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**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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**A thesis submitted for the degree of
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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

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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 gratitude 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.

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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

RO _x	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