3.1	2.8	3.1	3.3	3.3	3.4
138	2.8	2.7	2.5	2.5	2.6	3.0
146	3.2		3.2	2.6	2.8	3.1
Mean +/- SEM	3.1 +/- 0.2	3.0 +/- 0.2	3.0 +/- 0.2	3.0 +/- 0.3	3.1 +/- 0.4	3.5 +/- 0.4

Table 3.1.(iv) *Normal Saline Infusion: Plasma Potassium Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
225	3.6	3.9	4.1	4.0	4.1	3.6
226	3.0	3.9	4.2	4.1	4.2	3.9
228	3.5	3.9	3.9	3.8	3.1	3.2
233	3.3	4.0	4.0	4.0	3.8	3.9
Mean +/- SEM	3.4 +/- 0.1	3.9 +/- 0.0	4.1 +/- 0.1	4.0 +/- 0.1	3.8 +/- 0.2	3.7 +/- 0.2

Table 3.1.(v) *Normal Saline Infusion: Ra Glucose (µmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
112	9.6	10.1	10.9	9.7	9.5	9.1
116	9.0	9.7	10.2	10.4	11.1	10.9
133	13.2	14.4	15.1			17.8
135	10.7	10.4	9.9	10.3	10.2	10.2
138	6.3	5.7	5.8	5.5	6.2	6.4
146	10.6	9.9	9.2	9.8	9.0	9.1
Mean +/- SEM	9.9 +/- 1.0	10.0 +/- 1.1	10.1 +/- 1.2	9.1 +/- 0.9	9.2 +/- 0.8	10.6 +/- 1.6

Table 3.1.(vi) *Normal Saline Infusion: Glucose Clearance (ml/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
112	3.8	3.9	4.2	3.9	4.0	4.0
116	2.9	3.5	3.2	2.5	2.5	2.5
133	3.7	3.8	4.2			3.8
135	3.5	3.7	3.2	3.1	3.1	3.0
138	2.3	2.1	2.3	2.2	2.4	2.1
146	3.2	3.1	3.2	2.6	2.8	3.1
Mean +/- SEM	3.2 +/- 0.2	3.3 +/- 0.2	3.4 +/- 0.3	2.9 +/- 0.3	3.0 +/- 0.3	3.1 +/- 0.3

Table 3.1.(vii) *Normal Saline Infusion: Glycerol Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
225	0.21	0.39	0.29	0.30	0.29	0.27
226	0.24	0.24	0.25	0.22	0.21	0.26
228	0.44	0.41	0.23	0.27	0.23	0.26
233	0.50	0.33	0.23	0.27	0.24	0.21
235	0.38	0.35	0.39	0.35	0.33	0.33
243	0.23	0.35	0.29	0.29	0.28	0.29
Mean +/- SEM	0.33 +/- 0.05	0.35 +/- 0.02	0.28 +/- 0.03	0.28 +/- 0.02	0.26 +/- 0.02	0.27 +/- 0.02

Table 3.1.(viii) *Normal Saline Infusion: Plasma Free Fatty Acid Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
225	0.42	1.47	0.56	0.50	0.57	0.72
226	0.60	0.53	0.32	0.49	0.43	0.63
228	0.18	0.22	0.36	0.38	0.33	0.47
233	0.85	0.49	0.24	0.33	0.73	0.50
235	0.49	1.27	0.95	0.78	0.70	0.39
243	0.31	0.64	0.49	0.58	0.78	0.64
Mean +/- SEM	0.48 +/- 0.10	0.77 +/- 0.20	0.49 +/- 0.10	0.51 +/- 0.07	0.59 +/- 0.07	0.56 +/- 0.05

Table 3.1.(ix) *Normal Saline Infusion: Plasma Ketone Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
225	1.74	1.27	1.69	1.08	2.54	2.12
226	1.08	1.51	1.08	1.55	1.88	1.92
228	2.40	2.54	1.41	1.51	1.65	1.63
233	1.18	1.60	1.88	1.51	2.49	2.26
Mean +/- SEM	1.60 +/- 0.30	1.73 +/- 0.28	1.52 +/- 0.17	1.41 +/- 0.11	2.14 +/- 0.22	1.98 +/- 0.14

Table 3.1.(x) *Normal Saline Infusion: Plasma Triglyceride Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
225	0.2	0.5	0.3	0.3	0.3	0.2
226	0.1	0.1	0.1	0.1	0.1	0.1
228	0.1	0.2	0.1	0.1	0.1	0.1
233	0.3	0.3	0.3	0.2	0.2	0.2
Mean +/- SEM	0.2 +/- 0.0	0.3 +/- 0.1	0.2 +/- 0.1	0.2 +/- 0.0	0.2 +/- 0.0	0.2 +/- 0.0

Table 3.1.(xi) *Normal Saline Infusion: Ra Urea (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
112	17.8	17.3	17.1	16.3	15.6	15.4
116	15.2	15.2	15.6	14.9	14.5	14.7
133	16.3	15.6	15.5			15.9
135	18.7	18.3	18.2	18.2	17.6	17.6
138	16.0	15.5	15.7	15.3	15.2	15.4
146	14.7	14.7	14.7	14.7	14.9	14.4
Mean +/- SEM	16.5 +/- 0.6	16.1 +/- 0.6	16.1 +/- 0.5	15.9 +/- 0.6	15.6 +/- 0.5*	15.6 +/- 0.5*

Table 3.1.(xii) *Normal Saline Infusion: Net Protein Loss (g/kg.day)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
112	4.5	4.4	4.3	4.1	3.9	3.9
116	3.8	3.8	3.9	3.8	3.7	3.7
133	4.1	3.9	3.9			4.0
135	4.7	4.6	4.6	4.6	4.4	4.4
138	4.0	3.9	4.0	3.9	3.8	3.9
146	3.7	4.1	3.7	3.7	3.8	3.6
Mean +/- SEM	4.1 +/- 0.2	4.1 +/- 0.1	4.1 +/- 0.1	4.0 +/- 0.2	3.9 +/- 0.1*	3.9 +/- 0.1*

* $p < 0.05$ compared to 0 min.

Table 3.1.(xiii) *Normal Saline Infusion: Leucine Ra (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
112	7.8	7.0	6.2	6.4	7.0	7.3
116	7.0	7.0	7.0	6.4	5.9	6.4
133	8.1	6.8	6.6			8.5
135	7.4	6.6	8.3	7.8	5.5	6.1
138	5.5	5.9	4.4	5.8	8.0	5.4
146	11.3	10.4	9.5	9.8	9.0	9.5
Mean +/- SEM	7.9 +/- 0.8	7.2 +/- 0.6	7.0 +/- 0.7	7.0 +/- 0.7	7.1 +/- 0.6	7.2 +/- 0.6

Table 3.1.(xiv) *Normal Saline Infusion: Plasma Urea Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
225	4.1	4.2	4.3	4.2	4.2	4.1
226	5.4	5.6	5.4	5.2	5.1	5.1
228	3.3	3.2	3.2	3.2	2.9	3.2
233	7.1	7.3	7.6	7.9	7.6	7.4
Mean +/- SEM	5.0 +/- 0.8	5.1 +/- 0.9	5.1 +/- 0.9	5.1 +/- 1.0	5.0 +/- 1.0	5.0 +/- 0.9

Table 3.2.(i) *IGF-I Infusion 15 μg/kg.hr: Plasma Total IGF-I Concentration (μg/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	285	500	685	907	606	649
159	306	444	550	728	673	643
160	356	559	552	583	941	714
161	263	559	663	752	658	683
Mean +/- SEM	303 +/- 20	516 +/- 28*	613 +/- 36†	743 +/- 66†	719 +/- 75†	672 +/- 16†

Table 3.2.(ii) *IGF-I Infusion 15 μg/kg.hr: Plasma IGF-I Binding Form Concentration at t = 300 min (μg/l)*

	7 kDa		35-60 kDa		150 kDa	
Lamb	Concentration	% Total	Concentration	% Total	Concentration	% Total
158	57	9	471	73	121	19
159	68	11	503	78	140	22
160	66	9	569	80	79	11
161	75	10	510	75	104	15
Mean +/- SEM	67 +/- 4	10 +/- 0.5	513 +/- 20†	77 +/- 2	111 +/- 13	17 +/- 2

Table 3.2.(i) * $p < 0.05$, † $p < 0.01$ compared to 0 min.
Table 3.2.(ii) * $p < 0.05$ compare to normal saline infused controls

Table 3.2.(iii) *IGF-I Infusion 15 μg/kg.hr: Plasma Glucose Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	4.1	4.3	4.4	4.4	4.3	4.4
159	3.4	3.5	3.2	3.9	3.9	3.8
160	4.1	4.1	4.4	5.1	4.3	4.2
161	3.5	3.7	3.8	4.4	4.1	3.9
Mean +/- SEM	3.8 +/- 0.2	3.9 +/- 0.2	4.0 +/- 0.3	4.4 +/- 0.2	4.2 +/- 0.1	4.1 +/- 0.1

Table 3.2.(iv) *IGF-I Infusion 15 μg/kg.hr Plasma Potassium Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	4.0	3.5	3.5	3.6	3.7	4.3
159	3.5	4.3	4.3	4.8	4.0	4.2
160	3.3	3.2	3.2	3.2	3.0	3.0
161	3.1	3.2	3.3	3.0	3.0	3.3
Mean +/- SEM	3.7 +/- 0.3	3.6 +/- 0.3	3.6 +/- 0.3	3.7 +/- 0.4	3.4 +/- 0.3	3.7 +/- 0.3

Table 3.2.(v) *IGF-I Infusion 15 μg/kg.hr: Glucose Ra (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	13.1	13.0	13.5	13.9	14.6	14.3
139	11.2	11.4	10.9	12.5	12.8	13.3
144	13.7	13.8	14.9	17.1	15.6	14.8
145	13.2	12.3	13.2	15.5	16.6	13.6
Mean +/- SEM	12.8 +/- 0.5	12.6 +/- 0.5	13.1 +/- 0.8	14.8 +/- 1.0	14.9 +/- 0.8	14.0 +/- 0.4

Table 3.2.(vi) *IGF-I Infusion 15 μg/kg.hr Glucose Clearance (ml/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	3.2	3.0	3.1	3.2	3.4	3.3
139	3.3	3.3	3.4	3.2	3.3	3.5
144	3.3	3.4	3.4	3.5	3.6	3.5
145	3.8	3.3	3.5	3.5	3.8	3.5
Mean +/- SEM	3.4 +/- 0.1	3.3 +/- 0.1	3.4 +/- 0.1	3.4 +/- 0.1	3.5 +/- 0.1	3.5 +/- 0.1

Table 3.2.(vii) *IGF-I Infusion 15 μg/kg.hr: Glycerol Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	0.45	0.52	1.01	0.76	0.64	0.89
159	1.44	0.98	0.90	0.97	1.34	0.89
160	1.20	0.82	0.81	0.67	0.85	0.70
161	0.52	0.95	0.93	1.14	0.83	0.69
Mean +/- SEM	0.90 +/- 0.25	0.82 +/- 0.11	0.91 +/- 0.04	0.89 +/- 0.11	0.92 +/- 0.15	0.79 +/- 0.06

Table 3.2.(viii) *IGF-I Infusion 15 μg/kg.hr: Plasma Free Fatty Acid Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	1.33	0.78	2.13	0.72	0.65	0.42
159	0.56	0.86	0.85	0.59	0.65	0.42
160	0.42	0.73	0.88	0.93	0.25	0.43
161	0.48	1.62	0.69	0.24	0.32	0.31
Mean +/- SEM	0.70 +/- 0.21	1.00 +/- 0.20	1.14 +/- 0.33	0.62 +/- 0.15	0.45 +/- 0.10	0.37 +/- 0.04

Table 3.2.(ix) *IGF-I Infusion 15 µg/kg.hr: Ketone Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	1.02	1.24	1.28	1.35	1.35	1.10
159	2.53	3.14	3.53	3.38	3.74	3.54
160	1.44	1.42	1.09	0.84	1.13	1.03
161	1.34	1.06	1.05	1.07	1.16	0.90
Mean +/- SEM	1.58 +/- 0.33	1.72 +/- 0.48	1.74 +/- 0.60	1.66 +/- 0.58	1.85 +/- 0.63	1.64 +/- 0.63

Table 3.2.(x) *IGF-I Infusion 15 µg/kg.hr: Plasma Triglyceride Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	0.3	0.3	0.4	0.5	0.4	0.4
159	0.2	0.1	0.2	0.5	0.2	0.2
160	0.2	0.3	0.3	0.3	0.3	0.3
161	0.2	0.2	0.2	0.1	0.1	0.1
Mean +/- SEM	0.2 +/- 0.0	0.2 +/- 0.1	0.3 +/- 0.1	0.4 +/- 0.1	0.3 +/- 0.1	0.3 +/- 0.1

Table 3.2.(xi) *IGF-I Infusion 15 μg/kg.hr: Ra Urea (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	14.6	14.3	13.5	13.5	13.4	13.0
159	13.5	13.5	12.7	13.3	12.3	11.5
160	20.0	20.3	19.3	18.5	17.4	17.3
161	17.0	14.8	15.2	16.1	13.9	14.0
Mean +/- SEM	16.3 +/- 1.4	15.7 +/- 1.5	15.2 +/- 1.5	15.4 +/- 1.2	14.3 +/- 1.1*	14.0 +/- 1.2*

Table 3.2.(xii) *IGF-I Infusion 15 μg/kg.hr: Net Protein Loss (g/kg.day)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	3.7	3.6	3.4	3.4	3.4	3.3
159	3.4	3.4	3.2	3.3	3.1	2.9
160	5.0	5.1	4.9	4.7	4.4	4.4
161	4.3	3.7	3.8	4.0	3.5	3.5
Mean +/- SEM	4.1 +/- 0.4	4.0 +/- 0.4	3.8 +/- 0.4	3.9 +/- 0.3	3.6 +/- 0.3*	3.5 +/- 0.3*

* $p < 0.05$ compared to 0 min.

Table 3.2.(xiii) *IGF-I Infusion 15 μg/kg.hr: Leucine Ra (umol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	3.9	3.2	3.7	3.7	4.2	4.6
159	4.5	4.3	4.2	4.3	3.9	3.6
160	6.9	6.6	5.4	5.7	5.3	5.1
161	3.8	4.4	4.3	4.8	4.4	4.7
Mean +/- SEM	4.8 +/- 0.7	4.6 +/- 0.7	4.4 +/- 0.4	4.6 +/- 0.4	4.5 +/- 0.3	4.5 +/- 0.4

Table 3.2.(xiv) *IGF-I Infusion 15 μg/kg.hr: Plasma Urea Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
158	4.6	4.7	4.5	4.4	4.0	4.2
159	5.5	5.5	5.3	5.3	5.1	5.0
160	10.9	10.9	10.6	10.0	9.5	9.2
161	7.9	7.7	7.6	7.4	7.1	7.1
Mean +/- SEM	7.3 +/- 1.4	7.2 +/- 1.4	7.0 +/- 1.4	6.8 +/- 1.2	6.4 +/- 1.2	6.4 +/- 1.1

Table 3.3.(i) *IGF-I Infusion 50 μg/kg.hr: Plasma Total IGF-I Concentration (μg/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	219	904	1387	1606	1483	2528
139	259	701	1199	2057	1311	1298
144	420	946	1097	1292	1481	1559
145	261	746	1171	1481	2079	1526
147	179	1003	1471	1764	1473	1571
148	335	1060	1395	1561	1996	1334
Mean +/- SEM	279 +/- 35	893 +/- 58†	1287 +/- 61§	1626 +/- 107§	1637 +/- 130§	1636 +/- 185§

Table 3.1.(ii) *IGF-I Infusion 50 μg/kg.hr: Plasma IGF-I Binding Form Concentration at t = 300 min (μg/l)*

	7 kDa		35-60 kDa		150 kDa	
Lamb	Concentration	% Total	Concentration	% Total	Concentration	% Total
137	404	16	1684	66	430	17
139	38	3	1070	82	190	15
144						
145	37	2	1246	82	243	16
147	341	22	996	63	233	15
148	96	7	1264	85	102	8
Mean +/- SEM	240 +/- 54	10 +/- 0.3	1252 +/- 119*	76 +/- 5	240 +/- 54	14 +/- 2

Table 3.3.(i) † $p < 0.01$, § $p < 0.005$ compared to 0 min.
Table 3.3.(ii) * $p < 0.05$ compared to normal saline controls

Table 3.3.(iii) *IGF-I Infusion 50 μg/kg.hr: Plasma Glucose Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	1.8	1.8	1.6	1.2	1.2	1.0
139	2.5	2.4	2.2	2.0	1.7	1.6
144	2.3	2.2	2.1	2.3	2.2	2.0
145	2.2	2.4	2.3	2.1	1.8	1.3
147	3.5	3.2	2.5	1.9	1.8	1.7
148	3.6	3.5	3.8	3.6	2.9	2.1
Mean +/- SEM	2.7 +/- 0.3	2.6 +/- 0.3	2.4 +/- 0.3	2.2 +/- 0.3*	1.9 +/- 0.2†	1.6 +/-0.4§

Table 3.3.(iv) *IGF-I Infusion 50 μg/kg.hr: Plasma Potassium Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	3.9	3.7	3.3	3.2	2.9	2.9
139	3.7	3.3	3.2	3.8	3.4	3.2
144	3.6	3.3	3.2	2.9	3.0	2.9
145	3.9	3.7	3.7	3.5	3.2	3.2
147	3.7	3.6	3.1	3.1	2.9	2.9
148	4.0	3.7	3.9	3.5	3.4	3.2
Mean +/- SEM	3.8 +/- 0.1	3.6 +/- 0.1	3.4 +/- 0.1†	3.3 +/- 0.1§	3.1 +/- 0.1§	3.1 +/- 0.1§

* $p < 0.05$, † $p < 0.01$, § $p < 0.005$ compared to 0 min.

Table 3.3.(v) *IGF-I Infusion 50 μg/kg.hr: Glucose Ra (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	10.3	10.0	9.9	9.9	11.7	9.1
139	6.4	6.1	8.5	8.5	7.4	7.8
144	8.6	9.5	10.8	10.8	10.5	9.4
145	8.8	9.2	10.0	10.0	9.6	7.8
147	8.2	8.1	8.0	7.3	8.8	9.5
148	11.7	11.5	11.9	11.3	11.6	10.8
Mean +/- SEM	9.0 +/- 0.7	9.1 +/- 0.7	9.9 +/- 0.6	9.6 +/- 0.6	9.9 +/- 0.7	9.1 +/- 0.5

Table 3.3.(vi) *IGF-I Infusion 50 μg/kg.hr: Glucose Clearance (ml/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	6.4	5.7	6.3	8.3	9.8	9.1
139	2.9	2.7	2.8	4.3	4.4	4.9
144	4.0	3.9	4.5	4.7	4.8	4.7
145	3.7	3.6	4.0	4.8	5.3	6.0
147	2.3	2.5	3.2	3.8	4.9	5.6
148	3.3	3.3	3.1	3.1	4.0	5.1
Mean +/- SEM	3.8 +/- 0.6	3.6 +/- 0.5	4.0 +/- 0.5	4.8 +/- 0.7*	5.5 +/- 0.9§	5.9 +/- 0.7§

* $p < 0.05$, § $p < 0.005$ compared to 0 min.

Table 3.3.(vii) *IGF-I Infusion 50 µg/kg.hr: Plasma Glycerol Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	0.31	0.25	0.16	0.17	0.16	0.26
139	0.32	0.30	0.31	0.41	0.32	0.26
144	0.30	0.31	0.31	0.30	0.26	0.25
145	0.34	0.38	0.38	0.30	0.49	0.43
147	0.59	0.50	0.78	0.60	0.54	0.51
148	0.59	0.54	0.49	0.62	0.61	0.55
Mean +/- SEM	0.41 +/- 0.06	0.38 +/- 0.05	0.41 +/- 0.09	0.35 +/- 0.06	0.40 +/- 0.07	0.38 +/-0.06

Table 3.3.(viii) *IGF-I Infusion 50 µg/kg.hr Plasma Free Fatty Acid Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	0.98		0.77	0.64	0.71	0.61
139	0.36	0.40	0.41	0.42	0.60	
144	0.60	0.55	0.63	0.50	0.32	0.42
145	1.17	1.00	1.49	1.07	0.74	0.79
147	0.77	0.94	0.56	1.42	1.02	0.91
148	1.17	1.00	1.00	0.82	1.14	0.63
Mean +/- SEM	0.84 +/- 0.13	0.78 +/- 0.12	0.81 +/- 0.16	0.81 +/- 0.16	0.76 +/- 0.12	0.67 +/- 0.08

Table 3.3.(ix) *IGF-I Infusion 50 μg/kg.hr: Plasma Ketone Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	1.1	1.3	1.0	0.6	0.8	0.9
139	0.9	1.8	1.0	0.6	1.4	1.9
144	0.5	1.0	0.5	0.5	1.3	0.6
145	1.3	1.4	1.3	1.8	1.6	1.7
147	1.5	1.3	1.0	1.9	2.0	2.1
148	2.2	1.7	1.7	1.5	1.4	1.4
Mean +/- SEM	1.3 +/- 0.2	1.4 +/- 0.1	1.1 +/- 0.2	1.2 +/- 0.3	1.4 +/- 0.2	1.4 +/-0.2

Table 3.3.(x) *IGF-I Infusion 50 μg/kg.hr: Plasma Triglyceride Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	0.3	0.2	0.2	0.2	0.2	0.2
139	0.2	0.2	0.2	0.2	0.3	0.3
144	0.2	0.2	0.2	0.2	0.2	0.2
145	0.2	0.1	0.2	0.2	0.1	0.1
147	0.3	0.1	0.1	0.2	0.1	0.1
148	0.4	0.3	0.3	0.3	0.3	0.2
Mean +/- SEM	0.3 +/- 0.0	0.2 +/- 0.0	0.2 +/- 0.0	0.2 +/- 0.0	0.2 +/- 0.0	0.2 +/- 0.0

Table 3.3.(xi) *IGF-I Infusion 50 μg/kg.hr: Ra Urea (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	12.4	12.6	12.1	11.4	11.0	10.7
139	14.3		13.8	12.6	12.3	11.3
144	12.6	12.4	12.4	11.6	10.8	12.1
145	12.8	12.2	11.2	10.8	8.9	8.7
147	14.3	13.5	14.2	13.9	13.3	11.4
148	17.1	16.5	16.8	15.6	14.7	13.9
Mean +/- SEM	13.9 +/- 0.7	13.4 +/- 0.8	13.4 +/- 0.8	12.7 +/- 0.7†	11.8 +/- 0.8§	11.4 +/- 0.7§

Table 3.3.(xii) *IGF-I Infusion 50 μg/kg.hr: Net Protein Loss (g/kg.day)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	3.1	3.2	3.0	2.9	2.8	2.7
139	3.6		3.5	3.2	3.1	2.8
144	3.2	3.2	3.2	2.9	2.7	3.0
145	3.2	3.1	2.8	2.7	2.2	2.2
147	3.6	3.4	3.6	3.5	3.3	2.9
148	4.3	4.2	4.2	3.9	3.7	3.5
Mean +/- SEM	3.5 +/- 0.2	3.4 +/- 0.2	3.4 +/- 0.2	3.2 +/- 0.2†	3.0 +/- 0.2§	2.9 +/- 0.2§

† $p < 0.01$, § $p < 0.005$ compared to 0 min.

Table 3.3.(xiii) *IGF-I Infusion 50 μg/kg.hr: Leucine Ra (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	6.7	4.5	4.1	4.3	4.7	3.2
139	4.9	4.8	4.8	4.6	3.7	3.9
144	5.8	5.6	5.3	5.2	5.3	5.1
145	4.7	4.1	3.8	3.8	3.7	3.4
146	3.7	3.1	2.9	3.2	2.5	2.4
147	4.2	4.1	4.0	3.6	3.3	2.6
Mean +/- SEM	5.0 +/- 0.4	4.4 +/- 0.3	4.2 +/- 0.3*	4.1 +/- 0.3†	3.9 +/- 0.4†	3.4 +/- 0.4§

Table 3.3.(xiv) *IGF-I Infusion 50 μg/kg.hr: Plasma Urea Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
137	6.4	6.8	6.6	6.7	6.7	7.1
139	5.4	5.3	5.5	5.4	5.2	5.2
144	4.4	4.3	4.1	4.0	4.0	3.9
145	4.8	4.7	4.6	4.5	4.5	4.3
146	6.2	6.2	6.2	6.1	5.9	6.2
147	6.4	5.7	6.2	6.1	5.7	5.3
Mean +/- SEM	5.6 +/- 0.3	5.5 +/- 0.9	5.5 +/- 0.4	5.5 +/- 0.4	5.3 +/- 0.4	5.3 +/- 0.5

* $p < 0.05$, † $p < 0.01$, § $p < 0.005$ compared to 0 min.

Table 3.4.(i) *rhIGF-II Infusion 50 μg/kg.hr: Plasma IGF-I Concentration (μg/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	293	227	82	114	192	125
172	340	261	250	135	135	315
175	339	254	227	136	170	140
176	307	194	197	257	93	112
Mean +/- SEM	320 +/- 12	234 +/- 15	189 +/- 37*	160 +/- 33*	148 +/- 21*	173 +/- 48*

* $p < 0.05$ compared to 0 min.

Table 3.4.(ii) *rhIGF-II Infusion 50 μg/kg.hr: Plasma Glucose Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	3.7	3.8	3.9	4.0	4.2	4.3
172	3.2	2.9	2.7	2.6	3.9	3.8
175	3.3	3.3	3.5	3.2	3.0	2.5
176	3.8	3.8	3.6	3.3	3.0	3.1
Mean +/- SEM	3.5 +/- 0.2	3.5 +/- 0.3	3.4 +/- 0.3	3.3 +/- 0.3	3.5 +/- 0.3	3.4 +/- 0.4

Table 3.4.(iii) *rhIGF-II Infusion 50 μg/kg.hr: Plasma Potassium Concentration (mmol/l).*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	3.7	3.7	4.0	4.0	3.7	3.7
172	3.8	3.5	3.3	3.4	3.6	3.6
175	4.1	4.3	3.9	3.8	3.6	3.4
176	3.7	3.5	3.2	3.1	3.1	3.1
Mean +/- SEM	3.8 +/- 0.1	3.8 +/- 0.2	3.6 +/- 0.2	3.6 +/- 0.2	3.5 +/- 0.1	3.5 +/- 0.1

Table 3.4.(iv) *rhIGF-II Infusion 50 μg/kg.hr: Ra Glucose (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	11.9	12.3	12.8	14.2	15.8	14.7
172	12.9	15.3	12.2	12.0	19.0	18.1
175	13.4	13.7	15.4	13.8	13.6	12.7
176	15.8	16.4	14.9	14.3	12.8	14.6
Mean +/- SEM	13.5 +/- 0.8	14.6 +/- 0.8	13.8 +/- 0.5	13.6 +/- 0.5	15.3 +/- 1.4	15.0 +/- 1.1

Table 3.4.(v) *rhIGF-II Infusion 50 μg/kg.hr: Glucose Clearance (ml/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	3.2	3.2	3.3	3.6	3.8	3.4
172	4.0	5.3	4.5	4.6	4.9	4.8
175	4.1	4.2	4.4	4.3	4.5	5.1
176	4.2	4.3	4.1	4.3	4.3	4.7
Mean +/- SEM	3.9 +/- 0.2	4.3 +/- 0.4	4.1 +/- 0.3	4.2 +/- 0.2	4.4 +/- 0.2	4.5 +/- 0.4*

* $P < 0.05$ compared to 0 min.

Table 3.4.(vi) *IGF-II Infusion 50 μg/kg.hr: Glycerol Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	0.82	0.79	0.93	0.82	0.60	0.67
172	0.67	0.58	0.50	0.50	0.84	0.57
175	0.42	0.48	0.49	0.43	0.70	0.56
176	0.64	0.85	0.76	0.65	0.68	0.67
185	0.55	0.47	0.93	0.61	0.64	0.47
193	0.63	0.58	0.59	0.49	0.57	0.54
Mean +/- SEM	0.62 +/- 0.05	0.63 +/- 0.07	0.70 +/- 0.08	0.58 +/- 0.06	0.67 +/- 0.04	0.58 +/- 0.03

Table 3.4.(vii) *IGF-II Infusion 50 μg/kg.hr: Plasma Free Fatty Acid Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	0.53	0.95	2.36	2.34	0.31	0.76
172	0.59	0.70	0.62	1.47	1.97	0.29
175	0.24	1.14	0.66	0.61	0.29	0.54
176	0.39	0.95	0.57	0.89	0.40	0.49
185	0.96	0.75	1.48	0.61	0.39	0.47
193	0.94	1.27	0.92	0.70	0.62	0.53
Mean +/- SEM	0.61 +/- 0.12	0.96 +/- 0.09	1.10 +/- 0.29	1.10 +/- 0.28	0.66 +/- 0.27	0.51 +/- 0.06

Table 3.4.(viii) *rhIGF-II Infusion 50 µg/kg.hr: Ketone Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	1.43	1.58	1.91	1.40	1.14	1.15
172	1.02	0.92	0.92	1.45	0.93	0.69
175	0.12	0.81	0.79	0.85	0.56	0.37
176	1.17	1.39	1.46	1.12	0.84	0.99
Mean +/- SEM	0.94 +/- 0.29	1.18 +/- 0.19	1.27 +/- 0.26	1.21 +/- 0.14	0.87 +/- 0.12	0.80 +/- 0.17

Table 3.4.(ix) *rhIGF-II Infusion 50 µg/kg.hr: Plasma Triglyceride Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	0.2	0.2	0.4	0.3	0.1	0.2
172	0.3	0.3	0.3	0.4	0.5	0.2
175	0.2	0.3	0.2	0.2	0.2	0.1
176	0.1	0.2	0.1	0.1	0.1	0.1
Mean +/- SEM	0.2 +/- 0.0	0.3 +/- 0.0	0.3 +/- 0.1	0.3 +/- 0.1	0.2 +/- 0.1	0.2 +/- 0.0

Table 3.4.(x) *rhIGF-II Infusion 50 μg/kg.hr: Ra Urea (umol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	16.3	16.4	17.1	17.4	17.0	16.8
172	18.0	17.9	17.5	17.0	17.1	16.8
175	13.7	12.8	12.5	13.3	12.1	11.3
176	11.5	12.7	13.0	12.8	12.9	11.7
Mean +/- SEM	14.9 +/- 1.4	15.0 +/- 1.3	15.0 +/- 1.3	15.1 +/- 1.2	14.8 +/- 1.3	14.2 +/- 1.5

Table 3.4.(xi) *rhIGF-II Infusion 50 μg/kg.hr: Net Protein Loss (g/kg.day)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	3.8	4.1	4.3	4.4	4.3	4.2
172	4.5	4.5	4.4	4.3	4.3	4.2
175	3.5	3.2	3.2	3.4	3.0	2.8
176	2.9	3.2	3.3	3.2	3.3	2.9
Mean +/- SEM	3.7 +/- 0.3	3.8 +/- 0.3	3.8 +/- 0.3	3.8 +/- 0.3	3.7 +/- 0.3	3.5 +/- 0.4

Table 3.4.(xii) *rhIGF-II Infusion 50 μg/kg.hr: Ra Leucine (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	4.4	4.3	4.1	3.9	3.8	3.9
172	4.8	4.0	4.1	3.9	3.4	4.8
175	4.9	3.6	3.6	3.0	4.8	5.4
176	3.3	3.5	3.0	2.9	2.8	2.9
Mean +/- SEM	4.4 +/- 0.4	3.9 +/- 0.2	3.7 +/- 0.3	3.4 +/- 0.3	3.7 +/- 0.4	4.3 +/- 0.5

Table 3.4.(xiii) *rhIGF-II Infusion 50 μg/kg.hr: Plasma Urea Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
171	5.6	5.5	5.2	5.1	4.9	4.9
172	7.2	7.1	6.8	6.5	6.4	6.5
175	5.0	4.9	4.9	4.9	5.0	4.9
176	3.4	3.5	3.6	3.5	3.9	3.9
Mean +/- SEM	5.3 +/- 0.8	5.3 +/- 0.7	5.1 +/- 0.7	5.0 +/- 0.6	5.1 +/- 0.5	5.1 +/- 0.6

Table 3.5.(i) *Insulin Infusion 1 μg/kg.hr: Plasma IGF-I Concentration (μg/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	277	289	261	309	268	366
174	288	409	263	244	287	171
177	433	326	298	291	497	415
180	562	480	491	497	414	422
Mean +/- SEM	390 +/- 67	376 +/- 43	328 +/- 55	335 +/- 56	367 +/- 54	344 +/- 59

Table 3.5.(ii) *Insulin Infusion 1 μg/kg.hr: Plasma Glucose Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	3.4	2.7	1.7	1.7	1.6	1.4
174	3.4	2.5	1.5	1.3	1.4	1.6
177	3.6	2.4	1.8	2.0	2.0	1.6
180	4.1	2.9	2.6	3.4	4.1	3.4
Mean +/- SEM	3.6 +/- 0.2	2.6 +/- 0.1*	1.9 +/- 0.2†	2.1 +/- 0.5†	2.3 +/- 0.6†	2.0 +/- 0.5†

Table 3.5.(iii) *Insulin Infusion 1 μg/kg.hr: Plasma Potassium Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	3.9	3.9	3.7	3.3	3.4	3.4
174	4.0	3.7	3.5	3.3	3.4	3.1
177	3.9	3.7	3.6	3.3	3.3	3.1
180	3.6	3.4	3.4	3.4	3.2	3.3
Mean +/- SEM	3.9 +/- 0.1	3.7 +/- 0.1	3.6 +/- 0.1*	3.3 +/- 0.1§	3.3 +/- 0.1§	3.3 +/- 0.1§

* $p < 0.05$, † $p < 0.01$, § $p < 0.005$ compared to 0 min.

Table 3.5.(iv) *Insulin Infusion 1 μg/kg.hr: Ra Glucose (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	10.6	7.4	4.9	7.2	6.4	5.8
174	12.3	9.6	7.4	7.0	9.4	10.3
177	10.5	7.5	7.5	9.2	9.4	8.3
180	12.3	9.9	10.2	13.1	15.2	13.1
Mean +/- SEM	11.4 +/- 0.5	8.6 +/- 0.7	7.5 +/- 1.1*	9.1 +/- 1.4	10.1 +/- 1.8	9.4 +/- 1.5

Table 3.5.(v) *Insulin Infusion 1 μg/kg.hr: Glucose Clearance (ml/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	3.1	2.7	2.8	4.2	4.0	4.1
174	3.6	3.8	4.9	5.4	6.7	6.4
177	2.9	3.1	4.2	4.6	4.7	5.2
180	3.0	3.4	3.9	3.9	3.7	3.9
Mean +/- SEM	3.2 +/- 0.2	3.3 +/- 0.2	4.0 +/- 0.4	4.5 +/- 0.3*	4.8 +/- 0.7†	4.9 +/- 0.6†

* $p < 0.05$, † $p < 0.01$ compared to 0 min.

Table 3.5.(vi) *Insulin Infusion 1 μg/kg.hr: Plasma Glycerol Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	0.74	0.57	0.58	0.85	0.71	0.69
174	0.40	0.25	0.77	0.62	0.52	0.41
177	0.87	0.89	0.91	0.50	0.48	0.39
180	0.84	0.68	0.64	0.48	0.67	0.53
Mean +/- SEM	0.71 +/- 0.11	0.60 +/- 0.13	0.73 +/- 0.07	0.61 +/- 0.09	0.60 +/- 0.06	0.51 +/- 0.07

Table 3.5.(vii) *Insulin Infusion 1 μg/kg.hr: Plasma Free Fatty Acid Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	0.48	0.14	1.40	0.79	0.52	1.10
174	0.50	0.15	1.06	1.19	0.74	0.20
177	0.57	0.56	1.18	0.74	0.24	0.38
180	0.63	0.93	1.53	0.87	0.84	1.07
Mean +/- SEM	0.54 +/- 0.07	0.45 +/- 0.19	1.29 +/- 0.11	0.90 +/- 0.10	0.59 +/- 0.13	0.69 +/- 0.23

Table 3.5.(viii) *Insulin Infusion 1μg/kg.hr: Plasma Ketone Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	1.12	0.70	1.21	1.70	1.61	1.60
174	1.10	0.47	0.83	1.07	1.09	0.86
177	2.16	1.82	2.38	2.49	2.54	2.44
180	1.94	1.61	1.99	2.36	0.66	0.70
Mean +/- SEM	1.58 +/- 0.28	1.15 +/- 0.33	1.60 +/- 0.30	1.91 +/- 0.33	1.47 +/- 0.40	1.40 +/- 0.40

Table 3.5.(ix) *Insulin Infusion 1μg/kg.hr: Plasma Triglyceride Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	0.2	0.1	0.2	0.1	0.1	0.1
174	0.3	0.2	0.3	0.3	0.3	0.2
177	0.2	0.2	0.3	0.3	0.1	0.1
180	0.2	0.2	0.2	0.2	0.2	0.1
Mean +/- SEM	0.2 +/- 0.0	0.2 +/- 0.0	0.3 +/- 0.0	0.3 +/- 0.0	0.2 +/- 0.0	0.1 +/- 0.0*

* $p < 0.05$ compared to 0 min.

Table 3.5.(x) *Insulin Infusion 1 μg/kg.hr: Ra Urea (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	17.2	15.8	18.3	16.9	13.8	14.5
174	17.7	16.6	16.2	15.1	14.5	13.7
177	11.5	11.1	11.4	10.8	10.3	9.8
180	11.4	11.3	10.6	10.9	10.1	10.4
Mean +/- SEM	14.5 +/- 1.7	13.7 +/- 1.5	14.1 +/- 1.9	13.4 +/- 1.5	12.2 +/- 1.2*	12.1 +/- 1.2*

Table 3.5.(xi) *Insulin Infusion 1 μg/kg.hr: Net Protein Loss (g/kg.day)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	4.3	4.0	4.6	4.2	3.5	3.7
174	4.5	4.2	4.1	3.8	3.7	3.4
177	2.9	2.8	2.9	2.7	2.6	2.5
180	2.9	2.8	2.7	2.8	2.5	2.6
Mean +/- SEM	3.7 +/- 0.4	3.5 +/- 0.4	3.6 +/- 0.5	3.4 +/- 0.4	3.1 +/- 0.3*	3.1 +/- 0.3*

* $p < 0.05$ compared to 0 min.

Table 3.5.(xii) *Insulin Infusion 1μg/kg.hr: Ra Leucine (μmol/kg.min)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	4.7	4.8	4.5	3.6	3.4	4.2
174	5.2	5.1	3.2	2.8	3.1	2.7
177	4.0	4.5	6.6	5.3	5.1	4.7
180	4.6	5.2	5.0	5.2	4.3	4.5
Mean +/- SEM	4.6 +/- 0.2	4.9 +/- 0.2	4.8 +/- 0.7	4.2 +/- 0.6	4.0 +/- 0.5	4.0 +/- 0.5

Table 3.5.(xiii) *Insulin Infusion 1μg/kg.hr: Plasma Urea Concentration (mmol/l)*

Lamb	0 min	60 min	120 min	180 min	240 min	300 min
173	7.1	7.4	7.2	7.3	7.1	6.7
174	8.3	8.3	8.3	7.9	8.1	8.0
177	4.3	4.2	4.1	4.2	4.1	4.1
180	4.5	4.3	4.4	4.4	4.3	4.5
Mean +/- SEM	6.1 +/- 1.0	6.1 +/- 1.1	6.0 +/- 1.0	6.0 +/- 1.0	5.9 +/- 1.1	5.8 +/- 0.9

Table 3.6.(i) *Normal Saline: Fractional Synthesis Rate of Protein (%/day)*

	Adductor	Psoas	Diaphragm	Heart	Liver
112	0.7	0.7	1.0	3.3	15.0
116	0.9	1.2	1.5	3.6	16.9
133		1.2	1.8	3.2	7.0
135	0.7	0.8	1.2	1.7	7.9
138	0.7	0.6	0.9	2.5	8.4
146	0.8	0.7	1.6	3.0	11.2
Mean +/- SEM	0.8 +/- 0.1	0.9 +/- 0.1	1.3 +/- 0.1	2.9 +/- 0.3	11.0 +/- 1.7

Table 3.6.(ii) *rhIGF-I 15 µg/kg.hr: Fractional Synthesis Rate of Protein (%/day)*

	Adductor	Psoas	Diaphragm	Heart	Liver
158	0.8	1.0	1.5	5.1	15.4
159	0.7	0.8	1.3	4.0	12.8
160	0.7	0.7	1.1	4.2	12.3
161	0.6	0.5	1.0	2.8	9.6
Mean +/- SEM	0.7 +/- 0.0	0.7 +/- 0.1	1.3 +/- 0.2	4.0 +/- 0.5	12.5 +/-1.2

Table 3.6.(iii) *rhIGF-I 50 μg/kg.hr: Fractional Synthesis Rate of Protein (%/day)*

	Adductor	Psoas	Diaphragm	Heart	Liver
137	1.1	1.5	2.7	5.9	16.7
139	0.8	1.3	2.3	5.1	16.5
144	1.2	1.1	3.2	7.7	23.9
145	0.8	1.1	2.0	5.0	13.3
147	1.0	1.6	2.8	4.2	13.8
148	0.9	1.3	2.4	5.6	19.9
Mean +/- SEM	1.0 +/- 0.1*	1.3 +/- 0.1*	2.6 +/- 0.2§	5.6 +/- 0.5†	17.4 +/- 1.7*

Table 3.6.(iv) *rhIGF-II 50 μg/kg.hr: Fractional Synthesis Rate of Protein (%/day)*

	Adductor	Psoas	Diaphragm	Heart	Liver
171	0.6	0.8	1.4	3.3	11.4
172	0.6	0.8	1.1	3.2	11.1
175	0.7	0.7	1.5	4.9	10.4
176	0.5	0.6	1.3	3.8	10.4
Mean +/- SEM	0.6 +/- 0.0	0.7 +/- 0.0	1.3 +/- 0.1	3.8 +/- 0.4	10.8 +/-0.3

* $p < 0.05$, † $p < 0.01$, § $p < 0.005$ compared to normal saline infused controls.

Table 3.6.(v) *Insulin 1 μg/kg.hr: Fractional Synthesis Rate of Protein (%/day)*

	Adductor	Psoas	Diaphragm	Heart	Liver
171	0.7	1.1	1.6	5.2	19.1
172	0.8	0.7	1.0	2.7	10.8
175	0.8	1.0	1.6	4.9	15.8
176	0.6	0.6	1.5	3.9	11.8
Mean +/- SEM	0.7 +/- 0.0	0.9 +/- 0.1	1.4 +/- 0.1	4.2 +/- 0.6	14.2 +/- 2.0

Table 3.7.(i) *Plasma Total IGF-I Concentration (μg/l)*

Group 3 (TNF/Normal Saline)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AB	432	356	319	300	311	396
AD	386	366	404	340	344	347
AF	1137	855	999	715	839	873
AK	322	410	439	383	420	446
AM	280	384	418	298	265	300
Mean +/- SEM	511 +/- 159	474 +/- 96	516 +/- 123	407 +/- 79	436 +/- 104	472 +/- 103

Group 4 (TNF/IGF-I)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AA	446	1237	1974	1841	1984	2092
AC	463	1325	2487	2132	2854	2417
AE	591	1612	2389	2682	2746	3231
AJ	524	1085	1699	2174	1571	2190
AL	473	1180	1715	1688	1951	1669
Mean +/- SEM	499 +/- 26	1289 +/- 90*	2053 +/- 165§	2103 +/- 171§	2221 +/- 248§	2319 +/- 258§

* $p < 0.05$, § $p < 0.005$ compared to 180 min.

Table 3.7.(ii) *Plasma TNF and Insulin Concentrations.*

Group 3 (TNF/Normal Saline)

Plasma TNF(ηg/l)

	0 min	180 min	480 min
AB	<1	9,936	9,794
AD	6	9,743	8,597
AF	<1	8,438	7,860
AK	<1	11,681	8,277
AM	<1	7,920	5,928
Mean +/- SEM	1 +/- 1	9,544 +/- 656	8,091 +/- 629‡

Plasma Insulin (μg/l)

0 min	180 min	480 min
<0.15	0.46	0.98
<0.15	0.57	0.85
<0.15	<0.15	<0.15
<0.15	0.32	0.23
<0.15	0.35	0.34
<0.15	0.37 +/- 0.07	0.51 +/- 0.17

Group 4 (TNF/IGF-I)

Plasma TNF (ηg/l)

	0 min	180 min	480 min
AA	28	7,877	7,625
AC	<1	9,075	8,573
AE	<1	10,184	9,492
AJ	6	10,691	9,415
AL	<1	8,400	7,707
Mean +/- SEM	7 +/- 6	9,245 +/- 529†	8,559 +/- 398*

Plasma Insulin (μg/l)

0 min	180 min	480 min
<0.15	0.83	<0.15
<0.15	0.31	0.19
<0.15	0.39	<0.15
<0.15	0.89	<0.15
<0.15	0.60	<0.15
<0.15	0.60 +/- 0.12†	<0.16*

Significance: TNF

† $p < 0.0001$ Groups 3 & 4, t = 0 min *vs* t = 180 min (paired *t* test)

‡ $p < 0.07$ Group 3, t = 180 min *vs* t = 480 min (paired *t* test)

* $p < 0.02$ Group 4, t = 180 min *vs* t = 480 min (paired *t* test)

Insulin

† $p < 0.02$ Groups 3 & 4, t = 0 min *vs* t = 180 min (paired *t* test)

* $p < 0.03$ Group 4, t = 180 min *vs* t = 480 min (paired *t* test)

Table 3.7.(iii) *Plasma Cortisol and Glucagon Concentrations.*

Group 3 (TNF/Normal Saline)

Plasma Cortisol (ηmol/l)

	0 min	180 min	480 min
AB	25	197	175
AD	20	290	183
AF	30	414	231
AK	57	366	28
AM	123	277	174
Mean +/- SEM	51 +/- 19	102 +/- 13*	158 +/- 34*

Plasma Glucagon (μg/l)

0 min	180 min	480 min
101	180	191
63	70	110
65	103	181
91	180	125
80 +/- 9	133 +/- 28	152 +/- 20

Group 4 (TNF/IGF-I)

Plasma Cortisol (ηmol/l)

	0 min	180 min	480 min
AA	147	214	252
AC	30	299	230
AE	46	178	174
AJ	3	165	127
AL	28	298	136
Mean +/- SEM	51 +/- 25	76 +/- 10*	184 +/- 25*

Plasma Glucagon (μg/l)

0 min	180 min	480 min
68	119	163
65	104	113
95	112	104
50	130	81
70 +/- 9	166 +/- 6	117 +/- 17

Significance: * $p < 0.05$ Groups 3&4, t = 0 min *vs* t = 180 min (paired *t* test)
* $p < 0.05$ Group 4, t = 180 min *vs* t = 480 min (paired *t* test)

Table 3.7.(iv) *Glucose Concentration (mmol/l)*

Group 3 (TNF/Normal Saline)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AB	4.0	4.1	3.8	3.4	3.3	3.2
AD	4.2	4.0	4.0	3.6	3.2	2.9
AF	3.1	3.0	2.9	2.8	2.7	2.7
AK	4.0	4.0	3.9	3.2	3.4	3.3
AM	4.4	4.4	3.8	3.8	3.6	3.3
Mean +/- SEM	3.9 +/- 0.2	3.9 +/- 0.2	3.7 +/- 0.2	3.4 +/- 0.2†	3.2 +/- 0.2†	3.1 +/- 0.1†

Group 4 (TNF/IGF-I)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AA	4.4	4.3	4.1	3.6	2.8	2.3
AC	3.6	4.3	4.1	4.1	3.6	2.9
AE	4.5	4.7	4.1	3.1	2.7	2.1
AJ	4.1	4.1	4.0	3.7	3.1	2.6
AL	3.4	3.7	3.5	3.1	2.1	1.8
Mean +/- SEM	4.0 +/- 0.2	4.2 +/- 0.2	4.0 +/- 0.1	3.5 +/- 0.2	2.9 +/- 0.2†	2.3 +/- 0.2§

† $p < 0.01$, § $p < 0.005$ compared to 0 min.

Table 3.7.(v) *Ra Glucose (μmol/kg.min)*

Group 3 (TNF/Normal Saline)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AB	12.9	11.9	8.6	13.7	13.7	14.8
AD	13.2	13.0	14.2	13.3	10.9	12.6
AF	16.8	16.7	15.9	16.4	17.7	17.4
AK	8.5	8.6	8.7	8.7	10.8	8.8
AM	7.0	7.9	8.5	9.5	12.5	12.0
Mean +/- SEM	11.7 +/- 1.8	11.6 +/- 1.6	11.2 +/- 1.6	12.3 +/- 1.4	13.1 +/- 1.3	13.1 +/- 1.4

Group 4 (TNF/IGF-I)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AA	17.3	18.5	17.9	18.6	16.1	16.5
AC	12.4	14.5	14.4	14.4	13.6	13.8
AE	12.6	13.5	13.3	11.7	12.0	11.7
AJ	10.6	12.1	12.8	10.0	11.4	10.8
AL	7.1	8.8	8.4	8.0	7.7	7.7
Mean +/- SEM	12.0 +/- 1.7	13.5 +/- 1.6	13.4 +/- 1.5	12.5 +/- 1.8	12.2 +/- 1.4	12.1 +/- 1.5

Table 3.7.(vi) *Glucose Clearance (ml/kg.min)*

Group 3 (TNF/Normal Saline)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AB	3.2	2.9	2.3	4.0	4.2	4.6
AD	3.1	3.3	3.6	3.7	3.4	4.3
AF	5.4	5.6	5.5	5.9	6.6	6.4
AK	2.1	2.2	2.2	2.7	3.2	2.7
AM	1.6	1.8	2.2	2.5	3.5	3.6
Mean +/- SEM	3.1 +/- 0.7	3.2 +/- 0.7	3.2 +/- 0.6	3.8 +/- 0.6	4.2 +/- 0.6*	4.3 +/- 0.6*

Group 4 (TNF/IGF-I)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AA	3.9	4.3	4.4	5.2	5.8	7.2
AC	3.4	3.4	3.5	3.5	3.8	4.8
AE	2.8	2.9	3.2	3.8	4.4	5.6
AJ	2.6	3.0	3.2	2.7	3.7	4.2
AL	2.1	2.4	2.4	2.6	3.7	4.3
Mean +/- SEM	3.0 +/- 0.3	3.2 +/- 0.3	3.3 +/- 0.3	3.6 +/- 0.5	4.3 +/- 0.4†	5.2 +/- 0.6†

* $p < 0.05$, † $p < 0.01$ compared to 0 min.

Table 3.7.(vii) *Plasma Glycerol Concentration (mmol/l)*

Group 3 (TNF/Normal Saline)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AB	0.62	0.48	0.68	0.44	0.53	0.60
AD	0.38	0.32	0.42	0.37	0.36	0.30
AF	0.37	0.43	0.56	0.56	0.51	0.46
AK	0.21	0.16	0.15	0.09	0.10	0.13
AM	0.33	0.30	0.21	0.26	0.33	0.20
Mean +/- SEM	0.38 +/- 0.07	0.34 +/- 0.06	0.40 +/- 0.10	0.34 +/- 0.08	0.37 +/- 0.08	0.39 +/- 0.09

Group 4 (TNF/IGF-I)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AA	0.41	0.33	0.36	0.32	0.22	0.19
AC	0.64	0.62	0.48	0.69	0.61	0.46
AE	0.27	0.34	0.38	0.29	0.21	0.18
AJ	0.13	0.14	0.15	0.14	0.15	0.12
AL	0.42	0.42	0.36	0.26	0.23	0.26
Mean +/- SEM	0.37 +/- 0.09	0.37 +/- 0.08	0.35 +/- 0.05	0.34 +/- 0.09	0.28 +/- 0.08	0.24 +/- 0.06

Table 3.7.(viii) *Triglyceride Concentration (mmol/l)*

Group 3 (TNF/Normal Saline)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AB	0.3	0.2	0.1	0.1	0.2	0.1
AD	0.2	0.2	0.1	0.1	0.1	0.1
AF	0.3	0.2	0.2	0.2	0.2	0.2
AK	0.4	0.3	0.2	0.2	0.2	0.2
AM	0.4	0.3	0.2	0.2	0.2	0.2
Mean +/- SEM	0.3 +/- 0.1	0.2 +/- 0.0	0.2 +/- 0.0	0.2 +/- 0.0	0.2 +/- 0.0	0.2 +/- 0.0

Group 4 (TNF/IGF-I)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AA	0.2	0.2	0.2	0.2	0.1	0.2
AC	0.4	0.5	0.4	0.3	0.2	0.2
AE	0.2	0.2	0.2	0.2	0.1	0.1
AJ	0.2	0.2	0.2	0.2	0.1	0.1
AL	0.5	0.6	0.4	0.4	0.3	0.2
Mean +/- SEM	0.3 +/- 0.1	0.3 +/- 0.1	0.3 +/- 0.1	0.3 +/- 0.0	0.2 +/- 0.0	0.2 +/- 0.0

Table 3.7.(ix) *Plasma Potassium Concentration (mmol/l)*

Group 3 (TNF/Normal Saline)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AB	3.6	3.8	3.8	3.9	4.0	4.1
AD	3.7	3.7	3.6	3.5	3.6	3.6
AF	3.7	3.7	3.7	3.6	3.7	3.8
AK	3.9	4.1	4.2	3.8	4.2	4.2
AM	3.9	3.9	3.7	3.7	3.8	3.9
Mean +/- SEM	3.8 +/- 0.1	3.8 +/- 0.1	3.8 +/- 0.1	3.7 +/- 0.1	3.9 +/- 0.1	3.9 +/- 0.1

Group 4 (TNF/IGF-I)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AA	3.8	3.5	3.4	3.6	3.3	3.2
AC	3.1	3.2	3.0	3.1	2.9	3.1
AE	4.0	3.7	3.2	3.4	3.5	3.3
AJ	3.7	3.4	3.5	3.7	3.5	3.5
AL	3.8	3.6	3.4	3.2	2.5	2.2
Mean +/- SEM	3.7 +/- 0.2	3.5 +/- 0.1	3.3 +/- 0.1	3.4 +/- 0.1	3.1 +/- 0.2*	3.1 +/- 0.2*

* $p < 0.05$ compared to 0 min.

Table 3.7(x) *Plasma Urea Concentration (mmol/l)*

Group 3 (TNF/Normal Saline)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AB	6.3	6.4	6.5	6.7	7.4	7.7
AD	8.6	8.9	9.3	9.9	10.1	10.3
AF	12.9	13.2	13.5	13.8	14.0	14.5
AK	4.4	4.7	5.2	5.0	5.9	6.4
AM	7.0	7.1	7.0	7.3	7.7	7.8
Mean +/- SEM	7.8 +/- 1.4	8.1 +/- 1.5	8.3 +/- 1.5*	8.5 +/- 1.5†	9.0 +/- 1.4§	9.3 +/- 1.4§

Group 4 (TNF/IGF-I)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AA	8.3	8.8	8.8	8.7	8.8	9.0
AC	8.6	8.7	8.9	9.9	10.5	11.1
AE	6.8	7.0	7.4	7.2	7.6	8.2
AJ	5.9	5.9	5.8	5.7	5.7	5.9
AL	7.8	8.1	8.2	7.8	6.3	6.0
Mean +/- SEM	7.5 +/- 0.5	7.7 +/- 0.6	7.8 +/- 0.6	7.9 +/- 0.7	7.8 +/- 0.9	8.0 +/- 1.0

* $p < 0.05$, † $p < 0.01$, § $p < 0.005$ compared to 0 min.

Table 3.7.(xi) *Ra Urea (μmol/kg.min)*

Group 3 (TNF/Normal Saline)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AB	13.1	14.4	12.7	13.6	1 2.8	11.8
AD	17.4	16.3	15.6	15.3	15.1	15.0
AF	21.1	20.8	20.1	20.1	19.6	20.2
AK	12.1	11.9	11.9	11.8	12.3	12.1
AM	15.1	14.4	14.2	14.7	13.7	13.6
Mean +/- SEM	15.8 +/- 1.6	15.6 +/- 1.5	14.9 +/- 1.4	15.1 +/- 1.4	14.7 +/- 1.3*	14.5 +/- 1.5*

Group 4 (TNF/IGF-I)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AA	18.5	17.3	17.3	16.9	16.5	15.3
AC	21.0	19.0	19.4	17.8	18.6	16.3
AE	14.4	14.7	12.1	13.4	11.6	11.2
AJ	14.7	14.0	12.8	11.4	12.4	10.5
AL	15.4	14.8	14.4	13.6	12.8	11.7
Mean +/- SEM	16.9 +/- 1.3	16.0 +/- 0.9†	15.2 +/- 1.4†	14.3 +/- 1.4§	14.3 +/- 1.4§	13.0 +/- 1.1§

* $p < 0.05$, † $p < 0.01$, § $p < 0.005$ compared to 0 min.

Table 3.7.(xii) *Net Protein Loss (g/kg.day)*

Group 3 (TNF/Normal Saline)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AB	3.3	3.6	3.2	3.4	3.2	3.0
AD	4.4	4.1	3.9	3.9	3.8	3.8
AF	5.3	5.3	5.1	5.1	4.9	5.1
AK	3.0	3.0	3.0	3.0	3.1	3.0
AM	3.8	3.6	3.6	3.7	3.5	3.4
Mean +/- SEM	4.0 +/- 0.4	3.9 +/- 0.4	3.8 +/- 0.4	3.8 +/- 0.4	3.7 +/- 0.3*	3.7 +/- 0.4*

Group 4 (TNF/IGF-I)

Lamb	180 min	240 min	300 min	360 min	420 min	480 min
AA	4.7	4.4	4.4	4.3	4.1	3.8
AC	5.4	4.8	4.9	4.5	4.7	4.1
AE	3.6	3.7	3.0	3.4	2.9	2.8
AJ	3.7	3.5	3.2	2.9	3.1	2.6
AL	3.9	3.7	3.6	3.4	3.2	3.0
Mean +/- SEM	4.3 +/- 0.3	4.0 +/- 0.2†	3.8 +/- 0.4†	3.7 +/- 0.3§	3.6 +/- 0.3§	3.3 +/- 0.3§

* $p < 0.05$, † $p < 0.01$, § $p < 0.005$ compared to 0 min.

Table 3.8.(i) *Plasma Total IGF-I Levels During 90 Minute IGF-I Infusions (µg/l)*

	Basal				IGF-I 2.5 µg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
1A1	348	242	291	294	318	318	283
1B1	459	440	448	449	468	468	448
1A2	316		323	323	343	343	391
Mean +/- SEM	374 +/- 43	341 +/- 99	356 +/- 47	355 +/- 48	376 +/- 46	376 +/- 46	374 +/- 48

	Basal				IGF-I 20 ug/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AX	546	570	638	585	875	794	743
AY	884	850	762	832	780	865	843
AZ	467	721	742	643	912	713	968
Mean +/- SEM	632 +/- 128	714 +/- 81	714 +/- 38	687 +/- 129	856 +/- 39	791 +/- 44	851 +/- 65

	Basal				IGF-I 40 ug/kg.hr		
	- 20 min	- 10 min	0 min	Mean		+ 80 min	+ 90 min
AN	636	538	420	531	1131	1219	1470
AP	480	434	495	470	1074	1180	1063
AU	586	619	623	609	1487	1807	1311
Mean +/- SEM	567 +/- 46	530 +/- 54	513 +/- 59	537 +/- 40	1231+/-224*	1402+/-351*	1281+/-118*

	Basal				IGF-I 120 ug/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AO	526	530	556	537	2999	2595	2906
AQ	364	593	588	515	2716	2985	2847
AT	517	520	525	521	2228	2970	2537
Mean +/- SEM	469 +/- 53	548 +/- 23	556 +/- 18	524 +/- 7	2648+/-225§	2859+/-127§	2763+/-114§

Table 3.8.(ii) *IGF-I Binding Protein Forms During 90 Minute IGF-I Infusions (µg/l)*

2.5 µg/kg.hr	7 kDa		35-60 kDa		150 kDa	
	Conc	%	Conc	%	Conc	%
1A1	< 40	0	148	52	135	48
1B1	< 40	0	228	59	185	41
1A2	< 40	0	233	60	158	40
Mean +/- SEM	< 40	0 +/- 0	203 +/- 28	57 +/- 2	159 +/- 14	43 +/- 2

20 µg/kg.hr	7 kDa		35-60 kDa		150 kDa	
	Conc	%	Conc	%	Conc	%
AX	< 40	0	439	59	305	41
AY	< 40	0	520	62	323	38
AZ	< 40	0	594	61	374	39
Mean +/- SEM	< 40	0 +/- 0	518 +/- 45	61 +/- 1	334 +/- 21	39 +/- 1

40 µg/kg.hr	7 kDa		35-60 kDa		150 kDa	
	Conc	%	Conc	%	Conc	%
AN	265	18	871	59	334	23
AP	223	21	614	58	228	22
AU	127	10	919	70	265	20
Mean +/- SEM	205 +/- 41	16 +/- 3	801 +/- 95*	62 +/- 4	276 +/- 31	22 +/- 1

120 µg/kg.hr	7 kDa		35-60 kDa		150 kDa	
	Conc	%	Conc	%	Conc	%
AO	503	17	2029	70	374	13
AQ	790	28	1526	54	531	19
AT	238	9	2065	81	233	9
Mean +/- SEM	510 +/- 159	18 +/- 5*	1873 +/- 174§	68 +/- 8	379 +/- 86	14 +/- 3

* $p < 0.05$, § $p < 0.01$ compared to saline infused controls.

Table 3.8.(iii) *Glucose Concentration During 90 Minute IGF-I Infusions (mmol/l)*

	Basal				IGF-I 2.5 µg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
1A1	4.3	4.8	4.2	4.4		4.2	4.0
1B1	5.0	5.0	4.9	5.0		4.7	4.7
1A2	3.1	3.1	3.1	3.1		3.2	3.1
Mean +/- SEM	4.1 +/- 0.6	4.3 +/- 0.6	4.1 +/- 0.5	4.2 +/- 0.6		4.0 +/- 0.4	3.9 +/- 0.5

	Basal				IGF-I 20 µg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AX	4.0	3.9	3.8	3.9	3.8	3.7	3.7
AY	3.7	3.9	3.9	3.8	3.9	3.9	3.8
AZ	3.8	3.9	3.8	3.8	3.8	3.7	3.8
Mean +/- SEM	3.8 +/- 0.1	3.9 +/- 0.0	3.8 +/- 0.0	3.8 +/- 0.0	3.8 +/- 0.0	3.8 +/- 0.1	3.8 +/- 0.0

	Basal				IGF-I 40 µg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AN	3.6	3.5	3.4	3.5	3.8	3.7	3.7
AP	2.8	2.9	2.8	2.8	2.9	3.1	3.0
AU	4.2	4.5	4.1	4.3	5.3	5.4	5.3
Mean +/- SEM	3.5 +/- 0.4	3.6 +/- 0.5	3.4 +/- 0.4	3.5 +/- 0.4	4.0 +/- 0.7	4.1 +/- 0.7	4.0 +/- 0.7

	Basal				IGF-I 120 µg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AO	3.5	3.5	3.4	3.5	2.2	2.0	1.7
AQ	3.9	3.9	3.9	3.9	2.2	1.9	1.7
AT	3.1	3.1	3.1	3.1	2.7	2.4	2.4
Mean +/- SEM	3.5 +/- 0.2	3.5 +/- 0.2	3.4 +/- 0.2	3.5 +/- 0.2	2.4 +/- 0.2	2.1 +/- 0.2	1.9 +/- 0.2*

Table 3.8.(iv) *Glucose Ra During 90 Minute IGF-I Infusions (μmol/kg.min)*

	Basal				IGF-I 2.5 μg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
1A1	13.7	15.4	13.5	14.2		14.7	14.0
1B1	18.2	17.6	16.2	17.3		17.0	15.5
1A2	14.0	14.0	14.0	14.0		13.8	13.6
Mean +/- SEM	15.3 +/- 1.5	15.7 +/- 1.0	14.6 +/- 0.8	15.2 +/- 1.1		15.7 +/- 1.0	14.4 +/- 0.6

	Basal				IGF-I 20 μg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AX	16.6	16.8	18.3	17.2	16.6	16.3	16.0
AY	14.0	14.2	13.8	14.0	20.3	20.7	18.3
AZ	14.4	15.5	14.1	14.7	14.3	14.2	14.4
Mean +/- SEM	15.0 +/- 0.8	15.5 +/- 0.8	15.4 +/- 1.5	15.3 +/- 1.0	17.1 +/- 1.7	17.1 +/- 1.9	16.2 +/- 1.1

	Basal				IGF-I 40 μg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AN	12.7	12.3	13.2	12.7	12.5	13.0	12.5
AP	12.5	12.7	12.5	12.6	14.3	13.8	14.3
AU	20.2	21.2	17.7	19.7	20.3	22.2	22.0
Mean +/- SEM	15.1 +/- 2.5	15.4 +/- 2.9	14.5 +/- 1.6	15.0 +/- 2.4	15.7 +/- 2.4	16.3 +/- 2.9	16.3 +/- 2.9

	Basal				IGF-I 120 μg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AO	12.3	12.3	12.5	12.4	11.2	11.0	10.5
AQ	12.2	12.3	13.0	12.5	9.5	8.7	8.5
AT	13.7	13.7	13.7	13.7	13.0	13.8	13.5
Mean +/- SEM	12.9 +/- 0.4	12.8 +/- 0.5	13.1 +/- 0.3	12.9 +/- 0.5	11.2 +/- 1.0	11.2 +/- 1.5	10.8 +/- 1.5

Table 3.8.(v) *Glucose Clearance During 90 Minute IGF-I Infusions (ml/kg.min)*

	Basal				IGF-I 2.5 µg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
1A1	3.2	3.2	3.2	3.2		3.5	3.5
1B1	3.6	3.5	3.3	3.5		3.6	3.2
1A2	4.5	4.5	4.5	4.5		4.3	4.4
Mean +/- SEM	3.8 +/- 0.4	3.7 +/- 0.4	3.7 +/- 0.4	3.7 +/- 0.4		3.8 +/- 0.3	3.7 +/- 0.4

	Basal				IGF-I 20 µg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AX	4.2	4.3	4.8	4.4	4.4	4.4	4.3
AY	3.8	3.6	3.5	3.6	5.2	5.3	4.8
AZ	3.8	4.0	3.7	3.8	3.8	3.7	3.8
Mean +/- SEM	3.9 +/- 0.1	4.0 +/- 0.2	4.0 +/- 0.4	3.9 +/- 0.2	4.5 +/- 0.4	4.5 +/- 0.5	4.3 +/- 0.3

	Basal				IGF-I 40 µg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AN	3.5	3.5	3.9	3.6	3.3	3.5	3.4
AP	4.5	4.4	4.5	4.5	4.9	4.5	4.8
AU	4.8	4.7	4.3	4.6	4.1	4.1	4.2
Mean +/- SEM	4.3 +/- 0.4	4.2 +/- 0.4	4.2 +/- 0.2	4.2 +/- 0.3	4.0 +/- 0.5	4.0 +/- 0.3	4.1 +/- 0.4

	Basal				IGF-I 120 µg/kg.hr		
	- 20 min	- 10 min	0 min	Mean	+ 70 min	+ 80 min	+ 90 min
AO	3.5	3.5	3.7	3.6	5.1	5.5	6.2
AQ	3.1	3.2	3.3	3.2	4.3	4.6	5.0
AT	4.4	4.4	4.4	4.4	4.8	5.8	5.6
Mean +/- SEM	3.7 +/- 0.4	3.7 +/- 0.4	3.8 +/- 0.3	3.7 +/- 0.4	4.7 +/- 0.2	5.3 +/- 0.4	5.6 +/- 0.4*

Table 3.8.(vi) *Plasma Glycerol and FFA Concentrations During 90 Minute IGF-I Infusions (mmol/l)*

2.5 µg/kg.hr	Glycerol		Free Fatty Acid	
	0 min	90 min	0 min	90 min
1A1	0.20	0.14	1.64	1.75
1B1	0.25	0.27	1.89	2.40
1A2	0.35	0.23	1.53	1.19
Mean +/- SEM	0.27 +/- 0.04	0.21 +/- 0.04	1.69 +/- 0.11	1.78 +/- 0.35

20 µg/kg.hr	Glycerol		Free Fatty Acid	
	0 min	90 min	0 min	90 min
1A1	0.21	0.26	1.03	1.00
1B1	0.32	0.27	1.24	0.93
1A2	0.31	0.27	1.19	1.05
Mean +/- SEM	0.28 +/- 0.04	0.27 +/- 0.01	1.15 +/- 0.06	0.99 +/- 0.04

40 µg/kg.hr	Glycerol		Free Fatty Acid	
	0 min	90 min	0 min	90 min
1A1	0.42	0.32	2.54	1.47
1B1	0.31	0.25	0.59	0.55
1A2	0.16	0.23	1.09	0.68
Mean +/- SEM	0.30 +/- 0.08	0.27 +/- 0.03	1.41 +/- 0.59	0.90 +/- 0.29

120 µg/kg.hr	Glycerol		Free Fatty Acid	
	0 min	90 min	0 min	90 min
1A1	0.24	0.12	0.89	1.16
1B1	0.31	0.32	1.13	1.73
1A2	0.13	0.23	1.37	2.31
Mean +/- SEM	0.23 +/- 0.05	0.23 +/- 0.06	1.13 +/- 0.14	1.73 +/- 0.33

Appenidix 4
Future Studies

4.1 Human Growth Hormone

The study described in chapter 4 concluded that rhGH treatment may have clinical potential in improving the nutritional support of severely ill surgical patients because it was found to reduce the rate at which a group of these patients lost nitrogen. Recombinant growth hormone therapy in this context would be exceedingly expensive, and so unequivocal demonstration of its efficacy would be needed in order to justify the cost. This would require the performance of three studies: firstly, a double blind randomized prospective trial, to ensure the repeatibility of our initial non-randomized, non-blinded study. It would then be necessary to demonstrate that the improvements in biochemical indices seen after rhGH treatment (such as reduction in the rate of urea production) realize physiological improvements, such as enhanced respiratory muscle function or reduced post operative fatigue. The final step would be to determine whether rhGH treatment improves clinical end points, such as wound healing, postoperative complication rate, time to wean from ventilators, and days spent in hospital or mortality

I have helped in the design of a study of the effects of rhGH in parenterally fed surgical patients which combines the questions asked by the first two studies proposed above. This study is presently being performed in the Department of Surgery of Auckland Hospital. The protocol involves the selection of a group of severely septic patients defined by clear clinical and laboratory indices, who require parenteral nutrition. The study candidate undergoes a baseline study immediately prior to the

commencement of parenteral nutrition. This study involves a three hour primed constant infusion of ^{15}N urea and ^{13}C leucine, the performance of indirect calorimetry and measurement of voluntary muscle function by testing grip strength. The patients clinical state is then assessed by Elebute and Stoner's system for scoring sepsis[4]. A central line is then inserted and parenteral nutrition begun in the standard fashion. Over the next seven days the patient receives twice daily injections of either rhGH 10 IU or a placebo. Each day, the clinical state of the patient is graded, biochemical profile (urea, electrolytes, glucose and free fatty acids) determined and grip strength measured. On the final day of the study an isotopic infusion is repeated. When completed, this study should determine the effects of rhGH on pertinent biochemical, physiological and clinical indices in septic surgical patients receiving parenteral nutrition. If the data support the hypothesis that rhGH may have a potential role in the nutritional support of these patients, then the stage will be set for conducting a large study to determine the therapeutic efficacy of this hormone.

4.2 IGF-I

At the time of writing, rhIGF-I is undergoing Phase II clinical trials. Accordingly, the use of this very new agent in the context of clinical surgical research is some time hence. In the same manner as it has been shown to be effective in the treatment of Laron (GH receptor deficient and hence GH resistant) dwarfism, rhIGF-I may prove to be clinically more efficacious than rhGH therapy in severely septic patients, who have been demonstrated to be less GH responsive than normals[3], and in perioperative premature infants in whom GH receptors are yet to develop. The clinical potential of this differential response will be more clear when more is known of the GH and IGF type I receptor status in these two circumstances. Recombinant hGH offers practical advantages over rhIGF-I, as it can be

administered by relatively infrequent subcutaneous injections, whereas rhIGF-I will almost certainly demand a continuous infusion.

While further pharmacokinetic studies are being performed in normal human subjects, there are several lines of rhIGF-I/sepsis research which could be pursued in the interim. Recently, Fraker and colleagues reported that simultaneous treatment of normal rats with insulin and TNF protected them from the cachectic and tissue inflammatory consequences of TNF treatment alone[5]. The authors suggested several possible mechanisms for insulin's protection against TNF toxicity: i) insulin treatment leads to a marked hypoglycaemia and subsequent hyperphagia which protected the animals from the nutritional effects of the TNF, ii) insulin may alter the response of the macrophage, the primary endogenous source of TNF, to infused TNF or iii) insulin may change the response of other tissues to the effects of TNF. The doses of insulin used in this protocol were very large (2 IU/100 g s.c.,. b.d.), and it is conceivable that if the TNF protective actions were receptor mediated that they were a result of insulin cross reacting with the IGF type 1 receptor. If this were the case, then it can be hypothesized that the cytoprotective effects of insulin could be reproduced by administration of IGF-I. If the effect is IGF type1 receptor mediated, then it would probably be evoked by a lower dose of rhIGF-I than insulin, reflecting the relatively greater affinity of IGF-I for the IGF type 1 receptor. I am presently performing a study which is designed to investigate this hypothesis. The TNF model of Fraker and coworkers has been slightly adapted to enable insulin and rhIGF-I to be compared; in the previous study insulin was administered in the form of a zinc suspension which ensured continuous release. In my present protocol, both insulin and rhIGF-I are administered subcutaneously via *ALZET* minipumps. A hypoglycaemic and nonhypoglycaemic dose of rhIGF-I are being compared to the effects of an insulin infusion of the same daily dose as was used by Norton. A

further group of animals pair fed with the TNF treated group has been included for future GH/IGF-I receptor analysis so that the effects on the receptors of starvation can be separated from those of TNF.

In chapter 6, data were presented which demonstrated that rhIGF-I infusion had a pronounced acceleratory action on the rate of amino acid uptake by the heart. Others have reported *in vitro* findings which demonstrate that IGF-I has a positive inotropic effect on neonatal rat cardiocytes[10]. The question which presents itself is whether rhIGF-I infusion has a positive inotropic effect *in vivo*. If this proves to be the case, then rhIGF-I may have clinical potential in the treatment of heart failure. There exists an anecdotal report of severe cardiac failure responding to hGH treatment[2], and it is possible that this effect was mediated by IGF-I. A pilot study has been performed in an anaesthetized lamb in order to determine whether rhIGF-I has a positive inotropic action *in vivo*, and it would appear that bolus infusion increases the cardiac output. This observation remains preliminary and repeated studies are planned for the immediate future.

The pronounced effect which IGF-I had on the protein metabolism of the lamb heart suggests other clinical uses. Although adriamycin is one of the most potent chemotherapeutic agents against solid tumours, its usefulness is limited by a dose dependant cardiomyopathy which occurs following prolonged administration. I am at present performing a study designed to determine whether IGF-I infusion is capable of ameliorating the cardiotoxic effects of adriamycin. The protocol involves the chronic treatment of a large number of rats with adriamycin according to a dosage schedule described by Mettler *et al*, which produces cardiomyopathic changes in in nearly all animals six weeks after a 13 week treatment period[8]. Animals will be serially killed during the study period and their myocardial histology examined. As soon as the histological lesions typical of

adriamycin toxicity appear, two week *ALZET* minipumps will be inserted, filled with either rhIGF-I or the appropriate carrier (0.1M acetic acid). At the end of this period the animals will be killed, and autopsy evidence of congestive heart failure (pleural effusions, ascites, hepatomegaly, cardiomegaly) sought. The myocardial tissues will be examined histologically to determine whether rhIGF-I treatment slowed or reversed the development of cardiomyopathy.

IGF-II

The role of endogenous IGF-II remains to be clarified. Although there is evidence from *in vitro* and *in vivo* studies that IGF-II has growth promoting properties[7,9], we did not observe a reduction in the rate of net protein loss following IGF-II infusion in fasted lambs (chapter 6). The explanation for this may reside in the fall in plasma IGF-I levels induced by the IGF-II infusion, which probably resulted from the infused IGF-II displacing endogenous IGF-I from the IGF binding proteins. The subsequent increased rate of clearance of the more potent anabolic agent (IGF-II) may accordingly mask the weak anabolic action of IGF-II. It may be possible to unmask the growth promoting potential of IGF-II by simultaneously infusing sufficient IGF-I to maintain baseline IGF-I plasma concentrations.

The lipolytic action of growth hormone has been well described, and has been attributed to a direct action of the GH molecule. Recently, IGF-II has been demonstrated to possess lipolytic activity at very low concentrations[6], a result which leads to the speculation that IGF-II mediates the lipolytic response of GH. We have investigated this hypothesis by measuring the free fatty acid and glycerol concentrations during 300 minute IGF-II infusions of 15 and 50 μg/kg.hr into lambs, but were unable to demonstrate significant increases in either of these measurements over this

time. I am at present repeating this protocol with the additional step of administering an adrenaline bolus (1 μg/kg) 60 minutes prior to the end of the infusion in order to determine whether IGF-II increases the sensitivity to lipolytic stimuli, in the same manner as growth hormone[1].

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