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STUDY OF THE EFFECTS OF SUPRAPHYSIOLOGICAL GROWTH HORMONE ADMINISTRATION IN HEALTHY YOUNG ADULTS ON METABOLIC VARIABLES AND MARKERS OF SPORTING PERFORMANCE.

VOLUME 1 OF 1

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ABSTRACT

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STUDY OF THE EFFECTS OF SUPRAPHYSIOLOGICAL GROWTH HORMONE ADMINISTRATION IN HEALTHY YOUNG ADULTS ON METABOLIC VARIABLES AND MARKERS OF SPORTING PERFORMANCE.

By Catherine McHugh

Growth hormone (GH) administration in individuals with GH deficiency improves physical performance by increased stroke volume and systemic vascular resistance, substrate availability, erythropoiesis, and altered body composition (increased lean body mass and reduced body fat). This study has evaluated the performance of a methodology developed by the GH-2000 research team to detect GH abuse in athletes. This is based on the measurement of IGF-I and N-terminal pro-peptide of collagen type III (P-III-NP), in non-Caucasian amateur athletes. This study has also evaluated the effects of 28 days of supraphysiological GH administration in GH replete health individuals on markers of performance including exercise capacity (VO_2 maximum), heart rate, substrate utilisation, body composition, and insulin resistance. The study has examined if any changes are associated with changes in serum adiponectin, NEFA or IGF-I concentrations. 36 healthy non-Caucasian amateur athletes completed a double blind randomised placebo controlled trial of 28 days of self-administered subcutaneous nightly GH injections (0.1 IU/kg/day or 0.2 IU/kg/day) or placebo.

IGF-I and, P-III-NP concentrations were measured in serum samples obtained at day 0, 7, 14, 21, 28, 33, 42 and 84 of the trial. Maximal VO_2 was measured during by a treadmill running test at baseline and on day 28. Serial measurements of glucose, insulin, NEFA, were performed during a 75g oral glucose tolerance test (OGTT) on day 0 and 28. Indirect calorimetry was performed at 0, 60, 120 minutes to assess substrate utilization. Body composition was assessed by height, weight, 4-site skinfold thickness, bioimpedance analysis and air displacement plethysmosgraphy. Serum adiponectin was measured at baseline and day 28.

There was no evidence that ethnicity affected the response of IGF-I and P-III-NP to GH administration in non-Caucasian athletes compared with white European athletes. Fasting NEFA, glucose, insulin and insulin AUC during the OGTT and total body weight increased in men receiving GH while systolic blood pressure reduced. No change was seen in two measures of insulin resistance (HOMA and QUICKI), body composition or protein metabolism. There was a reduction in fat oxidation rates in men and women receiving GH, and a rise in men receiving placebo. Urinary nitrogen excretion fell in women receiving GH. In men receiving GH there was a correlation between AUC NEFA and AUC insulin on day 28 during OGTT, between IGF-I on day 28 and AUC insulin and AUC NEFA (correlation in both men and women receiving GH). There was also a correlation between IGF-I and P-III-NP and systolic and diastolic blood pressure changes on day 28 in men receiving GH. There was a correlation between IGF-I on day 2 and fasting fat oxidation in women receiving GH. There were no other correlations between changes in any other markers of insulin resistance or body composition and IGF-I, P-III-NP, adiponectin, glucose or other variables.

Conclusion

This study has shown that there is no evidence that non-Caucasian athletes respond differently to GH than white European athletes, at least in terms of the peak and maximal change in IGF-I and P-III-NP. While the study cannot exclude

small differences between ethnic groups, these data indicate that ethnicity should not have a major impact on the performance of the GH-2000 detection method.

There was evidence that GH may increase the supply of substrates (NEFA and glucose) and increase insulin resistance. GH use is associated with an increase in body weight, which in this study probably represented an increase in body water. There was evidence of anabolism, at least in women as shown by a fall in urinary nitrogen. Taken together these changes may explain why an athlete may gain a competitive advantage although this was not demonstrated in this study.

Table of contents

Table of tables

Table of figures

Declaration of Authorship

I, Catherine McHugh declare that the thesis entitled

STUDY OF THE EFFECTS OF SUPRAPHYSIOLOGICAL GROWTH HORMONE ADMINISTRATION IN HEALTHY YOUNG ADULTS ON METABOLIC VARIABLES

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

this work was done wholly or mainly while in candidature for a research degree at this University;

where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

where I have consulted the published work of others, this is always clearly attributed;

where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

I have acknowledged all main sources of help;

where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

parts of this work has been published before submission (McHugh, 2005a)(McHugh, 2005b, Holt, 2010, Holt, 2009).

Signed: ……

Date: ……

Acknowledgements

I would like to thank the US Antidoping Agency for their generous funding, as well as Professor Richard Holt and Professor Peter Sönksen who were my supervisors during this project.

My thanks to Dr Steve Wootton and Professor Christopher Byrne for their contribution to the planning and assessment of the metabolic studies in this thesis, Dr Nicola Englyst for her kind support and much needed trouble shooting ability, Adam Fityan, Jenny Bacon and the other medical students who assisted in the operation of this project.

And most importantly my beloved Rod Park for all his kindness, support and encouragement during the project and the writing up of this thesis. I would also like to thank my two wonderful children, James and Conor, who are my pride and my joy.

Personal contribution

The candidate was responsible for all the day to day running of the study, including obtaining ethical permission, data protection, medical supervision, subject recruitment, measurement and collection of data. All substrate utilisation, body composition, exercise capacity, and insulin resistance assessment were conceived and devised by the candidate after discussion with colleagues and a literature review. Adiponectin and glucose assays were performed by the candidate. The project was undertaken within a funded research study involving the measurement of insulin like growth factor-I and N-terminal pro-peptide of type III collagen in a double blind randomized controlled setting. The candidate was responsible for the addition of all insulin resistance, body composition, substrate utilisation and exercise capacity assessment components from conception, ethical approval to operational implementation.

NEFA analysis was kindly performed by the Institute of Human Nutrition in the University of Southampton, and insulin assays by Southampton University Hospitals Trust Endocrinology Laboratory. IGF-I and P-III-NP assays were performed in The Drug Control Centre. London.

Abbreviations

A acid ethanol extraction (AE), acid ethanol extraction and cryoprecipitation (AEC) adenine tri phosphate (ATP), area under the curve (AUC),

B basal metabolic rate (BMR), bioimpedance analysis (BIA), blood pressure (BP), body mass index (BMI), body surface area (BSA),

C carbohydrate (CHO), carboxyterminal cross-linked telopeptide of type I collagen (ICTP), confidence intervals (CI), C-terminal propeptide of collagen type I (PICP), coefficient of the variation (CV)

D diet induced thermogenesis (DIT), day (d)

E enzyme linked immunosorbant assay (ELISA), estimated energy expenditure (EEE), EthyleneDiamine Tetra Acetate (EDTA), euglycaemichyperinsulaemic clamp (HEC),

F fasting plasma glucose (FPG), fasting plasma insulin (FPI), fat free mass (FFM), Fédération International de Football Association (FIFA), gas chromatography (GC), free fatty acids (FFA),

G Gas chromatography/mass spectrometry (GC/MS), glucose (G), Growth hormone (GH), growth hormone binding protein (GHBP), growth hormone deficient adults (GHDA), growth hormone secretogogues (GHSs),

H human placental lactogen (HPL), human immunodeficiency syndrome (HIV)

I immunofunctional assay methodology (IFA), insulin (I), insulin-like growth factor-I (IGF-I), immunoradiometric assay (IRMA), insulin-like growth factor binding protein (IGFBP), insulin sensitive indices (ISI), International Amateur Athletics Federation (IAAF), intravenous glucose tolerance test (IVGTT),

K kilocalorie (kcal), kilo Dalton (kDa), kilogram (kg),

L litre (L)

M metabolic equivalent testing (MET), millimole (mmol), microgram (μg)

N nitrogen (N), non-esterified fatty acid (NEFA),

O oral glucose tolerance test (OGTT), oxygen (O_2)

P phosphocreatinine (PCr), pressure (P), physical activity rating (PAR),

Q Quantitative insulin sensitivity check index (QUICKI)

R radioimmunoassay (RIA), recombinant human GH (rhGH), resting metabolic rate (RMR), resting energy expenditure (REE), respiratory quotient (RQ),

S standard deviation (SD), standard error of the estimate (SEE), surface area artifact (SAA),

T triacylglyceride (TAG), time (t)

U Union CyclisteInternationale (UCI), United States Antidoping Agency (USADA)

V volume (V)

W World Anti-Doping Agency (WADA),

Word count

Abstract 638

Total word count 65,653

Chapter 1

Introduction

Growth hormone (GH) is the most abundant pituitary hormone and has diverse anabolic and metabolic effects and is known to play a crucial role in our metabolic wellbeing. For many years it was assumed that GH had no role beyond childhood but recently it has been appreciated that GH also has multiple metabolic and anabolic actions in adulthood. Disorders of deficiency or excess provide an insight into the importance and scope of this effect. Adult GH deficiency (GHDA) leads to the development of central obesity, insulin resistance, dyslipidaemia and premature cardiovascular disease. The similarities between GHDA and the metabolic syndrome have led to the hypothesis that abnormalities in the GH-Insulin-like growth factor I (IGF) axis may have an aetiological role in the metabolic syndrome. In contrast the GH excess seen in acromegaly is associated with premature cardiovascular death, cardiomyopathy, hypertension and diabetes.

There has also been considerable interest in the scientific literature recently in the potential therapeutic potential of GH and its ability to induce anabolism in catabolic patients in intensive care units and reduce the loss of muscle mass associated with ageing. Anecdotal evidence suggests it is abused by athletes to enhance sporting performance because of both its anabolic and lipolytic properties.

Much of the published data about GH comes from either people with GH deficiency, both before and after treatment with GH untreated or replaced population or from people with acromegaly. There are a number of inherent limitations in the extrapolation of data from studies of a GH deficient population to determine the physiological effects of GH in a healthy population.

Studies that show positive results are more likely to be published.

GH response to exercise or other stimuli is pulsatile and not mimicked by nightly GH injections.

Most studies are in GHDA over long term administration and exercise involves short pulses

GHD is a pathological state and studies are in adults with significant pathology and perhaps other hormone deficiencies; these individuals are not necessarily representative of the normal healthy athletic population.

Hence this study looks at the effects of GH in a normal healthy population.

1.1 Overview of the project

This project was funded by the United States Anti Doping Agency (USADA) to validate a test to detect GH abuse among athletes. The GH2000 project showed that a method based on the measurement of two GH markers, IGF-I and P-III-NP, used in conjunction with discriminant function formulae provided a sensitive and specific test to detect GH abuse among Caucasian athletes. The IOC expressed concerns about its use in non-Caucasian athletes at their meeting in Rome in 1999. The GH2004 project was established in Southampton in January 2003 to determine the validity of the GH2000 findings and the marker method of testing for GH usage in a non-Caucasian population. The GH2004 project followed a protocol that was similar to the GH 2000 project.

This study is part of that GH2004 project and involves a double blind randomised controlled trial of the administration of 2 doses of recombinant growth hormone (rhGH) (0.1 U/kg/day, 0.2 U/kg/day) or placebo to normal healthy volunteers for 28 days with serial measurement of IGF-I and P-III-NP for 84 days. The wash out period from 28 to 84 days was designed to determine the window of opportunity during which rhGH administration could be detected after the last dosing. Additional elements were added by the candidate to assess the effects of

GH on aspects of sporting performance. They include assessment of the following:

physical fitness with VO_2 maximum measurement with a treadmill running test, body fat and body composition estimation,

measures of substrate utilisation,

urinary nitrogen,

insulin resistance,

non esterified fatty acid levels before, during, and after an oral glucose tolerance test (OGTT)

basal and post glucose administration metabolic rate assessment.

Physical functioning is known to improve with replacement of GH in growth hormone deficient (GHD) individuals. These properties may be also evident with supraphysiological administration to normal individuals. Anecdotal evidence suggests that athletes have been abusing GH at least since 1982 but perhaps even longer, and they report a considerable performance advantage conferred by its use. Large scale clinical trials are not ideal to detect minor changes in metabolic measurements whereas athletes are trained to detect and analyse even small variations in performance. Exercising muscle requires efficient delivery of substrate, oxygen, and efficient uptake and utilisation of these substances. Possible effects that may confer a performance advantage include:

increased substrate availability by inducing hyperglycaemia, and increasing free fatty acid concentrations in serum,

improved delivery mechanisms by increased stroke volume secondary to cardiac muscle anabolism either directly or by the effects of IGF-I, and also by increased cardiac preload secondary to expansion of the blood volume by water retention

improved delivery systems by increased red cell mass secondary to erythropoiesis and changes in systemic vascular resistance

reduced whole body and visceral body fat

protein anabolism with increased muscle mass and tensile strength

reduced fatigue during and after exercise.

This thesis proposes that the changes seen in GHD are also seen in normal individuals receiving growth hormone and could potentially confer a performance advantage.

1.2 Physiology of growth hormone

Growth hormone (GH) is secreted from the anterior pituitary. The human adenohypophysis contains 5 to 10mgs of GH synthesized and stored in the somatotrophs. The approximate human GH rate of production is 0.4 to 0.5mgs per 24 hours (Hartman, 1991). 70% of circulating GH is in the form of a 22-kilo Dalton (kDa) polypeptide while 5 to 10% is in the 20 kDa isoform. Other isoforms, e.g. 17kDa and fragments of isoforms, make up a small proportion of circulating isoforms (McHugh, 2005b).

1.2.1 Regulation of growth hormone secretion

GH release is under the control of a number of hypothalamic hormones, and secretagogues. GH releasing hormone (GHRH) stimulates the synthesis and release of GH, while somatostatin modulates frequency and amplitude of pulses and is inhibitory in action. Growth-hormone secretagogues (GHSs) are small

synthetic molecules that stimulate the release of GH through a G-protein-coupled receptor in the pituitary. The natural ligand, ghrelin, is a hexapeptide released from the gastric mucosa and pituitary gland and also stimulates GH release from the somatotrophs(Kamegai, 2004). There a number of physiological stimuli to GH release. The most powerful external physical stimuli are exercise and sleep, while others include hypoglycaemia, hormonal agents as shown in table 1.1

Factors which stimulate GH release	**Factors which inhibit GH release**
GH releasing hormone	Somatostatin
Ghrelin	GH negative feedback
Sleep	insulin like growth factor-I
Hypoglycaemia	hyperglycaemia
reduced fatty acids	Obesity
increased amino acids	
Puberty	Senescence
Oestrogens	Progesterone
Androgens	glucocorticoids
α adrenergic agonists	β adrenergic agonists
Serotonin	serotonin antagonists
dopamine agonists	dopamine antagonists

Table 1.1 Regulation of endogenous GH secretion

1.2.2 Diurnal variation in growth hormone levels

Serum GH peaks in the post absorptive state, is highest 2 hours post hypothalamic pulse and during slow wave sleep. This generally occurs in the first hour of sleep (Van Cauter, 1998).

1.3 Metabolic effects of growth hormone

GH has metabolic effects throughout many body tissues both directly and indirectly through the production of GH-dependent proteins, the most important of which is IGF-I. A summary of GH actions is shown in figure 1.1.

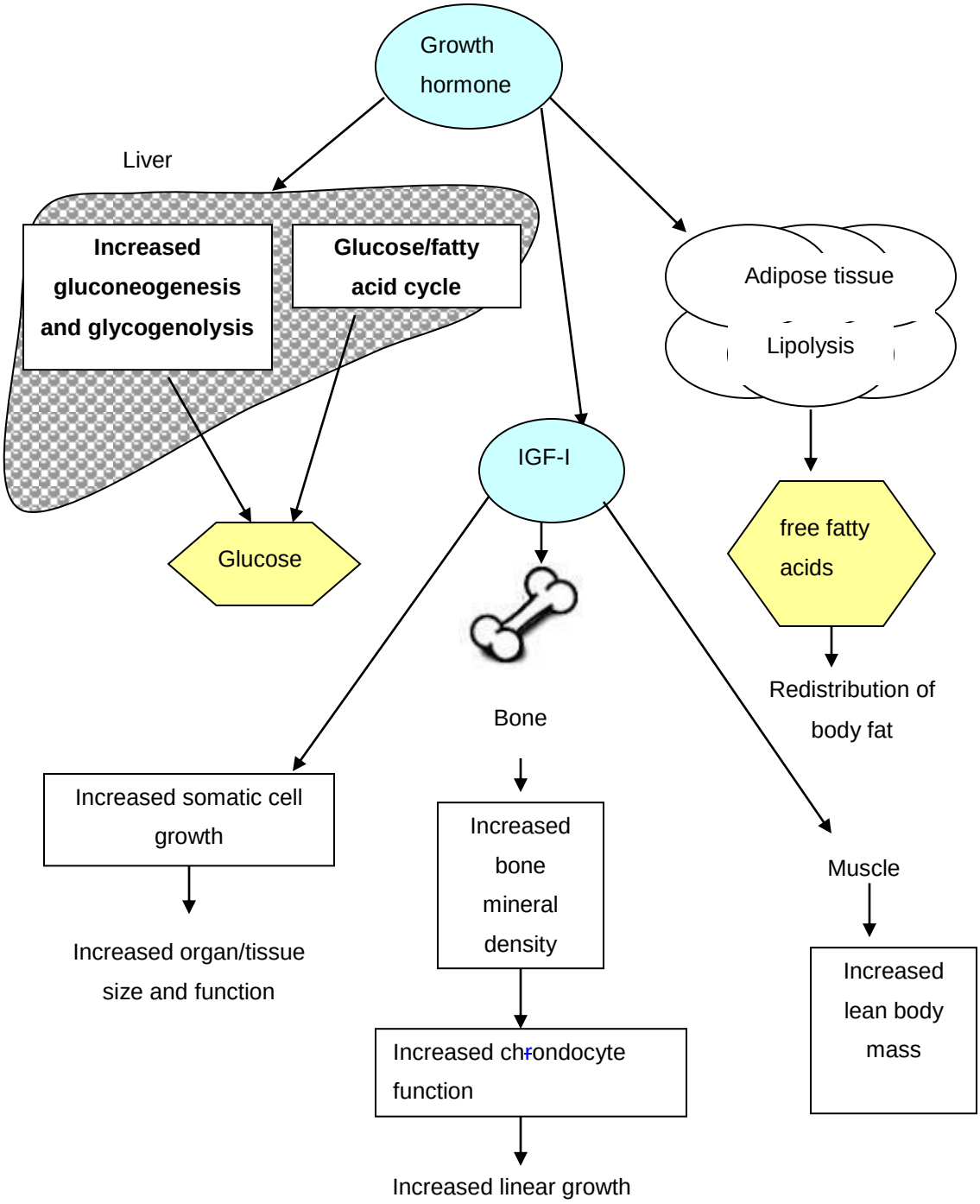


Figure 1.1 Summary of the actions of growth hormone. GH has direct effects on the liver to stimulate glycogenolysis and gluconeogenesis which increases circulating glucose. It induces adipose tissue lipolysis, redistribution of body fat

from central to peripheral depots and non esterified fatty acid (NEFA) production, which then enters the hepatic glucose/fatty acid cycle and further contributes to circulating glucose. GH stimulates hepatic and local IGF-I production which induces linear growth, increases bone mineral density, anabolism in muscle, as well as increases somatic cell growth and organ size

There is considerable inter-individual variation in GH action. Tissue delivery depends on the level of binding to growth hormone binding proteins (GHBP), tissue blood flow, renal clearance, and activity at tissue levels depends on GH receptor expression and post receptor signalling (Le Roith D, 2001).

Similarly at physiological concentrations the rate of elimination of free GH from plasma is controlled by total free GH concentrations, obesity, age and renalfunction within the physiological GH concentration range. At supraphysiological concentrations age is a negative predictor of clearance (Schaefer, 1996).

1.3.1 GH Receptor and the GH binding protein (GHBP).

The binding of GH to membrane receptors and subsequent cascade is explained in figure 1.2. GH binds to a single transmembrane receptor with two binding sites that undergo dimerization and bind a protein tyrosine kinase JAK (Janus associated kinase). This initiates a cascade involving phosphorylation of the JAK, the receptor and STAT kinases (signal transducers and activators of transcription) and their subsequent translocation to the nucleus and the activation of transcription factors (Lanning and Carter-Su, 2006).

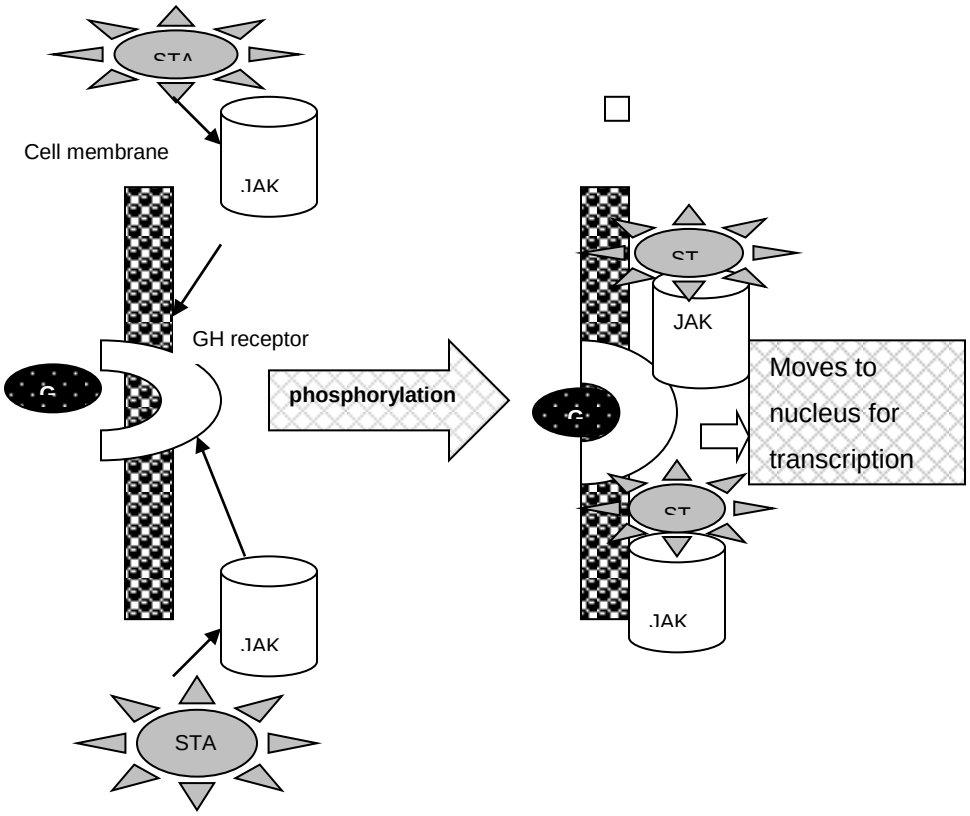


Figure 1.2 Metabolic action of growth hormone in cell.GH binds to a single transmembrane receptor with two binding sites that undergo dimerization and bind a protein tyrosine kinase JAK (Janus associated kinase). This initiates a cascade involving phosphorylation of the JAK, the receptor and STAT kinases (signal transducers and activators of transcription) and their subsequent translocation to the nucleus and the activation of transcription factors (Lanning and Carter-Su, 2006).

Half of the circulating GH is bound to GH binding proteins (GHBP) which are structurally identical to the extra-cellular component of the GH receptor and prolong the half-life of GH to approximately 20 minutes. There is considerable inter-individual variation in GHBP concentrations but levels in any one individual are low prenatally and rise in childhood to maintain a relatively constant level throughout adulthood (Veldhuis et al., 2006).

1.3.2 Effects on protein metabolism

The anabolic actions of GH in adults have been determined through physiological studies of protein turnover. GH increases total body protein turnover and muscle synthesis in adults with GH deficiency (Fryburg et al., 1991). Although IGF-I is produced widely throughout the body in response to GH, the liver produces the vast majority of circulating IGF-I (Liu and Barrett, 2002). Infusion of GH into the brachial artery in humans induces protein anabolism distal to the infusion site without augmentation of systemic IGF-I or whole body protein synthesis, demonstrating the importance of the local production and regulation of IGF-I (Flyvbjerg et al., 1991).

Insulin is required for anabolism with GH; insulin inhibits the breakdown of protein while GH and IGF-I increase protein synthesis. GH and testosterone act through separate mechanisms and have additive effects on anabolism. This has not been lost on athletes who use cocktails of anabolic agents to gain the maximal effect.

1.3.3 Effects on lipid metabolism

While GH is considered an anabolic hormone, it is more accurately termed a partitioning agent as GH induces lipolysis in adipose tissue and leads to a reduction in fat mass at the same time as it increases protein synthesis and lean body mass. It inhibits lipoprotein lipase and stimulates hormone sensitive lipase activity. This is directly opposite to the actions of insulin on these molecules.

Lipoprotein lipase is synthesised in adipose tissue and attaches to the vascular endothelium where it lyses passing chylomicrons and VLDL to produce FFA and glycerol for storage in adipose tissue as triacylglycerol. GH induces release of FFA and leads to increased glycerol and non-esterified fatty acid (NEFA) concentrations that compete with glucose in the fatty acid/glucose cycle the liver and contribute to the acute increase in insulin resistance and resulting increase in hepatic glucose output (Randal PJ, 1963). NEFA levels are inversely proportional to circulating glucose concentration. The glucose fatty acid cycle was proposed in 1963 by Randle. He described a regulatory process whereby glucose elevation induces insulin secretion which in turn suppresses NEFA release from adipose depots. This reduces competition within skeletal muscle for substrate utilisation and allows use of circulating glucose as a fuel source in preference to NEFA. GH stimulates hormone sensitive lipase which also leads to increased NEFA production. This process is inhibited by insulin.

1.3.3.1 Free fatty acids, GH and obesity.

Obesity is associated with high circulating FFAs and direct infusion of FFA leads to a reduced GH production. FFAs are thought to inhibit GH release by facilitating other inhibitory factors rather than directly suppressing GH pituitary secretion. Infusion of arginine or pyridostigmine, which inhibits somatostatin release, followed by the addition of Acipimox , an antilipolytic agent, leads to a greater fall in GH concentration compared with arginine stimulation alone. This does not

occur with Acipimox alone (Cordido, 1996). FFA are slow to return to normal after weight loss and may thus be important in the slow return of GH secretion to normal (Kreitschmann-Andermahr, 2010, Cordido, 1996).

1.3.3.2 Adiponectin

Adiponectin is a 28 kDa protein produced by adipocytes and production is stimulated by insulin. It has anti-atherogenic properties and is abundant in the circulation accounting for 0.01% of circulating protein molecules (Ouchi, 2000). It consists of an N-terminal collagen like domain, a C-terminal globular domain which has structural similarities with complement factor C1q and TNF superfamily (Yu-Yahiro et al., 2001). There are 2 known adiponectin receptors; AdipoR1 is highly expressed in skeletal muscle and AdipoR2 is expressed in the liver (Yamauchi and Mechanic, 1988).

A recombinant form of the C-terminal domain has been shown to induce fatty acid oxidation and weight reduction in mice but the recombinant whole molecule of adiponectin resulted in less weight loss or fatty acid oxidation (Yamauchi and Mechanic, 1988, Fruebis, 2001). Adiponectin is negatively correlated with BMI but interestingly women have higher levels when compared to men matched for BMI although women have inherently a higher percentage body fat (Kern et al., 1989). Obese individuals have lower levels and weight loss raises adiponectin(Ouchi, 2000). Subjects with type 2 diabetes have lower circulating adiponectin when compared to people without diabetes of similar BMI (Ouchi, 2000). Levels are inversely related to tumour necrosis factor alpha levels (TNF-α) (Kern et al., 1989) and it has been shown to inhibit the attachment of monocytes to vascular endothelium as seen in early atherosclerosis (Arita, 1999) and TNF-α induced expression of endothelial adhesion molecules. Adiponectin concentration is not influenced by food intake (Ouchi, 2000).

In this study the markers of insulin resistance used were glucose and insulin at intervals during OGTT, area under the curve of glucose and insulin during the OGTT, HOMA, QUICKI, insulin/glucose ratio, and adiponectin concentrations before and after 28 days of rhGH administration.

Since GH exerts a significant effect on fat metabolism, it has been suggested that adipocytokines, such as adiponectin, may play an important role in mediating the effects of GH on glucose homeostasis (Herrmann, 2005). Adiponectin is inversely related to adiposity and insulin resistance (Herrmann, 2005, Siemińska, 2005, Ronchi, 2004, Lam, 2004), and is thought to protect against diabetes. The effect of GH on adiponectin in healthy individuals is unknown. GH may increase adiponectin through a decrease in fat mass but by contrast may increase adiponectin through its effect on insulin sensitivity.

The aim of the current aspect of this study is to assess the change in insulin resistance and serum adiponectin in healthy young adults in response to 28 days of two doses of rhGH. The short term administration period in this study allows an assessment of IS before major changes in body composition occurred.

In animal studies, adiponectin administration increases muscle lipid oxidation rates and reduces plasma NEFA via lipoprotein lipase inhibition. This is associated with increased hepatic insulin sensitivity and suppression of hepatic glucose output and even hypoglycaemia (Combs, 2001).

As these actions oppose GH, there has been interest in the effects of GH on adiponectin secretion. Cross-sectional studies of GHD adults and children have suggested that adiponectin concentrations are no different from healthy controls and the administration of GH did not affect adiponectin concentrations (Joaquin, 2008, Hana, 2004, Schmid, 2005, Ciresi, 2007). By contrast, adiponectin concentrations were increased, and inversely correlated with triglyceride levels in prepubertal children with Prader-Willi syndrome. Following treatment with rhGH, adiponectin concentrations increased further (Festen, 2007).

Adiponectin has been reported to be increased, unchanged and decreased in patients with acromegaly but where changes were reported, these do not appear to be related to the development of insulin resistance (Siemińska, 2005, Ronchi, 2004, Lam, 2004).

1.3.4 Effects on glucose metabolism

In the 1930s it became evident that dogs post hypophysectomy had improved diabetic control (Houssay and Biasotti, 1930). GH directly antagonises the actions of insulin, as shown in figure 1.4. Increased hepatic gluconeogenesis and glycolysis lead to increased non-oxidative glucose turnover and hepatic glucose output. GH is released in association with glucagon in the hypoglycaemic state and is in fact required to maintain optimal pancreatic islet cell function (Nussey and Whitehead, 2001). GH administration increases hepatic glucose output and reduces peripheral uptake of glucose. GH also inhibits glycogen synthase via lipid accumulation accompanied by peripheral insulin resistance via inhibition of glucokinase, GLUT 2 mRNA and glucokinase most notably in skeletal muscle (Valera et al., 1993).

Hypoglycaemia and dietary intake of amino acids and ghrelin produced by gastric tissue stimulate GH release from the pituitary. In contrast a fall in FFA, administration of glucocorticoid or tri-iodothyronine inhibits GH release. Increases in circulating GH induce NEFA and glycerol production which increases blood glucose by stimulation of hepatic gluconoegenesis and glycogenolysis. NEFA also enter muscle and act as substrates for Krebs cycle with citric acid production and feedback inhibition of glucose uptake into muscle. In contrast glucose ingestion induces insulin release. This stimulates glucose uptake into tissues via GLUT 4, inhibits glycogen phosphorylase and stimulates glycogen synthetase leading to glycogen storage. Insulin increases glucose

uptake into muscle and peripheral tissues, and inhibits gluconeogenesis and glycogenolysis. Thus insulin and GH have opposing actions.

1.3.4.1 Molecular aspects of growth hormone actions on glucose metabolism.

Post receptor insulin and GH/IGF-I signalling overlap (Fernando et al., 2005). The liver is the major site of GH induced IR and the stimulation of phosphatidylinositol 3-kinase (PI3K) pathway is thought to be involved in hepatic insulin resistance. Skeletal muscle insulin resistance is mediated by an increase in the p85 subunit of the PI3K system, which has a negative effect on insulin signalling (Fernando et al., 2005). GH inhibits serine phosphorylation of the insulin receptor subunit-1 (IRS-1). This thus has a negative effect on insulin signalling and induces the suppression of cytokine signalling (SOCS-1 and SOCS-2) which in turn modulates IRS, in addition to suppression of adiponectin(Fernando et al., 2005).

1.3.4.2 Co-ordination of the feed-fast metabolic changes

Insulin production is augmented after eating (post prandially) and increases glucose transport into hepatocytes. GH production is higher during periods of fasting (post absorptive) and increases fat metabolism as glucose levels fall.

Insulin inhibits hormone sensitive lipase reducing NEFAs release into the circulation. The rising glucose and insulin levels post prandially also stimulate muscle glycolysis which produces glycerol-3-phosphate that facilitates re-esterification of fatty acids which are retained in adipose tissue.

Excess GH post prandially, for example in patients with acromegaly or diabetes, induces insulin antagonism and loss of its glucose lowering effect with resultant hyperglycaemia and higher circulating NEFAs.

1.4 Sporting Performance

1.4.1 Definition of Sporting Performance

Sporting performance is a complex mulitfactorial biological and psychological entity and to some extent sport specific. The physical and mental requirements for shooting are vastly different then weight lifting for example. This is further highlighted by the performance enhancing agents chosen in each sport. Shooting athletes reputably benefit from the bradycardic and sympatholytic effects of beta blockers whereas weight lifters use agents for their anabolic properties.

There are a number of markers of sporting performance including VO_2, heart rate, serum lactate concentrations, muscle strength, muscle circumference, body fat, lean body mass, resting RQ, and BMR.

1.4.2 Brief history of doping

Substance abuse in sport is not a modern phenomenon and dates back as far as Ancient Graeco-Roman times when figs were used to enhance performance. The term “doping” comes from the word “dop” which is a substance made from grape skins used by Zulu warriors to enhance battle prowess (World Anti-doping Agency, 2005). The first recorded drug related fatality occurred in 1886 when Andrew Linton died during the Bordeaux-Paris cycle race allegedly from an

overdose of strychnine, heroin and a compound known as “trimethyl”. The International Olympic Committee set up a Medical Commission in 1967 and the official list of prohibited substances was published. The first systematic testing began at the 1972 Olympic Games in Munich with the analysis of over 2000 urine samples by gas chromatography (GC) with nitrogen-selective detection for stimulants. In 1983 at the Pan American Games systematic urinary screening was first introduced but it was not until 1994 in the Lillehammer XVII Olympic Winter Games that blood testing was used for the first time in an attempt to detect blood doping (Veldhuis et al., 2006).

The prohibited substance list has been reclassified with the setting up of the World Antidoping Agency (WADA) in 1999 and banned substances are now classified according to their mode of action.

Doping is defined in the World Anti-Doping Agency (WADA) code as “the administration of or use by an competing athlete of any substance foreign to the body or any physiological substance taken in abnormal quantity, or taken by an abnormal route of entry into the body with the sole purpose of increasing in an artificial and unfair manner their performance in competition.”

An athlete is considered to have violated the regulations if the prohibited substance is discovered in the athlete’s bodily fluid, or if the athlete attempts to use a prohibited substance or method, fails to submit a sample once requested or fails to make themselves available for out-of-competition testing, unless the athlete can demonstrate that the presence of the substance is caused by a physiological or pathological condition (World Anti-doping Agency, 2005).

1.4.3 Growth Hormone Abuse in Sport

Antidoping authorities introduced GH testing using the isoform method (described later) at the Athens Olympic Games in 2004, and has been used in Turin 2006 and Bejing 2008 (Barroso, 2009). There were no positive tests during those games. The first adverse analytical finding was that of Terry Newton who was an English rugby league player, and tested positive during out of competition testing in February 2010. He later admitted to taking rhGH.

The Underground Steroid Handbook was published as far back as 1983, where GH was described as “the most expensive, most fashionable and least understood of the new athletic drugs” (Duchaine, 1983). It first came to prominence in the general media when Ben Johnson was stripped his 100m gold medal from the Seoul Olympic Games and he subsequently admitted to having taken a cocktail of drugs which included GH. Yuan Yuan, a member of the Oriental National Swim Team was forced to withdraw from the 1999 Swimming World Championships when 13 vials of human GH was discovered in her suitcase. The grand jury testimony of Tim Montgomery (previous 100-metre world record holder) contained an admission that he had received an 8-week supply of GH and a steroid compound known as “the clear” (Fainaru-Wada and Williams, 2004). Both pituitary-derived and both short-acting and discontinued depot recombinant human GH (rhGH) are widely available on the internet.

1.4.4 Why is GH abused?

Anecdotal evidence strongly suggests that GH is widely abused for its anabolic and lipolytic properties in the sporting community. Some of the anabolic actions of GH are mediated through IGF-I and this has been discussed earlier in this chapter.

GH and testosterone are both anabolic and act synergistically albeit through separate mechanisms. There have been reports of cocktails of a number of anabolic agents being used by athletes to gain the maximal effect.

There have been suggestions that the banning of GH is encouraging its use (Rennie, 2003). However, there are a number of arguments to the contrary. Firstly, athletes look for an individual response while clinical trials look at mean changes in larger populations/cohorts. Secondly similar arguments were made about anabolic steroids and it is only recently that clinical trials have shown what athletes have known for a long time that anabolic steroids enhance performance (Hartgens and Kuipers, 2004). Thirdly research into the effects of GH by clinical endocrinologists has fallen at least a decade behind athletes. Athletes are highly trained to know their performance and evaluate small changes in response to changes in training. By contrast clinical trials are designed to evaluate relatively large changes and the numbers needed to detect a 1% change in performance would be huge yet the margin for winning an Olympic Gold medal is usually less than this. Finally, athletes use a cocktail of drugs that are individually tailored to their requirements. In contrast, clinical trials are designed to evaluate only one or two interventions at a time with all other things being equal. Athletes provide a new paradigm for examining the potential benefits of new anabolic agents that clinical trials are not capable of.

1.4.5 Physiological aspects of GH which could potentially affecting sporting performance

In healthy subjects GH has been shown to lead to reduced body fat 0.9 kgs, increased lean body mass of 2.1 kgs in a meta-analysis of studies of healthy adults in a meta-analysis by Liu et al with mean GH administration dose of 36mcg/kg/day for 20 days (Liu, 2008). It has recently been shown to increase sprint capacity by Meinhardt et al (by 0.71kJ) after GH administration at a dose of 2 mgs/day for 8 weeks (Meinhardt, 2010). It did not increase biceps or quadriceps strength on 1 repetition maximum strength testing (Yarasheski, 2008). Further markers of exercise capacity are shown in table 1.3. Basal metabolic rate increased, a higher resting heart rate (3.8 bpm) and higher exercising HR (Lange et al., 2002, Hansen, 2005) occurred after GH treatment. Resting respiratory exchange ratio or respiratory quotient was reduced indicating preferential use of lipids as a metabolic fuel source over carbohydrates. RQ during and after exercise were not significantly different.

Results of qualitative summary of exercise capacity from meta-analysis by Liu et al., 2008			
Marker of exercise capacity	Published paper	Comparison between GH and non GH treated groups	p value for comparison between groups
Plasma lactate level	(Hansen, 2005)	Higher in GH group	<0.001
	(Irving, 2004)	Higher in GH group	0.07
	(Lange et al., 2002)	Higher in GH group	<0.001
Heart rate	(Hansen, 2005)	Higher in GH group	<0.02
	(Irving, 2004)	Higher in GH group	0.12-1.0
	(Lange et al., 2002)	Higher in GH group	<0.001
	(Ehrnborg, 2005)	Higher in GH group	0.08
Plasma free fatty acid level	(Hansen, 2005)	Higher in GH group	<0.0
	(Lange et al., 2002)	Higher in GH group	<0.001
	(Healy, 2003, Healy, 2006)	Higher in GH group	<0.05
Respiratory exchange ratio or respiratory quotient	(Hansen, 2005)	Similar in both groups	0.64
	(Lange et al., 2002)	Similar in both groups	>0.76
	(Healy, 2003)	Similar in both groups	Reported non significant but no p value reported
	(Graham, 2007a)	Lower in GH group	>0.05
Bicycling speed	(Lange et al., 2002)	Higher in GH group	0.39
	(Lange et al., 2002)	Lower in GH group	0.44
VO_2max	(Ehrnborg, 2005)	Higher in GH group	0.76
	(Graham, 2007a)	Higher in GH group	>0.05
Power output	(Ehrnborg, 2005)	Higher in GH group	0.84
Energy expenditure	(Healy, 2003, Healy, 2006)	Similar in both groups	Reported non significant but no p value reported
Maximum inspiratory pressure (non exercising)	(Graham, 2007a)	Higher in GH group	<0.05

Table 1.3 Summary of markers of exercise capacity from meta-analysis by Liu et al., 2008

Plasma lactate concentration was shown to be higher and is associated with reduced stamina and earlier exhaustion and raised plasma glycerol can lead to reduced lactate clearance(Lange et al., 2002, Billat, 2003).

Free fatty acids were higher during exercise due to lipolysis (Lange et al., 2002). There was an increase in resting maximal inspiratory pressure (Graham, 2007b, Graham, 2007a, Graham, 2007c).Conversely there was no change in power output, cycling speed, exercising energy expenditure or VO_2 maximum after GH administration (Ehrnborg, 2005, Berggren, 2005).

1.4.6 Effect of exercise on growth hormone

The GH/IGF axis exerts significant effects on exercise performance. Exercise induces a GH pulse and is considered one of the most potent stimulating agents for GH secretion (Sutton and Lazarus, 1976). GH concentrations rise 10 to 20 minutes after the onset of exercise, peak either at the cessation or a short time after cessation of exertion and have been reportedly elevated for up to 120 minutes after the end of exercise (Viru, 1992, Raynaud, 1981, Lassarre, 1974).

Both acute and chronic physical exercise are potent stimulae for GH secretion (Dall et al., 2000). It has been postulated that many of the health benefits of exercise are mediated by GH and exercise is second only to sleep as a physical stimulus for GH release.

The exact physiological mechanism of GH regulation during exercise is unknown and a number of likely agents have been postulated. Serum pH was assessed in one study with alkaline infusion leading to a reduced GH pulse after exercise (Elias, 1997). The pulse is also reduced in cold external temperatures and is proportionally reduced according to core body temperature (Christensen, 1984,

Wheldon. A., 2006). Lactate infusion does not increase GH secretion (Luger and G.P., 1992) but the intensity of exertion up to the lactate threshold does influence GH secretion so lactate seems a likely candidate albeit early in exertion it reaches its peak (Pritzlaff and Hartman, 1999). Exercise is known to increase so GH dependent proteins such as GHBP, IGFBP-3, total IGF-I, acid labile subunit, and reduce IGFBP-1 (Bang and Hall, 1990, Wallace et al., 1999). There are no changes in free IGF-I and all the above proteins rise in parallel and iGFBP-3 proteolysis rates are unchanged so it is likely that exertion causes an expulsion of performed complexes into the circulation (Gibney, 2007). It has been postulated that IGF-I is used for its reparative properties post exercise and/or IGFBP-1 reduced post exercise hypoglycaemia (Gibney, 2007). It is likely that endogenous GH pulses exert a delayed effect on lipolysis in the post exercise period or during prolonged low intensity exercise (Gibney, 2007)

It is thought that hypothalamic pathways also regulate GH pulses during exercise. This is likely to be via somatostatin and GHRH actions, but the exact mechanism is unknown. Possible mechanisms include cholinergic activation, temperature changes, anaerobic glycolysis, lactate formation (Roemmich JN, 1997) and changes in fuel metabolism and substrate utilization, predominately glucose and fatty acids may play a role as well as opiates, serotonin, dopamine control of GH release (Thompson et al., 1993, Schwinn et al., 1976).

Factors leading to a greater rise in GH include gender, body habitus, physical fitness, magnitude and duration of exercise (Lassarre, 1974, Bunt et al., 1986, Kanaley, 1999, Felsing, 1992, Snegovskaya, 1993), whereas age and cold ambient temperatures reduce the magnitude of the rise in GH (Hagberg and B., 1988). The age related changes may result from adverse body composition changes associated with ageing (Veldhuis and D.A., 1995, Veldhuis, 1991). A study comparing lean and obese, young and older men reported age and

exercise capacity measured by maximal oxygen consumption (VO_2 maximum) determine GH concentrations with exercise and not body fat. Hence physical training and not maintenance of low body fat determines GH concentrations with ageing (Holt et al., 2001). Conversely fitness programs have not been shown to increase the GH response to exertions (Sutton and Lazarus, 1976, Kanaley, 1999).

A study by Pritzlaff et al showed a greater GH concentration with each GH pulse as the magnitude of exertion increased but no change in the frequency of pulses or elimination half life of GH (Pritzlaff and Hartman, 1999). GH levels correlate with exercise duration and number of repetitions of exercise bouts when intensity is constant (Kanaley, 1997, Wideman, 2006). Women have higher resting GH concentrations but both genders have a similar incremental rise in GH secretion with exercise (Wideman and A., 1999).

1.4.7 Psychological aspects of GH use

There is undoubtedly a motivational effect of the use of ergogenic agents to enhance athletic performance. The placebo effect may be a significant part of the beneficial effects on performance. Anecdotally athletes describe a feeling of enhanced ability to resume training on days following vigorous training sessions.

Studies of GH replacement in GHD adults have shown improved mental state, including mood and energy levels, normalisation of sleep patterns and improvement in overall quality of life scores (Astrom, 1990, McGauley, 1989). GHD Adults who have low self esteem especially underestimation of body size showed improvement in self esteem, anxiety, and depression scores after rhGH replacement (Riva, 1995). Improvements after 1 month of therapy have been shown in energy levels, emotional lability, and mental fatigue (Mahajan, 2004).

Reintroduction of rhGH in previously treated individuals after interruption of therapy demonstrated that IGF-I is negatively correlated with measures of fatigue, tension, depression and anxiety, and positively with feeling of increased vigour and energy(Stouthart, 2003).

Conversely individuals with depression have blunted physiological GH secretion in response to GH releasing hormone and also blunted physiological responses to GH itself (Harro, 1999). Fibromyalgia has also shown to be associated with low IGF-I and reduced hypothalamic pituitary axis functioning. Depression is a recognised symptom of this condition. The role of rhGH replacement in such patients is under review (Yuen, 2007).

1.4.8 Challenges of Detecting GH Abuse

There are a number of important considerations when measuring serum GH for detection of illicit abuse in athletes. Care must be taken to distinguish endogenous from exogenous GH by determining concentrations of the different isoforms of GH, exclusion of pathological conditions such as acromegaly. Exercise induces a GH burst which must be taken into consideration in post competition testing (Wallace et al., 2000, Weltman et al., 1992). Repeated sampling over 24 hours would not be feasible in a track side setting (Hartman, 1991). GH is not reliably detectable in urine which makes it essential that a serum sample is obtained track side as is currently common practice to detect blood doping and erythropoietin (Albini et al., 1988). Other potential issues include molecular measurement problems such as the difficulty distinguishing pituitary derived from exogenous GH, the heterogeneity of GH, binding proteins, cross reactivity with other pituitary polypeptide hormones such as prolactin, potential monoclonal antibody cross reactivity in commercial immunoassays, and the short half life of circulating GH.

1.4.9 Methodologies for Detecting GH

There are two possible methods of detecting GH abuse: the marker method and the isoform method. Both methods are subject to the WADA requirement that 2 separate immunoassays are used which detect separate and different epitopes of the peptide (World Anti-doping Agency, 2005).

1.4.9.1 GH Isoforms

The isoform method relies on the determination of the ratio of 22 kDa GH to total GH concentrations. This was previously known as “direct method”. There are a number of isoforms of circulating GH. The 22 kDa is the most abundant comprising 75% of circulating GH isoforms, the others are collectively known as the “non 22 kDa” and primarily include the 20 and 17 kDa isoforms. Recombinant GH consists of 22 kDa isoform alone and leads to a marked reduction in endogenous “non 20 kDa” isoforms by negative feedback mechanisms. This method of detection is based on a high ratio of 22 to non 22 kDa isoforms (Bengtsson et al., 1999). Wu et al have developed ultrasensitive chemiluminescent assays with the use of a monoclonal antibody against 20 kDa and 22 kDa GH isoforms and show that despite higher 20 and 22 kDa concentrations in women compared to men, there was no gender difference in the ration of 20 kDa to 20 kDa + 22kDa in the basal GH state (Wu, 2010). There are a number of disadvantages with the isoform method. Firstly, it has a short window of opportunity for detection because GH has a T½ of 36 hours and returns to baseline within 48 hours of administration (Wu et al., 1990). It cannot detect GH which is derived from either animal or human pituitaries, detect IGF-I use or that of GH secretagogues.

1.4.9.1.1 GH Isoform Assays

WADA has published guidelines for the application of GH differential isoform assays in order to harmonise testing strategies across all WADA accredited laboratories (World Antidoping Agency, 2010). This involves the use of two monoclonal antibody tests to determine the relative abundance of 22 kDa GH over other isoforms. Laboratories are supplied with two kits ("1" and "2") by CMZ-AssatGmBH Germany. The initial sample may be assayed using either kit but the confirmation assays must be performed using both kits. The immunoassay is a dual-antibody sandwich-type system with GH specific monoclonal antibody precoated on the tube surface for "capture" of GH and a detection antibody labelled with acridinium ester. This ester allows luminescence when excited with energy from the luminometer instrument. Each of the kits has a "recombinant" and "pituitary" antibody". The former antibody preferentially captures 22 kDa GH and the latter the non 22 kDa GH. The results are expressed as a ratio of the recombinant/pituitary GH concentrations for each kit.

Laboratories must comply with validation procedures in order to be accredited by WADA for testing and must validate the assay on site and must comply with the following measurements:

Limit of quantification of 0.05 ng/mL or less

Intra-assay CV not higher than 10%

Inter-assay CV not higher than 20%

1.4.9.2 GH dependent markers

This method involves detection of GH abuse by measuring the serum levels of a number of markers of GH in conjunction with specific equations, “discriminant functions” to detect GH abuse. Two markers in particular have been shown to be both sensitive and specific compared to single marker analysis. The GH 2000 group in London identified two groups of potential markers in particular, IGF-I and IGFBPs, and markers of bone and collagen turnover and mineralization. From these markers, IGF-I and P-III-NP were chosen (Wallace et al., 2000).

IGF-I has a number of properties which make it an ideal biochemical marker of GH usage. It has little diurnal or day-to-day variation (See and Wilson, 1988), rises post GH administration in a dose-dependent uniform fashion by 2 to 4 fold (low and high dose respectively), has a low basal scatter (Dall et al., 2000, Holt, 2010) and minimal change with exercise. IGFBP-3 has a less uniform rise dose dependent curve with GH administration than IGF-I (Kniess et al., 2003). Total IGF-I is more useful as free IGF-I is less GH responsive (Longobardi et al., 2000).

1.4.9.2.1 Insulin like growth factor-I

1.4.9.2.1.1 Structure and synthesis

Insulin like growth factor-I (IGF-I) is a 70 amino acid straight chain peptide and has structural homology with insulin. In 1963 Froesch demonstrated that most insulin-like activity in humans was not determined by insulin and in studies involving insulin antibodies showed suppression of insulin-like activity by only 10%; the agents responsible for this phenomenon are now known as IGF-I and IGF-II (Froesch et al., 1963). The IGF receptor binds insulin but with less affinity that IGF and the reverse is true of the insulin receptor (Megyesi et al., 1975). IGF-I consists of 4 domains; A and B domains in common with the insulin

molecule, C similar to proinsulin and a unique carboxyterminus(Blundell et al., 1978).

The IGF-I gene is located on the long arm of chromosome 12 (Sussenbach et al., 1991). After cellular mRNA production two propeptides are cleaved to produce the mature IGF-I molecule.

IGF-I is produced ubiquitously throughout the body at a rate of approximately 10mg per day. Production in the intrahepatic tissues is largely regulated by GH but extrahepatic production is also controlled by a combination of GH, thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), insulin and local growth factors, such as platelet-derived growth factor (Holly and Wass, 1989). None of its cellular sources have storage capabilities for IGF-I, hence around 95% of IGF-I circulate in the bound form. The majority of circulating IGF-I is in a 150 kDa ternary complex binding IGF-I, IGFBP3 orIGFBP5, 85kDa glycoprotein acid labile subunit (ALS) (Baxter, 1994, Rechler, 1993, Ooi, 1999). Remaining circulating IGF-I is bound to IGFBPs 1, 2, 4, or 6 in a 50 kDa compound that are incompletely saturated with IGF-I molecules (Jones, 1995, Stewart, 1996).

IGF-I is anabolic in action but this is dependent on the availability of insulin and amino acid supplies. In states of plentiful amino acids and insulin supply, there is stimulation of protein synthesis and inhibition of breakdown with IGF-I infusion but no evidence of this anabolism in states of deficient amino acids or insulin (Jacob R, 1996).

A raised BMI has been shown to be associated with a depressed IGF-I but not IGFBP-3 (Gapstur, 2004). However, some studies have shown free serum IGF-I and BMI to have a positive correlation (Katz et al., 2000). This is thought to be related to the reduced binding capacity of IGF-I by decreased IGFBP3 levels due to raised insulin levels seen in obesity (Gapstur, 2004). IGF-I was reported to be

inversely related to visceral adiposity (Poehlman, 1990) but conversely studies have reported no association between subcutaneous fat, waist circumference, visceral adiposity and IGF-I levels (Landin-Wilhelmsen et al., 1994, Goodman-Gruen and Barrett-Connor, 1997). This is likely to be related to lower GH levels seen in obesity (Gapstur, 2004, Chang et al., 2002). Insulin is inversely related to IGFBP1 and IGFBP 2 levels but positively correlates with serum free IGF-I levels (Musey et al., 1993, Krajcik et al., 2002).

1.4.9.2.1.2 Insulin like growth factor binding proteins

IGFBP1 is the only acute regulator of IGF-I bioavailability and it is hepatic in origin. It has a role in transportation of IGF-I between the circulation and interstitial space and controls free IGF-I levels at metabolically active sites, most notably its growth promoting actions. However IGFBP 1 has considerable interindividual variation and rises after exercise (Suikarri et al., 1989, Koistinen et al., 1996).

IGFBP2 is present in serum and foetal cerebrospinal fluid and is a 34 kilodalton protein. It is inhibitory to the actions of IGF-I and IGF-II.

IGFBP3 is the most abundant of all the IGFBPs in the serum and forms a complex with IGF-I, IGF-II and acid labile subunit. Proteolysis of this complex is necessary to liberate IGF-I and allow it to become metabolically active. Production is stimulated by a protein diet, GH, IGF-I and insulin (Clemmons et al., 1989). It has thus transporting and storage functions but also a role in modulating IGF-I stimulated cell growth (Conover, 1992a) as well as tissue repair capabilities (Baxter, 1994). IGFBP3 rises with GH administration but has a less

uniform and dose dependent curve than IGF-I (Kniess et al., 2003). The assays for IGFBP3 provide technical facility and do not require separation of IGFBP3 from the IGF-I molecule complex as mentioned previously. In vivo circulating levels of IGFBP3 are of the range 1–5mg/L allowing ease of detection and should allow good assay sensitivity (See and Wilson, 1988). IGFBP3 levels are less effected by age and nutritional status than IGF-I reflecting the stabilising effect of IGF-II with which it is complexed (Thorner et al., 1998).

IGFBP-4 is found primarily in liver tissue and involved in IGF transportation and is inhibitory to the actions of IGF-I (Jones, 1995).

IGFBP-5 is produced in the kidney, IGFBP-6 is present in cerebrospinal fluid and greater binding affinity for IGF-II.

IGF-I and IGFBP-3 are known to correlate positively with height in children (Rasmussen, 1995) but not in Caucasian adults (Stokes et al., 2004). However, some publications demonstrate a correlation between height and IGF-I in Japanese men (Teramukai et al., 2002) but to a lesser extent in black men (Gapstur, 2004). IGF-I levels were lower and IGFBP-3 higher in women compared to men.

1.4.9.2.1.3 IGF-I and glucose homeostasis

IGF-I is insulin like in structure, post translational receptor signalling, and actions (Murphy, 2003). In terms of glucose metabolism it is less potent than insulin with lower affinity for insulin receptors. IGF-I and IGF-II are present in the circulation in greater quantities but as both are largely bound to IGFBPs, they have less biological effect (Hintz et al., 1972, Burgi et al., 1996). Infusion of IGF-I leads to

enhancement of peripheral glucose uptake in excess of that of insulin but it has less effect than insulin on suppression of hepatic glucose output (Laager et al., 1993). This may be by virtue of the greater density of IGF receptors in muscle and peripheral tissue compared to insulin receptors, differences in post receptor signalling and IGFBPs peripherally as well as hybrid receptors which have structural similarity to insulin receptors and are peripherally located but preferentially bind IGF-I (Murphy, 2003, Siddle et al., 1994). Hybrid IGF/insulin receptors are present in abundance in skeletal muscle and more functionally similar to insulin receptors, but preferentially bind IGF-I. These receptors are increased in the hyperinsulinaemic environment of type 2 diabetes while insulin receptors are down-regulated (Federica, 1998).

1.4.9.2.1.4 IGF I, GH and obesity

Obese individuals have higher IGF-I especially in BMI range of 30 to 35 kg/m^2(Schneider, 2006). Conventional wisdom is that the hyperinsulaemia of obesity leads to impaired hepatic IGFBP production and so a higher percentage of free IGF-I is available which feeds back to inhibit GH secretion (Frystyk, 1999). However, concerns have emerged about the reliability of the earlier chromatographic and immunoradiometric methods of free IGF measurement (Frystyk, 2004). More recent studies using ultrafiltration methods using IRMA suggest that there has been overestimation of the free components of IGF-I (Rasmussen, 1995) and weight loss was not shown to alter free IGF even though it normalised GH levels.

1.4.9.2.2 N-terminal pro-peptide of type III collagen

The effect of GH on bone is also evident by looking at changes in collagen markers after GH administration. N-terminal pro-peptide of type III collagen (P-III-NP) is secreted by fibroblasts. In clinical practice P-III-NP is a marker of the metabolic turnover of collagen and is raised in conditions of hormonally induced

growth (Trivedi et al., 1989), hepatic fibrosis, lung fibrosis, ovarian and colonic neoplasia (Böger, 1996), alcoholic liver disease and is reduced in ErhlosDanlos syndrome (Steinmann et al., 1989). It is marker of reocclusion post thrombolysis in the case of anterior wall myocardial infarction and is used as an important prognostic indicator. It is both a marker of wound healing in cardiac transplantation as well as a marker of transplant rejection (Conover, 1992b).

1.4.9.2.2.1 Effects of growth hormone on bone metabolism

GH has direct effect on bone growth. GH affects bone metabolism via GH receptors on osteoblasts and local production of IGF-I (Sjogren et al., 2001). It stimulates proliferation of cartilage in the growing epiphyseal plate, stimulates linear growth, increased bone mass and mineral content and the number of bone modelling units (Nelson, 2006). Green et al proposed a dual-effector hypothesis in bone growth; GH binds to membrane receptors in the prechondrocyte stimulating differentiation into an early chondrocyte, which in turn secretes IGF-I locally to further stimulate differentiation into the mature chondrocyte form (Koller et al., 1998). Injection of GH into the epiphyseal plate of hypophysectimised rats induces limb growth. GH also influences bone metabolism through increased calcium and 1,25 vitamin D absorption from the intestine (Isgaard et al., 1999, Sjogren et al., 2001, Healy et al., 2003). This is mediated via GH effects on renal 25(OH) D 1-α hydroxylase (Halloran and Spencer, 1988).

Patients with osteoporosis have been shown to have lower IGF-I concentrations with normal GH secretion and it is postulated that there is hepatic GH resistance in such patients (Patel, 2005). There is evidence of osteopenia in adults with GH deficiency and this can be reversed by rhGH therapy (Gomez et al., 2000). IGF-I is considered a marker of bone mineral density in men with osteoporosis (Ljunghall et al., 1992).

Both acute and endurance exercise induces a biphasic bone reaction, with an initial phase of bone resorption followed by a period of bone formation. Both mechanical stress and endocrine factors such as endogenous GH and testosterone are important in this re-modelling process (Conover, 1996).

1.4.9.2.2.2 Use of bone markers for detection of growth hormone

Bone and soft tissue markers can be used to detect GH use. N-terminal pro-peptide of type III collagen (P-III-NP) is a marker of the formation of type 3 collagen largely in soft tissues and exhibits little day-to-day, diurnal or gender variation in basal concentrations and rises in a dose dependent fashion following GH administration (Wallace et al., 2000). It rises following injury but not in sufficient quantities or duration to effect the detection of GH (Erotokritou-Mulligan, 2008). C-terminal propeptide of collagen type I (PICP) rises with GH use and is involved in the early scaffold of collagen formation and callus formation with bone remodelling. Carboxyterminal cross-linked telopeptide of type I collagen (ICTP) is a marker of bone resorption. It has a short phase rise of 9.7% particularly in males (Dall et al., 2000).

Markers of bone mineralisation include osteocalcin and bone alkaline phosphatase (table 1.4). Osteocalcin concentrations rise is non dose dependent manner after GH with and men having a greater magnitude rise (Longobardi et al., 2000). There is a small rise 60 minutes post exercise. Bone specific alkaline phosphatase (ALS) is posture dependent and rises slowly after GH administration (Wallace et al., 1999). Leptin is an adipocytes hormone and rises response to GH but changes are too variable for reliable use (Kniess et al., 2003).

Marker	Disappearance T½ (hours)
Osteocalcin	770
C-terminal propeptide of collagen type III	433
N-terminal pro-peptide of type III collagen	696
Carboxyterminal cross-linked telopeptide of type I collagen	248
IGF-I	89.5
IGFBP3	179
ALS	119

Table 1.4 Disappearance half-lives of markers of GH.

(Wallace et al., 2000, Longobardi et al., 2000).

1.4.9.3 Discriminant function analysis

A number of equations have been developed to improve the GH detection methods. Use of a single marker does not convey sufficient specificity to detect rhGH abuse and so a combination of markers is used in discriminant function analysis to improve sensitivity and specificity.

The GH 2000 team developed the marker method for the detection of GH abuse in sport. They looked at a number of possible biochemical markers but IGF-I and P-III-NP were found to be the most discriminatory when used in conjunction with

the discriminant function analysis formulae which they developed. The formulae are gender specific to allow for the gender differences in response to GH. They also include an age term to reflect the fall of the markers with age. They were developed during their double blind randomised controlled GH administration trial (Dall et al., 2000, Longobardi et al., 2000) and calibrated against an elite athlete population studied in cross section (Healy, 2005)

Male score = -6.586 + 2.905 X log (P-III-NP) + 2.1 X log (IGF-I) – 101.737/age

Female score = -8.459 + 2.454 X log (P-III-NP) + 2.195 X log (IGF-I) – 77.666/age

The mean normal score is 0. The detection cut off in elite athletes has been set at 3.7 which is equivalent to a false positive rate of approximately 1 in 10,000. This is higher than the values used in medicine because a lower false positive rate is needed in view of the serious implications of a positive test.

Kicman advocated the use of the ratio of IGF-I to IGFBP-2, and IGFBP-3 to IGFBP-2 (Kicman et al., 1997). There is a significant elevation of the product of IGF-I x P-III-NP and IGF-I x IGFBP-3 in a GH administration study (Kniess et al., 2003).

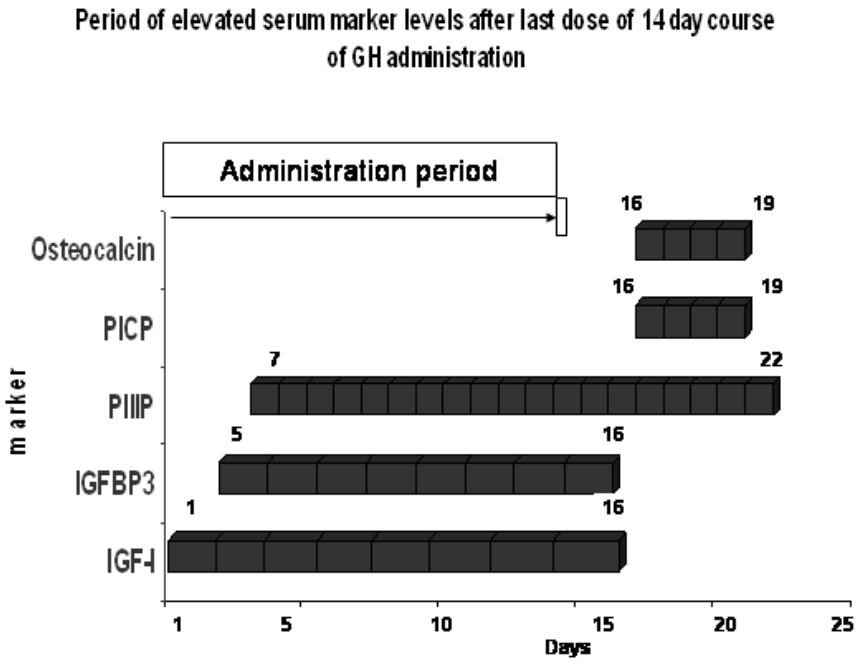


Figure 1.3. Period of elevated serum marker levels after the last dose of a 28-day course of rhGH.

(Kniess et al., 2003).

1.4.9.4 Creation of Appropriate Reference Ranges for GH detection

A positive drug test in the sporting world can be career ending and it is therefore imperative that the false positive rate of all antidoping tests be lower than the acceptable rate applied to current medical investigation of 2SD from the mean. With 2SD, 5% of the “normal” population lies outside the range. WADA has accepted a false positive rate of 1 in 10,000 tests.

Setting the “normal ranges” for testing results needs to be cognisant of false positive results, and take into account natural differences in GH and GH markers between athletes and the non athletic population, as well as differing sporting disciplines and the rise in GH and its markers seen after acute and chronic exercise.

Total IGF-I and IGFBP-1 rise post exertion unlike free IGF-I (Wallace et al., 1999). Bone remodelling is also seen after long term resistance training but less so with endurance sports. In the shorter term, exercise induces a metabolic acidosis leading to stimulation of osteoclasts and inhibition of osteoblastic activity (Woitge et al., 1998). Post competition levels of GH markers in elite athletes are different from standard reference ranges (Erotokritou-Mulligan, 2009, Healy, 2005)

Similarly any reference range must be gender specific and allow for the lower GH peaks and higher troughs seen in women and relative GH resistance (See and Wilson, 1988, Nussey and Whitehead, 2001).

GH secretion rises through childhood and early adulthood and there is a peak at puberty. It is important that pubertal staging be recorded and applied to tests in adolescent athletes (Guha, 2010). Subsequently GH and IGF-I and IGFBP3 declines by approximately 14% per decade in both athletic and non athletic populations (Corpas et al., 1993). Differences between sporting disciplines in GH markers is largely accounted for by body habitus, age of competitors, and gender of competitors (McHugh, 2005b).

1.4.9.5 Standardisation of assays

Standardisation of assays is crucial for GH abuse detection and there are calls for WADA to develop its own assay to avoid heterogeneity. There are a number of commercial assays currently available and use different dissociation methods, calibrants, antibodies and have differing normal ranges and detection rates (Banfi et al., 1990, Sjogren et al., 2001). In this study samples had to be reassayed and conversion factors had to be determined in order to compare the GH-2000 data to the GH-2004 data. A comparison of IGF-I assays (Nichols RIA, Mediagnost RIA, R&D ELISA) demonstrated a variability due to IGF-I dissociation procedure, calibration standards, IGF-I concentrations, and antibody heterogeneity (Abellan et al., 2005).

High affinity antibodies should be used wherever possible as IGFBPs have a similar affinity and compete with conventional antibodies. IGFBPs should be dissociated and separated from the IGF-I prior to assay, e.g. by acidification (Deyssig, 1993). Acid ethanol extraction followed by a cryo- precipitation is a refinement of the size exclusion method giving a considerably improved IGF-I recovery of 90-95%.

1.5 Ethnicity

1.5.1 Principles

Subjects in this study were non Caucasian because the study was funded to determine the validity of a test for GH abuse among non Caucasian athletes. However, the term race is a socio-cultural concept with no scientific basis (Witzig, 1996) and was constructed on the basis of determinants such as skin colour, geographical origins, and language. It has no fixed definition in the literature with the Oxford dictionary listing 5 definitions. Ethnicity has been defined as “group of

people within a cultural system who desire or are given special status based on traits such as religion, culture, language, or appearance" (Thomas, 1993). Some sociologists today view it as a method of perpetuating racism and antisocial feelings. The term ethnicity allows for more sociocultural influences and is generally preferred but they are both sociological constructs and subject to ethnocentric biases" (Senior and Bhopal, 1994). A declaration statement by the American Association of Physical Anthropology in 1996 (American Association of Physical Anthropology, 1996) was as follows;

"pure races, in the sense of genetically homogenous populations do not exist in the human species today, nor is there any evidence that they have ever existed in the past."

It can be seen how tenuous the subdivisions truly are by assessing their historical perspective. In 1758 Von Linne was the first author to classify Homo Sapiens in a manner similar to animals (Von Linne, 1956). He classified humans into races as follows:

Europeans ; "fair…gently, acute, inventive… governed by laws"

Americans; "copper-coloured…obstinate…content, free…regulated by customs"

Asiatic; "sooty…severe, haughty, covetous…governed by opinions"

African; "black…crafty, indolent, negligent…governed by caprice"

In this study ethnic groupings were self-selected by the volunteer from the following; Afro-Caribbean, Indo-Asian, Oriental, Mixed race and details of the ethnic origin of parents and grandparents were recorded in order to ascribe groupings more accurately for the purposes of analyses.

It is crucial to define racial/ethnic subdivisions in all scientific or epidemiological reporting. In the U.S.A. the term Asian is used to refer to an Oriental population, but in the UK, Oriental are referred to as Oriental and Asians are regarded as individuals from India, Pakistan and Bangladesh (Witzig, 1996). This highlights

the importance of defining exactly what constitutes each race and terminology for race. The 1990 Census in the US reported 250 self-selected ethnic groups (U.S. Dept of Commerce, 1992).

Approximately 0.012% of genetic heterogeneity can be attributed to race (Pate et al., 1995). Within a race there can be up to 85% variation in genetic makeup (Rose et al., 1984). Grouping by colour does not allow for changes in skin pigmentation throughout life (Rensberger, 1990), groupings by geography do not take migratory patterns into account, and groupings by language are only valid if language is culture-specific (King, 1981). Descendants of migrants do not necessarily maintain genetic homogeneity with the migrant population because of natural mutations and if interbreeding occurs between populations the descendants are not genetically similar to either population (Cavalli-Sforza and Feldman).

For the purposes of this study, ethnicity was self-reported into one of the following categorises: Afro-Caribbean, Indo-Asian (individuals with ancestry from Indian subcontinent or the Middle East), Oriental, and mixed race. Details were recorded of ethnic origin of the subject, and that of their parents and grandparents. Nationality was also recorded. The nationality and country of residence was recorded to determine the sociocultural origin of the subject.

1.6 Effects of ageing on growth hormone

The pattern of GH secretion throughout life is maintained but the amplitude of secretion is highest during late puberty and the neonatal period. There is an age related decline in GH levels with a loss of 14 % per decade of life and thus by 60 years of age the average person has GH levels equivalent to that of a young GH deficient adult. The age related decline in GH has been termed the somatopause. The state of transient insulin resistance of puberty is attributed

largely to the effects of the increase in GH secretion at this time. Reduction in the secretion of IGF-I and IGF binding protein-3 (IGFBP-3) follows the fall in GH (Corpas et al., 1993).

1.6.1 Performance enhancement in ageing adults

There is an age related decline in GH concentrations and restoration of serum levels, or supraphysiological levels, could potentially confer advantages in body strength and performance in elderly individuals. Rudman et al showed no improvement in strength or functional ability despite improved increased lean body mass, reduced body fat, improved skin thickness and bone density after rhGH of 6 months duration alone or in combination with exercise training (Rudman et al., 1990, Papadakis et al., 1996, Marcus, 1990, Taaffe, 1994, Taaffe, 1996). Combination therapy with testosterone has been shown to result in minor increases in muscle strength, lean body mass and exercise capacity (VO_2 max) after 26 weeks therapy (Blackman, 2002). Another trial titrating to physiological levels of GH and testosterone demonstrated synergistic effects with increased muscle mass, reduced body fat and improved exercise capacity in the combination group with increased lean body mass in the group treated with GH alone (Giannoulis, 2006, Blackman, 2002).

1.7 Effects of gender on growth hormone

There are gender related differences in body fat distribution and the incidence of cardiovascular disease which may be in part attributable to the differences in GH patterns that exist between the sexes. Women are relatively GH resistant when compared to men and have lower peaks of GH than men, higher resting GH and require higher replacement doses to induce clinical and biochemical improvement in states of GH deficiency (Møller et al., 1996). Studies of waist to hip ratios demonstrate a greater reduction in men after rhGH administration than in women (Bengtsson et al., 1999). GH deficient women required higher doses of rhGH per kilogram in order to reach target therapeutic IGF-I levels indicating

lower IGF-I responsiveness among women (Cuneo et al., 1998, Johansson et al., 1999, Drake et al., 1998). Women with GHD require one third to one half more GH replacement than men (Drake et al., 1998).

Women have a lower IGF-I concentration for a given GH concentration in acromegaly and in GH administration studies (Parkinson et al., 2001, Wallace et al., 1999) and administration of GH exogenous could be detected by IGF-I alone in 86% of men but only 50% of women which has implications for the detection of illicit GH use in the sporting community (Dall et al., 2000). Women have a reduced GH suppression on OGTT, and up to 3 times higher mean GH concentrations over 24 hours (Chapman, 1994, Pincus, 1996, van den Berg, 1996). There appears to be a hormonal basis for the gender difference and administration of oral oestrogen after the menopause lowers basal IGF-I, increases GH and reduces IGF-I rise post GH administration (Lieberman, 1994, Weissberger, 1991). There is a doubling of serum GH in the periovulatory stage of the menstrual cycle but no difference in the follicular phase (Weissberger, 1991, Faria, 1992).

1.8 Recombinant GH

1.8.1 Properties of recombinant GH

Bioengineered recombinant GH is currently available commercially with an amino acid sequence identical to endogenous 22kDa GH and replaces cadaveric pituitary GH which was available for clinical use up to 1983 but is still available on the black market. Unscrupulous commercial companies market cadaveric and animal pituitary GH with their ensuing risk of cross infection most notably

HIV or Creutzfeld Jacob disease; prion concentrations are naturally highest in the pituitary gland (Mills, 1990).

Administration is via a subcutaneous or intramuscular injection once daily. Nightly injections do not mimic normal GH pulses (Lucidi et al., 1998).

Clinical indications for legitimate usage include

hypopituitary states,

isolated GH deficiency,

short stature children,

gonadal dysgenesis,

chronic renal insufficiency in children,

The anabolic properties of GH have led to considerable interest in use of GH in the elderly and catabolic patient populations. Recent clinical trials report benefits in muscle wasting, debilitation and recovery as well as perception of wellbeing associated with ageing and in intensive care unit (Barle, 2001). Administration of GH to the elderly population is associated with a 10% increase in lean body mass, a 15% reduction in overall adiposity and a 2% increase in bone mineral density as well as improvements in cardiovascular function, immune function, and cognition (Lamberts et al., 1997, Papadakis et al., 1996, Degerblad et al., 1990, Frayne, 1996).

Depot preparations are no longer available through official channels, e.g. Nutropin depot (Colao and W., 2004).

Contraindications to administration include the presence of malignancy, serious illness post renal transplant, growth promotion in children with closed epiphyses and in pregnancy (Mehta, 2000) .

1.8.2 Pharmacokinetics of Saizen®

Saizen® is a product of Serono Merck and contains recombinant human growth hormone produced in a genetically transformed murine cell line with a GH gene insertion. The bioavailability of Saizen following subcutaneous administration in healthy subjects is 60 to 90% with a volume of distribution in steady state of 7L, with a total metabolic clearance rate of approximately 15L/hr with negligible renal clearance and elimination half-life of 2 to 4 hours. Serum concentrations reach maximum levels at 4 hours and return to baseline at 24 hours. Subcutaneous administration provides a peak GH level at 3.5 to 4 hours post administration. Recommended product storage following reconstitution is in a refrigerator at 2 to 8C for a maximum of 28 days. Products not reconstituted can be stored for up to 3 years at room temperature (Serono).

1.8.3 Adverse events of exogenous GH administration.

There have been a number of multicentre trials examining the effects of rhGH replacement in GHDA. The KIMS study (Pharmacia, Upjohn International Metabolic Database) was a pharmaco-epidemiological study of 6,000 patients

given rhGH post marketing with an average 2.8 years follow-up. 32 developed overt diabetes, but notably the majority of these had independent diabetes risk factors, such as family history, obesity, impaired glucose tolerance in the pre treatment phase and the diabetes rates were consistent with the age-related increase in the incidence of diabetes in the general population. Eli Lilly published HypoCSS (Hypopituitary Control and Complications Study) which reviewed 1881 rhGH patients over a mean duration of two years and found no increased incidence of diabetes compared to the untreated GHDA controls (Harman, 2001).

Further analysis of KIMS data in GHDA who are over 65 years of age has demonstrated that there are improvements in LDL and total cholesterol in both genders. Men showed reductions in diastolic blood pressure and waist/hip ratio unlike age matched women (Monson JP, 2003).

Adverse events can be classified as follows;

Metabolic; mild transient fluid retention manifesting as peripheral oedema

Musculoskeletal; arthralgia, myalgia, growth deceleration due to high affinity antibody to GH formation occurs in 0.04% of children (Blethen et al., 1996).

Endocrine; gynaecomastia, pancreatitis

Dermatological; mild skin rashes, especially at injection sites.

Tumour formation is rare. One study of 2922 cases representing 9004 patient years reported no cases of *de novo* tumour formation but a recurrence rate of 1.1%/patient year for leukaemia in children previously treated, 2.2%/patient year for solid cranial tumours and 3.8%/patient year for craniopharyngiomas (Cowell and Dietsch, 1995).

Incidence of reported side effects of rhGH administration Symptom	% of patients experiencing this symptom (total = 1034)
Headache	3
Parasthesia	1.3
Dizziness	0.77
Migraine	0.54
Convulsions (of whom 4 out of the 6 patients had pre-existing epilepsy)	0.46
Carpal tunnel syndrome	0.31
Tremor	0.15
Leg cramps	0.15
Fatigue	0.69
Depression	0.54
Asthenia	0.31
Anxiety	0.23
Insomnia	0.23
Emotional lability	0.15
Increased sweating	1.07
Decreased body weight	0.31
Flushing	0.23
Tendonitis	0.23
Increased body weight	0.15
Sleep apnoea	0.15
Snoring	0.15
Dryness of the mouth	0.08

Table 1.2 Incidence of adverse events in patients receiving GH during the KIMS study of 1034 patients followed over 818 patient years. (Bengtsson et al., 1999).

1.9 Purpose of the study

The marker method of detection of GH abuse in athletes was shown to be both sensitive and specific for use in the detection of GH abuse among athletes and involved the measurement of IGF-I and P-III-NP and the use of equations developed by the GH2000 team in London but there were questions raised at the IOC committee meeting as to the validity of this marker method in an ethnically diverse population. This study aimed to determine if there were any ethnic differences in these markers and thus in use of the marker method and these equations.

In addition measures of sporting performance were assessed to determine if GH conferred any performance enhancing benefit on athletes as has been anecdotally reported. Measures included exercise capacity,

Much of the research in the literature involves GH administration to GHDA individuals. Studies also involve assessment of acromegaly as a state of GH excess but in both GHDA and acromegaly the hormonal abnormality is likely to be long standing prior to presentation. This study provides an opportunity to study the effects of administration in young healthy individuals and provide some insight into the early changes in changes in body composition and insulin resistance in response to GH administration.

1.10 Aims of the study

The aims of this study are as follows:

To determine if the reference ranges for IGF-I and P-III-NP for elite athletes from non-white European ethnic groups also apply to white European elite athletes.

To determine if IGF-I and P-III-NP concentrations differ between non Caucasian athletes and Caucasian athletes after GH administration and determine if any differences are of sufficient magnitude to invalidate the GH2000 test/formulae for detection of GH abuse among athletes.

To determine if GH could enhance potential markers of performance by inducing changes in body composition, substrate utilisation, free fatty acids, and exercise capacity.

To assess the effects of 28 days of rhGH administration in healthy young adults on insulin resistance determined by glucose and insulin concentrations during rapid sampling OGTT, HOMA, QUICKI, insulin to glucose ratio, and adiponectin concentrations.

To determine if changes in insulin resistance are induced by changes in body fat distribution mediated by adiponectin or NEFA.

1.11 Hypothesis

This thesis hypothesises that

There is no ethnic difference in the response of IGF-I and P-III-NP to exogenous GH administration in athletes.

The equations developed by the GH2000 team are valid in non Caucasian athletes to detect GH abuse.

Short term supraphysiological GH administration leads to changes in parameters of sporting performance, i.e. body fat reduction, increased exercise capacity, a fall in carbohydrate utilisation and rise in fat utilisation, increased fatty acid concentrations, and a fall in urinary nitrogen excretion.

Short term supraphysiological GH administration in healthy volunteers will reduce insulin sensitivity by changes in body fat mediated by free fatty acids and/or adiponectin.

Chapter 2

Principles and Methodology

This chapter will outline the principles and methodology used in many aspects of this study. Methodology unique to a specific study will be detailed in the methodology section of that individual chapter.

2.1 Overview of procedure

Subjects were recruited locally by means of posters, exhibition stands at local gyms, sporting or ethnic-specific events, clubhouses, press releases, television documentaries by the Discovery Channel, BBC (Meridian), Channel 4 and Southampton T.V. We also contributed articles to the local press including The ECHO Newspaper and “AND Magazine”. Posters were displayed in local and university gym halls and the candidate attended university freshers’fayres, sporting events and training sessions of university teams.

Recruitment was extremely challenging in view of the predominantly Caucasian population in Hampshire and the transient and often unreliable nature of the student population, delays with ethical approval, distribution delays in the GH supply company, and personnel changes within the team mid-project. Legal difficulties regarding ownership of any possible commercialisation after the project led to a 6 month delay in obtaining the drug/placebo during which time 5 subjects dropped out. The funding for this project was provided by the United States Antidoping Agency (USADA) in order to develop the validity of using IGF-I and P-III-NP as a test for rhGH abuse among non Caucasian athletes and it was within that setting that this project was undertaken.

Subjects were requested to attend the Southampton Wellcome Trust Clinical Research Facility (WTCRF) after a 12 hour overnight fast and in a rested and relaxed state. They were specifically requested not to use an active method of transport, e.g. cycling.

An intravenous cannula was inserted into the antecubital fossa and a further 45 minutes was allowed to elapse. A baseline venous blood sample was collected from the cannula into a fluoride oxalate tube (1ml) for glucose analysis, a lithium heparin sample (5 mls) for insulin and non-esterified fatty acid (NEFA), and a clotted sample (5 ml) for adiponectin, IGF-I and P-III-NP.

Any blockage in the cannula was treated with normal saline flushes initially and with intravenous heparin flushes (Hepsal) if absolutely necessary. If heparin was used, the cannula was flushed with normal saline to minimize sample contact with heparin because of its effects on NEFA. Only water consumption was allowed throughout the OGTT.

75gms of glucose (Polycal®) was given orally to the volunteer and further venous blood samples were taken for glucose measurement from the cannula at 5, 10, 15, 20, 25, 30, 35, 40, 60, 90,120 minutes post glucose administration and insulin at 0, 30, 60, 90, 120 minutes, and NEFA at 5, 10, 15, 20, 30, 40, 60, 90, 120 minutes.

Subjects were asked to void their bladder and all urine was collected, measured and a 30mls sample retained for urinary nitrogen analysis in a universal container. This was performed pre OGTT, and again after the OGTT.

Subjects then underwent indirect calorimetry under a ventilatory hood before glucose administration (time 0) and again at 60 and 120 minutes post glucose administration during the OGTT.

Subjects were then asked to perform a running treadmill test according to a Bruce protocol to exhaustion with measurement of VO_2 maximum by indirect calorimetry. Verbal encouragement was given by the candidate.

Details of weight, gender, and unique candidate identifier number were sent to SeronoMerck™ for randomisation and a dosing pack was subsequently returned to the candidate. The candidate then gave the pack to the subject and instructed the subject in administration of the GH or placebo. The subject commenced nightly subcutaneous injections and returned on day 7, 14 and 21 for IGF-I and P-III-NP sampling by venesection, recording of side effects and then returned on day 28 for a repeat of the tests performed on day 0.

Serum IGF-I and P-III-NP concentrations were taken on day 0, 7, 14, 21, 28, 33, 42, 84. No other data were collected after day 28.

The procedure is represented diagrammatically in figure 2.1 and 2.2.

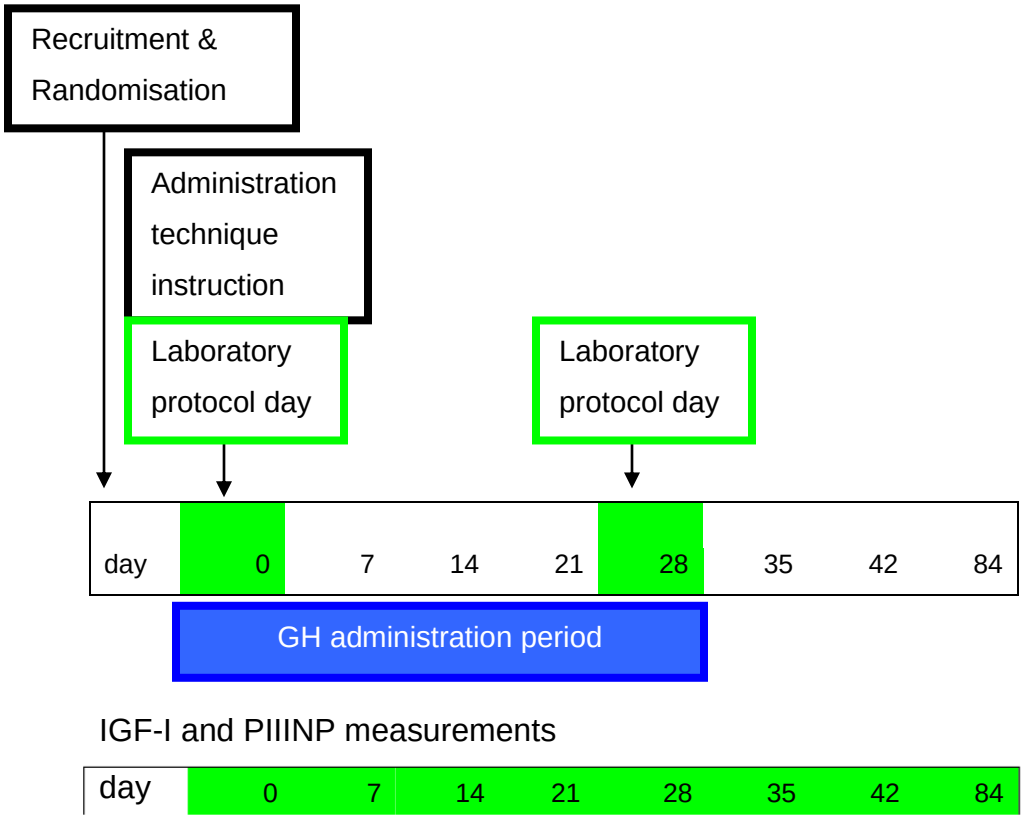


Figure 2.1 Diagrammatic representation of study protocol.

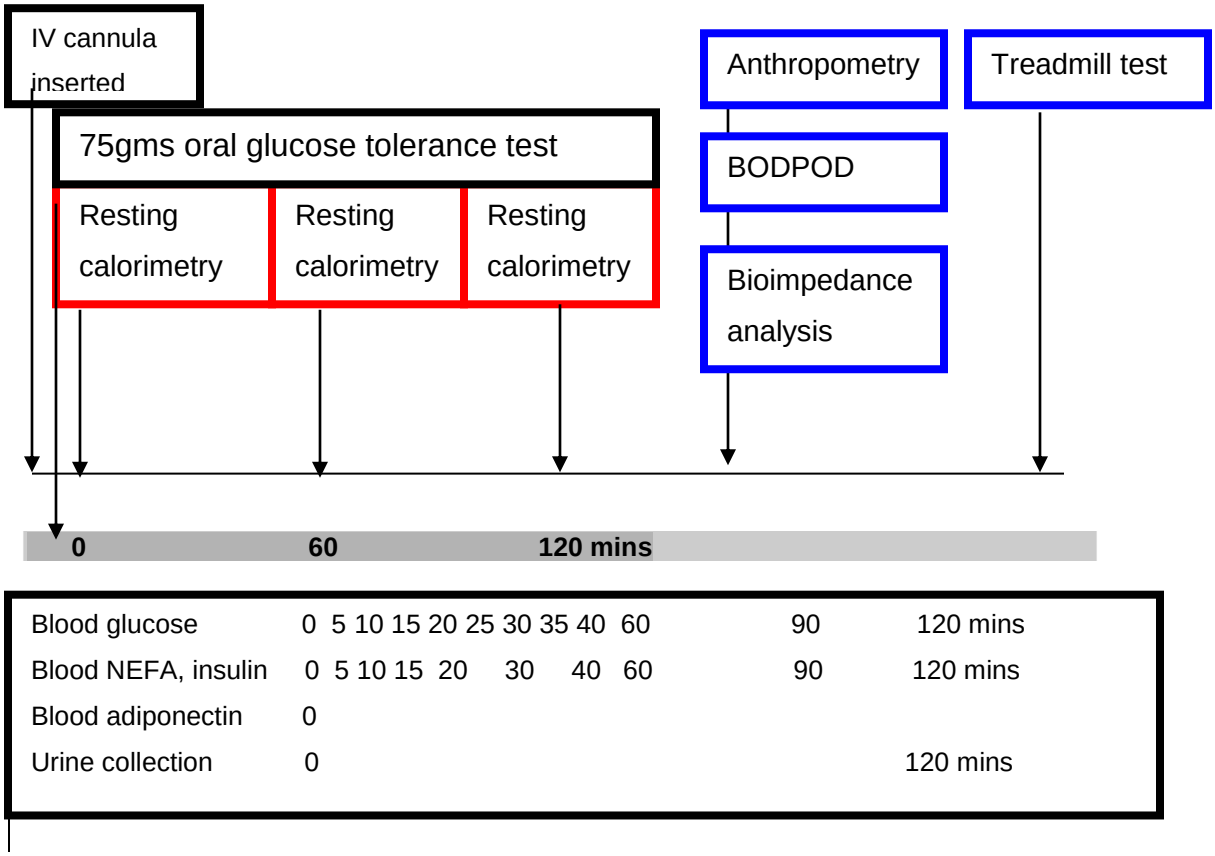


Figure 2.2 Diagrammatic representation of laboratory day study protocol.

2.2 Subjects

66 subjects were recruited in total but only 36 completed the study. This discrepancy was caused by problems with the supply of growth hormone resulting in a number of delays of several months' duration after which subjects were no longer in a position to start the study, often for personal reasons, college holidays or having finished their studies and left Southampton. Recruitment difficulties are discussed further in chapter 7.

No subjects discontinued the drug or placebo for side effects or any other reason. Four subjects were excluded from the analyses because of protocol violations; there were unacceptable delays (>4 days) in the post GH assessment because of personal reasons of the subjects.

2.2.1 Inclusion criteria

In order to be included in the study subjects must fulfil the following criteria:

Non Caucasian or mixed race ethnic origin.

18 to 40 years of age.

Routinely participate regularly in sporting activities at least twice per week for 1 year. The duration of exercise must exceed 30 minutes. Details were recorded of the type of sporting activity undertaken, frequency and also the competitive level of the athlete: Club (level 6), local (level 5), regional (level 4), national (level 3), international (level 2) and Olympic (level 1).

2.2.2 Exclusion criteria

Subjects were excluded from the study if they had any of the following:

Pre-existing endocrine illness likely to affect the GH/IGF-I axis.

Any injury within the last 84 days of sufficient severity to cause the subject

to abstain from sporting activity for 3 days or more.

Pregnancy or failure to take reliable contraception.

2.3 Consent

All subjects gave signed informed consent in accordance with the regulations of the Southampton and Isle of Wight local Research and Ethics committee and the study was conducted within their guidelines and with prior approval. The study was regulated by the Research and Development Department of Southampton University Hospitals NHS Trust. Data were collected and stored in accordance with the UK Data Protection Act 1998.

2.4 Statistical analysis

Demographic data were compared between all groups using an unpaired t test after logarithmic transformation. Other variables were analysed using log transformed paired t tests comparing day 0 and day 28 values using SPSS 14.0® and a one way ANOVA comparing the treatment group with the placebo group where data were available for both groups. Results are presented as mean value ± standard error of mean (SEM).

This sample size for this project was chosen to determine with reasonable accuracy the size of any differences between age-adjusted mean GH scores for different ethnic groups. This would allow determination of whether different ethnic groups would require different cut-off points. It was part of the larger aim of the funded study to determine the validity of a test for GH abuse among athletes using IGF-I and P-III-NP. The calculated number of participants was 90. Difficulties arose in recruitment as cited earlier and the study did not reach the desired 90 volunteers and the study was underpowered.

2.5 Randomisation

Randomisation was performed by SeronoMerck™. Subject weight, gender and unique identifier were provided to SeronoMerck™ on enrolment. Randomization was performed at their head office and packs were forwarded to the candidate for use. No knowledge of groupings was provided to any other operatives in Southampton. The randomisation code was broken and details provided upon completion of the study and finalisation of the database. Subjects were randomly assigned to receive either placebo or GH in either low (0.1U/kg/day) or high doses (0.2U/kg/day). Packs received appeared identical in every way including visual appearance of pens, accessories, liquid in vials, dosing regimens (both had low and high dosing regimens) and labelling.

2.6 Candidate instruction

2.6.1 Overview of Subject Instruction Procedure and administration technique.

While attending the WTCRF on day 0 each individual was weighed and a candidate pack was ordered and upon receipt of same from SeronoMerck™ the candidate attended either in WTCRF or other convenient location and the subject was instructed in person by the candidate. Using a demonstration pen kit and placebo vials, subjects were instructed in the reconstitution of vials and powder, and subcutaneous administration techniques.

Subjects were instructed by the candidate in the technique of self-administration using the Click.easy®8mg pen provided by Serono Merck™. The device contains a pen and a reconstituting device. The Saizen® cartridge is the powdered vial marked Saizen® "powder for reconstitution" and the diluent vial contains a clear liquid.

The procedure was as follows:

Remove the Click.easy®8mg pen from the packaging, place vertically on a hard surface with the powdered Saizen® cartridge on the bottom and the diluent vial on top. Turn the diluent cartridge clockwise and it descends until the intervening needle punctures the powdered vial and the diluent is delivered into the powdered vial. Allow to stand for 2 minutes until the powder is completely dissolved. Invert the device (do not shake or it will foam) and the Saizen® cartridge is now on the top. Pull the diluent plunger downwards and the mixture now moves into the Saizen® cartridge. Remove excess air by pushing the plunger upwards and expelling air. Turn the device and separate the Saizen® cartridge. Discard and carefully dispose of the remaining device. Insert the reconstituted Saizen® cartridge into the pen device by unscrewing the mid-section and inserting the Saizen® cartridge into the hollow chamber. Reattach the chamber and attach a disposable needle to the end of the pen and replace the cap for safety.

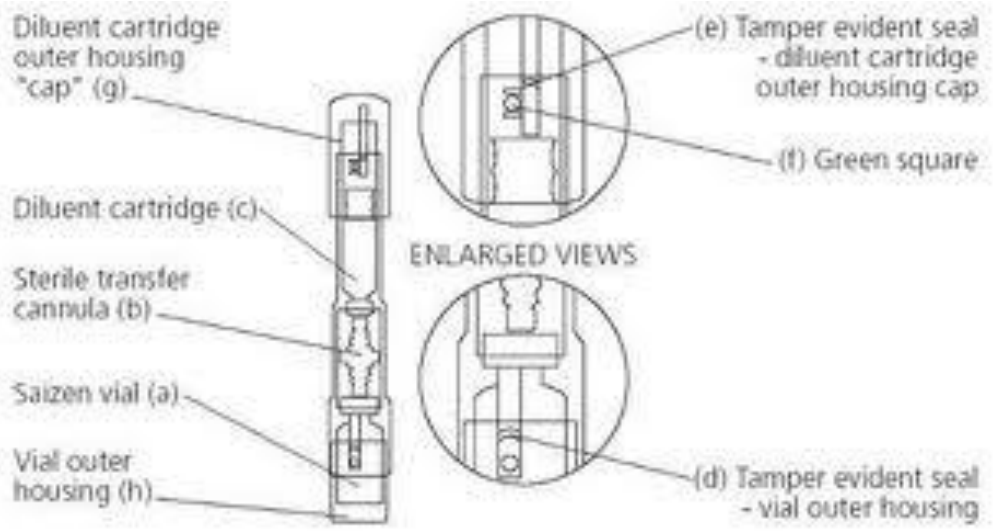


Figure 2.3 SaizenClick.easy; administration instruction diagram.

The device should be stored in a refrigerator at 4 to 8°C but may be removed up to 30 minutes before delivery to allow the solution to be at room temperature upon administration in order to minimise pain at the injection site.

Subjects performed a number of administrations witnessed by the candidate until deemed competent in self-administration. The candidate then provided packs to the subject and they were instructed to administer their dosing regimen nightly. GH/placebo was self-administered for 28 days.

2.7 Compliance with treatment

Compliance was assessed by direct questioning and requesting the subjects to return empty vials for counting at the end of the trial. This also allowed us to receive any unused vials and prevent any entering the open market. Problems with compliance assessment are discussed in chapter 7.

2.8 Body Composition Assessment

All assessments were undertaken adhering to the Wellcome Trust Clinical Research Facility (WT-CRF) standard operating protocols.

2.8.1 Height

One measurement of height was taken for each subject, using a wall mounted stadiometer (SECA™, model 220) when on-site in the Wellcome Trust Clinical Research Facility or using a portable stadiometer (Leicester Portable Stadiometer™) for off-site measurements. Please see figure 2.4. Both are validated every 6 months using standard measures conforming to weights and measures standards (U.K. and Paris). Subjects were asked to remove their shoes, stand on a platform placed on a flat surface with their feet together, asked

to “stand tall” with their arms by their sides and to relax their shoulders in order to minimise lumbar lordosis. Their head was placed in the Frankfurt plan, whereby the lower border of the orbit of the eye is on a horizontal plan with the upper margin of the external auditory meatus. The L-shaped head-piece was lowered onto the top of the subjects head and measurements were taken in centimetres (Cameron, 2000, Malozowski et al., 1993, Fidanza, 1991).

2.8.2 Weight

Subjects were weighed on an electronic scales (SECA™) placed on a flat surface, asked to remove outer clothing, have an empty bladder, stand still on the platform, stand in the centre of the platform, place their slightly feet apart and distribute weight evenly on both feet.

2.8.3 Body Mass Index (BMI)

BMI is the calculation of weight (kg)/ (height (m))^2 (Keys et al., 1972). It allows comparison between individuals and estimation of the appropriateness of weight for height.

2.8.3.1 Assumptions

BMI estimations make the assumption that all individuals have the same proportions of muscle, bone, water, etc. and this is not necessarily always the case. Individuals with a higher proportion of body weight coming from muscle, e.g. body builders, will have a higher BMI and be categorised in the obese range when in fact their fat content is not excessive. This is particularly important in assumptions made for muscular athletes.

Trunk-to-limb ratios differ among individuals and persons with shorter legs relative to their trunk height will have higher BMI.

2.8.4 Mid upper arm circumference

The subject was asked to stand as before, with their back facing the observer, arm flexed to 90º, and palm facing upwards. The tip of the acromium process was palpated and marked with a skin marker. A tape measure was dropped from this point to the horizontal plane of the olecranon process and the length noted. The tape measure was of non stretchable material, had a leader before the zero line and was checked 3 monthly for wear and tear leading to inaccuracies (Fidanza, 1991). A further mark was placed at the midpoint of this acromio-olecranon distance. The arm was then allowed to relax to the extended position and a tape placed around the circumference at the mid acromio-olecranon point with the tape measure at a right angle to the long axis of the arm. The tape was not held so tight so as to indent the skin. One measurement was taken to the nearest 0.1 centimetres and the value recorded (Fidanza, 1991, Callaway et al., 1988, Cameron, 2000).

2.8.5 Waist measurement

Waist measurements were taken with a plastic hand held measuring tape at the midpoint of the distance between the lowest rib and the iliac crest both palpated from the side of the subject. The tape is passed horizontally around the circumference. The measurement is made at the end of expiration with the subject relaxed (Fidanza, 1991). One measurement is taken to within 0.1 cm.

2.8.6 Hip measurements

The tape was placed horizontally around the subject. Measurement were started at the waist point and moved down sequentially with a measurement taken approximately every inch until the widest point is reached. The largest reading was recorded. Measurements were performed with only tight fitting shorts (Fidanza, 1991, Callaway et al., 1988).

2.8.7 Assessment of skinfold thickness

Anthropometry was performed on subjects and an estimation of body fat made using the Harpenden Calliper™ (Holtain Ltd, Bryberian, Crymmych, Pembrokeshire). The callipers have 0.2mm divisions and exert a constant pressure of 10g/mm³ and are held for 5 seconds (Dempster and Aitkens, 1995) and measurements are thus of compressed fat. The non-dominant side was used in all instances. The average of 3 measurements was taken for each site. If a measurement was greater than 10% above or below the other readings, the measurement was repeated twice more to ensure reproducibility.
Measurements were taken at 4 sites for inclusion in the equation. These sites are easily located with bony landmarks and where skin and fat is easily lifted from the underlying muscle; triceps, biceps, subscapularis, suprailiac.

2.8.7.1 Assumptions

Fat compressibility is constant – subcutaneous fat undergoes a two stage process during the process of skinfold measurement. Finger compression by the

observer induces greater compressive force than the subsequent 10g/mm² pressure of the calliper blade so the fat begins to slowly re-expand (Fidanza, 1991). There are gender differences in fat compressibility with women's fat having greater compressibility than that of men.

Skin thickness is negligible or a constant fraction of skinfold. In fact the dermis is not a constant fraction of the skinfold measurement but this finding is of undetermined significance (Fidanza, 1991).

Adipose tissue patterning is fixed. Sampling of only one area may lead to a misrepresentation of the subcutaneous cutaneous adipose tissue (SCAT) but areas are highly intercorrelated and so inclusion of more than 3 sites in the analysis does not necessarily reduce the error of the estimate (Fidanza, 1991).

Adipose tissue has a constant fat fraction. On post mortem evidence the fat fraction is usually 60 – 70% but varies according to weight, fat status, age, and gender (Fidanza, 1991). Adipose tissue in a middle aged man has a higher percentage of fat cells than a younger men.

A fixed proportion of internal to external fat. This was not confirmed on post mortem examination by Alexander 1964 (Alexander, 1964).

2.8.7.2 Skinfold assessment technique

2.8.7.2.1 Triceps skinfold technique

A mark was made at the most prominent point of the triceps muscle with the arm in the anatomical position. The subject was asked to stand as before, their back facing the measurer and arm flexed to 90º, palm facing upwards and the mid acromio-olecranon point was located from the earlier measurement for the mid arm circumference and marked. A tubular fold of skin was lifted up vertically between the thumb and index finger of each of the observer's hands at a distance of 1 to 2 cm above and below the where the two marks intercept. It was manipulated to ensure the subcutaneous fat comes away from the muscle and the callipers applied at a point on blade breadth from the apex of the skinfold and held in position for 5 seconds and then removed.

Due care was taken not to twist the callipers when reading the dial. This was repeated three times with the removal of the callipers and release of the skin for 5 seconds between each measurement. The average value was recorded (Fidanza, 1991)

2.8.7.2.2 Biceps skinfold technique

A similar method is applied as with the triceps fold using the most prominent part of the biceps muscle with the arm in the anatomical position. The arm was then flexed to 90º with the palm facing upwards and 3 measurements made at this point as for the triceps measurements (Fidanza, 1991). Please see figure 2.4.

Figure 2.4 Subject undergoing biceps skinfold measurement.

2.8.7.2.3 Subscapular skinfold measurement.

The observer stood behind the subject and the subject was asked to allow his/her arms to relax by their sides. The lowermost point of the scapula was palpated and the skin was marked. An oblique tubular skinfold was picked up in the natural crease of the skin and the callipers applied (Fidanza, 1991).

2.8.7.2.4 Suprailiac skinfold measurement

The subject stood with their arms and the observer stood behind them. A mark was placed at the highest point of the iliac crest. The subject raised their arm and the lowermost apex of the axilla palpated, a tape measure was dropped from this

point to the iliac crest mark and a mark placed where this mid axillary line could intersect with a line through the horizontal plane of the mark placed at the iliac crest. A measurement of a tubular skinfold in the natural crease of skin was taken on three consecutive measurements (Fidanza, 1991). In this study a skilled nurse operator performed all anthropometric measurements. 5 serial observations were recorded on 3 individuals to determine the coefficient of the variation (CV).

2.8.8 Bioimpedance Analysis

Fat is relatively anhydrous and is a poor conductor with a high resistance to passage of current. Thus fat yields a high impedance or resistance to flow, whereas lean tissue has lower impedance. A low level excitation current is used 500 to 800μA at 50 kHz. The resistance offered by fat measured at a constant frequency of 50 kHz reflects the volume of water in the extracellular fluid, muscle and water compartments (Kushner, 1992). The excitation current is passed through the distal electrode and the fall in voltage due to impedance is measured at the proximal electrode (Bodystat Inc., 2000).

Bioimpedance assessments were made using the Bodystat 1500® sport and fitness machine (Bodystat® Inc.). A bioimpedance analysis measurement reflects the degree of resistance to the flow of current in the body. Resistance can be measured, body water calculated and body fat extrapolated using standard equations based on the resistance of body constituents. Human tissues act as conductors and/or insulators of electrical current flow. The lean body mass consists of 73% water and the electrolyte content allows it to conduct an electrical current.

Bioimpedance analysis offers similar predictive accuracy to skinfold thickness testing but has the advantages of not requiring a high technical ability from the operator, can be used in obese individuals (Gray et al., 1989) and is less

uncomfortable and intrusive for the subject (Heyward and Stolarczyk, 1996). The Bodystat 1500® uses specific unpublished equations which incorporate factors for differing ages, gender, and physical activity levels (Chumlea et al., 1988).

2.8.8.1 Assumptions

Bioimpedance analysis assumes that the human body is a perfect cylindrical unit, with uniform length and cross sectional area. In fact the body can be more accurately divided in to 5 cylinders; a head, 4 limbs and a trunk cylinder. Each area provides individual and unique resistance to electrical passage (Kushner, 1992).

At a fixed signal frequency the impedance to current flow is directly proportional to the height of the body (conductor) and inversely related to the cross sectional area of the cylinder (body).

Impedance= p(length/cross-sectional area)

Where p is a constant and assumed to be the specific resistance of the tissues. In life this resistance is not constant and varies among individual tissues and over time within the same tissues, because of fluxes in states of hydration and electrolyte components (Kushner, 1992). The resistance of the trunk is 2-3 times that of the limbs and the limbs of an adult are more resistant than those of a child (Fuller and Elia, 1989, Chumlea et al., 1988)

2.8.8.2 Technique

The subject was fasting for 12 hours, rested for 5 minutes, asked to void bladder, and avoid alcohol and diuretic usage for 48 hours prior to testing. The subject lay supine on a non-conductive surface, legs at >45° angle from each other with no limbs touching, including thighs. The areas to be used were wiped with an alcohol swab, an adhesive electrode sticker and the paired red electrode are placed proximal to the metacarpophalangeal joint of the third digit on the right hand and the partner black electrode is placed between the heads of radius and ulna on the same side. On the right foot an electrode conductor sticker and a red electrode were placed proximal to the second metatarsophalangeal joint and a black electrode was placed between the heads of tibia and fibula on the same foot. Please see figure 2.5.

The subject was asked to lie still and relaxed for the duration of the measurement. Details of height and weight, and age were entered into the machine and measurement performed.

Figure 2.5 Subject undergoing bioimpedance analysis.

2.8.8.3 Sources of error

The possible sources of error are as follows:

Instrumentation; BIA machines differ; in this study, the same machine were used for serial or comparable data.

Technical; The bio impedance machinery is easy to operate and there is little inter-observer error if electrode placement is accurate (Jackson et al., 1988). The proximal electrode placement is crucial and a deviation of 1cm in positioning can lead to a 2% error in resistance (Elsen et al., 1987). Differences between using ipsilateral or contralateral measurements are generally small (Graves et al., 1989, Lukaski et al., 1985).

Environmental; Subjects should be supine on a non conductive surface. The room temperature must be 22°C as a lower temperature (14 °C) reduces skin temperature to 24°C from the normal 33°C and increases total body resistance by 35Ω (Caton et al., 1988).

Subject factors; It is estimated that 3.1 to 3.9% variation in resistance is due to variations in day-to-day state of hydration of the body (Jackson et al., 1988). Dehydration has been shown to increase resistance by approximately 40 Ω (ohms), and lead to up to a 5kg underestimation of FFM (Life Measurement Inc.). In one study a measurement within 4 hours of a meal reduced the resistance by 13 to 15 Ω and can over predict FFM by 1.5kgs (Deurenberg et al., 1988). Follicular compared to premenstrual staging induces a small reduction of approximately 5 to 8Ω in BIA, and an increase of 7Ω during menses compared to follicular stage. Neither were large enough to have an effect in FFM estimation (Gleichauf and Rose, 1989). Some women experience variations of up to 2 to 4

kgs(Bunt et al., 1989), owing to changes in intracellular water, total body water and the ratio of extra to intra cellular water and body weight (Mitchell et al., 1993).

Auto calibration is performed by the Bodystat 1500® prior to performance of the test. Operator calibration was performed weekly. Electrodes are attached to a calibrator unit, which consists of a 500Ω resistor. A test sequence is performed. The impedance should be in the range 496-503 indicating a variation of 0.5% (Bodystat Inc., 2000). If readings are outside this range the batteries must be replaced and if the error persists then contact is made with the manufacturer. This did not arise during the period of the study.

2.8.9 Air displacement plethysmography

Air displacement plethysmography was performed using the BOD POD® (Life Measurement Inc, Concord, CA). (Life Measurement Inc.). The BOD POD® (Life Measurement Inc, Concord, CA) utilizes the principles of whole body densitometry and uses the two-compartment model of body density to derive body fat percentages by the application of standard equations for calculation of %body fat and % lean tissue. Body mass is determined by weighing the subject on an electronic scales, which is calibrated prior to use with the application of a 20 kg weight and acceptable results are within 0.02kgs of the actual weight. Subject measurement is averaged over 5 seconds to minimise errors introduced by movement artefact or ballistic effects of heart rate (Daniels and Alberty, 1967). Volume is measured by air displacement plethysmography and body density calculated on the basis that density = mass/volume.

2.8.9.1 Technique

The BODPOD® has two chambers; a plethysmograph chamber of known volume separated from a reference chamber, also of known volume, by a membrane that oscillates upon changes in pressure and consequently volume in the chambers. Measurements are made of the frequency of the oscillations. Tiny oscillations of the diaphragm induce volume changes in the chambers and thus pressure changes. By measuring the ratio of pressure changes in the chambers an unknown volume can be determined. The subject was rested with no exercise or eating for 1 hour prior to measurement, void bladder and wear only a swimming hat and snug fitting swimming trunks and removed all jewellery.

The subject was weighed on a calibrated electronic scale, and then seated in the sealed compartment of the BOD POD® with instructions to sit as still as possible and breathe normally. Three tests were performed lasting 40 seconds each. The subject was requested to open the BODPOD® door in between each test in order to equilibrate the air inside the compartment with atmospheric air.

The measurement of body volume by the BOD POD® undergoes 3 steps.

Calibration of the chamber using a 2-point process. The initial phase is calibration of the chamber to establish baseline with the chamber empty and then with 48 litres removed into the calibration cylinder communicating with the test chamber by a valve mechanism. The second calibration is within the reference cylinder to establish range.

Estimation of raw body volume. Most of the air in the chamber is adiabatic thus Poisson's Law is used to determine the raw volume. Adiabatic means is defined as a net transfer of heat to or from the working fluid of zero. Thus this process does not take account of surface area artefact (SAA) or thoracic volume.

Estimation of thoracic volume. Air in the lungs is isothermal and thus is 40% more compressible than adiabatic air so the lung volume is measured as being 40% larger than it actually is in life. The BOD POD® software predicts the mid-tidal thoracic volume from equations based on pulmonary function data on functional residual capacity (FRC) (Crapo et al., 1982). This value is defined as FRC+ (tidal volume/2).

Air in contact with the body skin can be estimated by surface area artefact (SAA)

SAA (L) = k (L/cm²) x body surface area (cm²)

Body surface area 71.84 x weight (kgs)$^{0.425}$x height (cm)$^{0.725}$

It has a typical value of approximately –1.0 L for an adult of average size (Fields et al., 2002). k is a constant derived empirically by the manufacturer. Body surface area (BSA) is calculated from the equations of Dubois and Dubois (Dubois and Dubois, 1916). The BOD POD® then calculated the corrected body volume using the following equation (Siri, 1961) .

Corrected body volume (L) = raw body volume (L) – SAA (L) + 40% thoracic volume (L)

Thus body density is calculated Density = Mass/volume and this value is then used in conjunction with standard equations for estimation of body fat and lean percentage. The Siri equation is accepted for use in the Caucasian population and Schutte for Afro-Caribbean subjects (Schutte et al., 1984, McCrory et al., 1995).

The Siri equation is as follows

%fat = ((4.95/body density) – 4.5) x 100

where the density of fat is 0.90kg/l and density of fat free mass is 1.10kg/l

%fat free mass = 100 - % fat.

Fat mass (kgs) = (%fat)(body mass)/100

Fat free mass (kgs) = body mass –fat mass

The Schutte equation is more suitable for Afro American populations and is as follows

%fat = (4.374/body density – 3.928) x 100

Published literature on the reliability of BOD POD® measurement of inanimate objects reports it to be excellent. A CV of 0.025% was reported with 20 repeated measurement of a 50.039L cylinder (Dempster and Aitkens, 1995). In adult subjects the reported mean of CV's in published literature ranges from 1.7% to 4.5% within one day and 2.0% to 2.3% between days. When the BOD POD® was compared to hydrostatic weighing for measuring % body fat the CVs did not differ considerable 1.7% and 2.3% respectively (McCrory et al., 1995).

Two studies of body volume calculations of BOD POD® compared to HW reported better precision with BOD POD® (0.07, 0.11 L) than HW (0.15, 0.16 L) but it should be noted that the HW made actual calculation of intra thoracic volume and this incurred variation is not experienced in the BOD POD® intra thoracic volume which is derived from the standard equations(Davies, 2009, Dewit et al., 2000).

2.8.9.2 Sources of error

Increased body heat and moisture can lead to underestimation of body fat (Higgins et al., 2001). Facial and body hair can lead to an underestimation of body fat due to trapped isothermal (Higgins et al., 2001). Wearing of a hospital gown during measurement has been shown to increase body fat by 5.5% compared to underwater weighing (without the gown). In the same study a one or two piece swimsuit was not shown to cause a difference in measured body fat percentage (Higgins et al., 2001). Subject motion leads to measurement error.

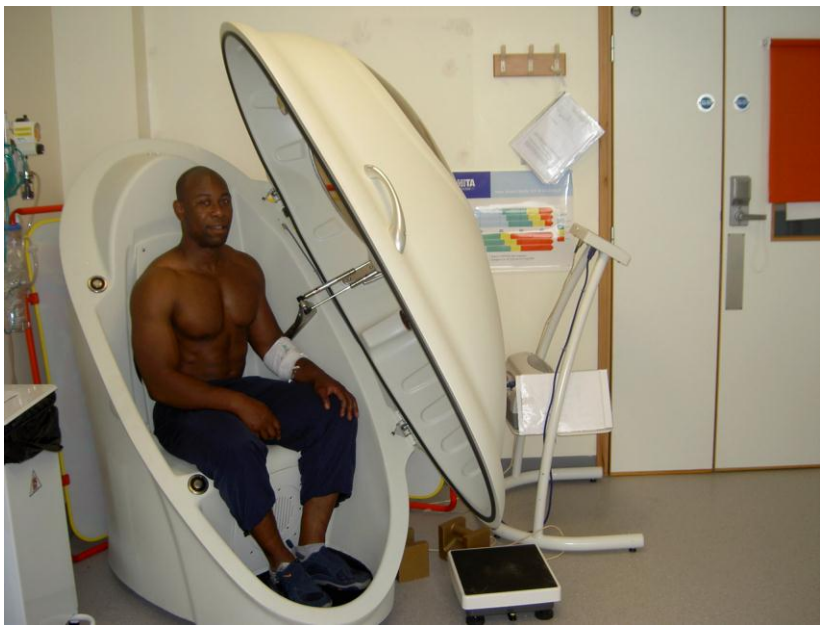


Figure 2.6 Volunteer in BODPOD®.

Volunteers were clothed only in a tight fitting swim suit to reduce distortion of volumes. For the purposes of display he is shown here in shoes and tracksuit. Please see figure 2.6.

Problems with the BODPOD® are discussed further in chapter 7.

2.9 Blood Pressure Measurement

Systolic and diastolic blood pressure recordings were performed on subjects.

2.9.1 Technique

The subject remained sitting for 3 minutes prior to taking the reading, the arm was supported by the observer and measurements were taken with the cuff level at mid sternal level (O'Brien et al., 2001). One measurement was taken of resting brachial blood pressure in the non-dominant arm using an electronic sphygmomanometer (Dynamap® 1846 S X).

2.9.2 Sources of error

Biological: forearm circumference, position and diameter of artery, surrounding tissue anatomy as well as vasomotor/anxiety factors (Forstner, 1999).

Technical: inappropriate cuff size, arm positioning, operator skills and failure to standardize technique or calibrate equipment (Steppan, 2001).

2.10 Measurement of exercise capacity

2.10.1 Principles of measurement of exercise capacity

Maximal oxygen consumption (VO_2 maximum) is a measure of physiological exercise capacity. In this study it was assessed during maximal graded exertion using a Bruce protocol (table 2.3) on a treadmill (Woodway®, USA) with indirect calorimetry using the Cortex Metasoft 3 Metalyser® (Cortex Biophysix, Germany). VO_2 maximum has been attained when further graded increases in workload fail

to produce further oxygen uptake (Lieberman, 1994, Snell and Mitchess, 1984), and occurs immediately prior to attainment of maximal exercise level. Oxygen uptake (VO_2) at rest is approximately 0.25L/min.

The maximum oxygen consumption per minute when exercising at sea level is called the VO_2 maximum and is calculated as follows:

VO_2 (L/min) = pulmonary minute ventilation (L/min) x (inspired fraction of oxygen – expired fraction of oxygen)(Wasserman et al., 1987).

or

VO_2 (L/min) = maximal cardiac output (L/min) x (arterial oxygen content– venous oxygen content)

where maximal cardiac output is maximal stroke volume (mls/beat) x maximal heart rate (beats/min) (Wasserman et al., 1991). VO_2 max can be predicted from standardised equations in individuals unable to undergo maximal exercise testing from performance during walking. However, the error rates are higher than with formal testing (Thompson et al., 2001). It can also be predicted without calorimetric measuring of oxygen consumption concentrations by age and activity concentrations and duration of exercise (Schutte et al., 1984).

Progression to VO_2 max is a multiphasic curve. There are 3 phases to VO_2 kinetics seen with onset of light to moderate exercise below the individual's lactate threshold. Phase 1 is termed the cardiodynamic phase and occurs just

after the onset of exercise. It is characterised by a rapid rise in pulmonary VO_2 and reaches a transient plateau in 15 to 20 seconds, due to the rapid delivery of deoxygenated blood for alveolar gas exchange. Phase 2 and 3 reflect the oxidative or metabolic dynamics of working muscle. After phase 1 there is a rapid exponential phase 2 rise in VO_2 representing the increase in VO_2 of metabolically active tissues, e.g. muscle. Phase 2 lasts 5-10 minutes approximately. When this reaches maximum oxygen consumption there is a plateau defined as phase 3 (Mole and Hoffmann, 1999).

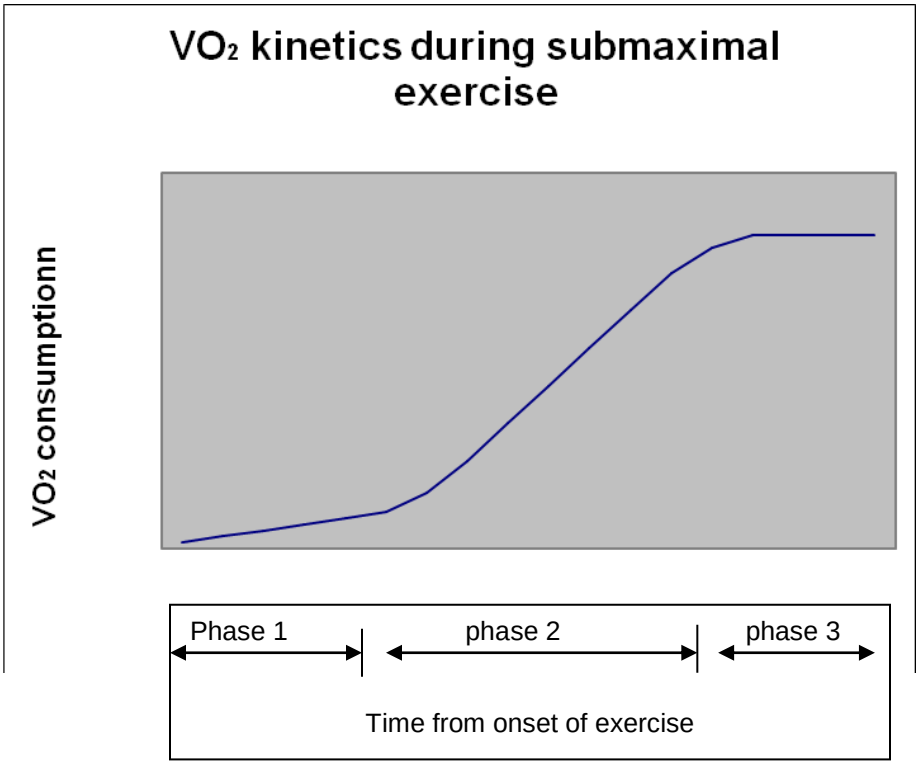


Figure 2.7 VO_2 kinetic curve during submaximal exercise to below the lactate threshold.

(Mole and Hoffmann, 1999).

Other determinants of muscle ability to sustain endurance performance include capillary density, enzymatic action, mitochondrial number and size, type of muscle fibre, as well as motivational and psychological factors (Holoszy and Coyle, 1984). Maximal oxygen uptake (VO_2 max) has been shown to be a marker for cardiovascular fitness and this has a high correlation with the duration of exercise on standardised protocol testing (Schutte et al., 1984). A quicker dynamic oxygen response to exercise reflects a higher degree of aerobic efficiency, with less reliance on anaerobic and lactate producing mechanisms with the onset of exercise (Edwards, 1950).

Physical exercise involves a complex interaction of cardiovascular, pulmonary and cellular metabolic adaptations. This increases oxygen usage by working muscles as a result of increased extraction of circulating oxygen facilitated by vasodilatation of peripheral vascular beds, vasoconstriction of non-active vascular beds, increased cardiac output, increased pulmonary ventilation and blood flow (Wasserman et al., 1991). The ATP concentration in working muscle also plays a critical role in setting oxygen demand which controls and maintains oxygen delivery by neuronal mechanisms (Raven and Potts, 1998). The contribution of muscle mass to this VO_2 is seen in experiments on leg oxygen consumption which has been shown to contribute approximately 80% of whole body maximum oxygen consumption (Odland et al., 1998).

Cardiovascular fitness is an important regulator of the GH/IGF-I axis via GH, IGF-I and the binding proteins (Eliakim et al., 2001). Adolescent men with higher VO2 max have increased GH pulsatility with higher mean GH peaks but no difference in IGF-I concentrations compared to less fit matched controls (Eliakim et al., 2001). Lower GHBP may lead to reduced hepatic sensitivity to endogenous GH. IGFBP4 is negatively correlated with VO_2 max as it inhibits IGF-I (Eliakim et al., 2001).

2.10.2 Factors influencing maximal oxygen uptake capacity

A number of factors influence the predicted and actual VO_2 maximum.

i. Habitual physical activity level

Physical activity level is a very important determinant of VO_2 maximum and endurance training increases VO_2 max (Lieberman, 1994). Cross sectional analysis reports an average VO_2 maximum of 40ml/kg/min in sedentary populations, 60 to 70 mls/kg/min in county champions, and 80 mls/kg/min in world class athletes. The rate of improvement is dependent on intensity, frequency and duration of the training program, age, gender, and baseline VO_2 max prior to commencing training (Caton et al., 1988). VO_2 max can rise by 15 to 30% in the first 2 to 3 months of training and declines slowly to pre training concentrations within approximately 6 months of cessation of training with a more rapid decline in skeletal muscle than whole body VO_2 max.

The increase in VO_2 max with training is primarily due to the increasing cardiac output but also the adaptive responses of muscle to improve oxygen extraction. There is a right shift in the oxygen-haemoglobin dissociation curve so that for a given VO_2 the athlete has improved oxygen diffusion capacity thought to be due to increased erythrocyte concentration of 2,3-diphosphoglycerate (Snell and Mitchess, 1984). There is an initial rise in plasma volume followed by an increase in haemoglobin concentration due to increased sympathetic activity and dehydration associated with training (Convertino, 1991). By the third week of endurance training there is a decline in plasma catecholamine response to exercise with a decline in resting and submaximal heart rate.

Endurance runners of world class competitive standard have average heart rates of 30 to 40 beats per minute (Wasserman et al., 1987). This is likely to be due to reduced sensitivity of cardiac β receptors (Brundin and Cernigliaro, 1975), increased cardiac vagal tone (Ravissin, 2002), increased filling volume due to increased plasma volume (Convertino, 1991). Training does not change maximal exercise heart rate but maximal stroke volume is increased due to a greater preload induced by the expansion in plasma volume associated with training, and increased left ventricular compliance(Gledhill et al., 1994).

Maximum diastolic blood pressure is reduced thought to be secondary to an increased capacity for muscle vasodilatation (Snell et al., 1987). There is also an increased muscle vascularity and capillary density in trained muscles thought to contribute to the increased diffusing and oxygen extraction capacity of trained muscle cells (Costill et al., 1976). The maximal pulmonary ventilation and breathing rate of trained athletes is higher which can induce arterial hypoxaemia limiting VO_2 max. Tidal volume is unchanged but the trained athlete has an improved tolerance to higher concentrations of lactic acidosis than untrained individuals. The sweating threshold is lowered with training and athletes experience a relative heat acclimatization allowing greater ability to dissipate heat (Banfi et al., 1994).

During training there is initially an imbalance between the oxygen delivery, fibre activation and energy metabolic needs of the tissues, which is perceived as an “error” message by peripheral chemo and mechano receptors that transmit afferent messages to the central cardiovascular regulatory system. In muscle this signal process is due to monitoring of the phosphorylation state of the muscle by chemoreceptors, in the respiratory system it is monitored by the partial pressures of oxygen and carbon dioxide by baroreceptors and chemoreceptors, while

cardiovascular monitoring is achieved through variations in cardiac output and peripheral vascular resistance monitored by baroreceptors.

ii. Genetics

There is an ongoing debate about the contribution of genotype, phenotype and training to attainment of sporting excellence and VO_2 max. There is certainly a high degree of hereditability in physical attributes contributing to fitness. Monozygous twin studies report 20-40% hereditability in VO_2 max, 50% in maximum heart rate attained and 70% for physical working capacity (Bouchard, 1985). Maternally inherited mitochondrial DNA is thought to contribute to differences in VO_2 max, maximal heart rate, and blood lactate between individuals (Lesage et al., 1985).

There are undoubtedly individual differences and certain individuals have a predisposition to success at certain sports both by genetic endowment of the above variables as well as physical makeup. This predisposition is not a guarantee of success and training and appropriate conditioning are critical.

iii. Age

Maximum achievable heart rate declines with age 0.5 to 1 beat/minute for every year of life after 20 years. The VO_2 max declines by 0.3 to 0.5mls/kg/min per year (Astrand, 1960). Age also is associated with a reduction in arteriovenous oxygen differences, and cardiac output. However, on cross sectional analysis there is also an increasing tendency to sedentary lifestyle with increasing age but individuals who maintain a high level of physical activity as they get older have been shown to have improved aerobic capacity compared to age and gender matched sedentary individuals (Raven and Mitchell, 1980).

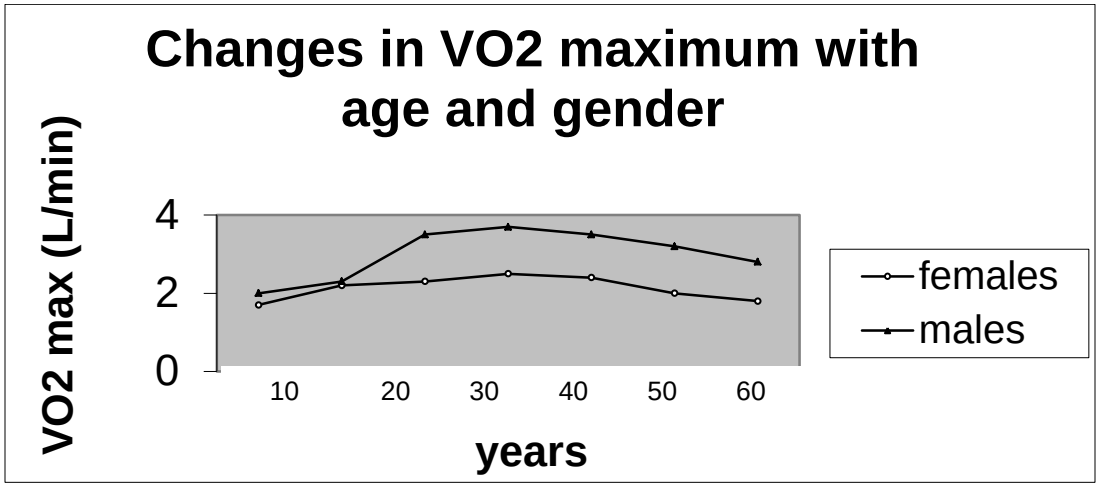


Figure 2.8 Changes in VO_2 maximum with age in women and men

(Hermansen, 1974).

iv. Gender

There is a linear increase in VO_2 max with increasing height, weight and age in both boys and girls before puberty and they have almost equivalent VO_2 max levels (Franssila_Kallunki et al., 1992). Subsequently boys gain muscle mass and girls gain fat and as adults VO_2 max in women is approximately 20% lower than that of men. When matched for lean body mass this difference is approximately 5%. Clearly there are other factors such as the lower haemoglobin concentration seen in women which reduces oxygen carrying capacity (Astrand, 1960).

Children up to the age of 12 years show no gender difference in VO_2 max but by 16 year boys have a 50% higher VO_2 max than girls of the same age. In adulthood the gender difference is thought to be as a result of higher percentage body fat and thus lower relative muscle mass and a lower circulating

haemoglobin concentration than men. This difference applies to trained or untrained individuals (Woodson, 1984).

v. Body size and composition

These are vital components of VO_2 maximum and variations in body size are thought to account for 70% of differences in VO_2 max (Wyndham and Hegns, 1969). The mechanical load of carrying a higher body mass increases the energy demands on the body. However, the composition of this higher load is important as muscle provides a more metabolically active energy source than fat. Thus VO_2 max is often expressed per unit of body surface area, FFM, body mass or limb volume (Thompson et al., 2001).

Vi.Sporting discipline specificity

There is sporting discipline specificity in energy transfer systems utilised and VO_2 max needed. The quantity of muscles activated during the sport is an important factor in the VO_2 max of athletes (Sharp et al., 2002). Athletes have energy transfer capacities specific for their sport. Swimming involves upper body exercise while running involves lower body exercising thus protocols should be specific for the sport in question (Thompson et al., 2001). Long distance runners have a different metabolic profile than middle distance runners as seen in a significantly higher VO_2 max in the long distance athletes (Boileay et al., 1982).

2.10.3 Factors limiting maximal oxygen consumption.

i. Cardiac output.

Cardiac output is the major limiting factor to VO_2 max. During exercise the skeletal vascular bed is expanded beyond the ability of the heart to increase cardiac output to fill this excess capacity. In fact maximum VO_2 is produced with

engagement of one third of the skeletal mass indicating that cardiac output is a limiting factor. .

ii. Pulmonary function.

Arterial desaturation during exercise indicates that pulmonary function can be a limiting factor to attaining VO_2 max, however, in general pulmonary blood flow and capillary blood volume increase to maintain alveolar-arterial oxygen equilibrium (Dempsey et al., 2002).

iii. Skeletal muscle oxygen utilisation capacity.

The movement of oxygen from circulating haemoglobin to muscle mitochondria by diffusion is another limiting factor to VO_2 maximum. Pathological conditions leading to a reduction in this diffusing capacity are associated with a reduction in VO_2 max. Skeletal muscle utilisation of oxygen is also likely to be influenced by the training status of the individual. Carbon dioxide release at the alveolar border in early exercise is slower initially than oxygen consumed thought to be due to the high solubility of carbon dioxide in tissues compared to oxygen (LeBlanc et al., 1984, Whipp and Ward, 1990). After the onset of exercise some carbon dioxide is initially stored especially during low intensity exercise thus alveolar gas changes may not be truly representative of tissue dynamics (Edwards, 1950).

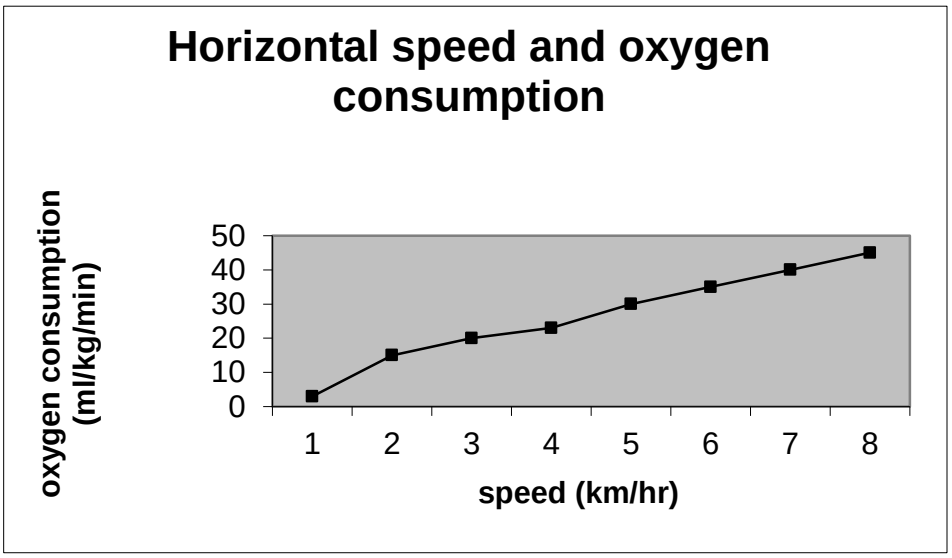


Figure 2.9 Relationship between horizontal speed and oxygen consumption

(Falls and Humphrey, 1976).

2.10.4 Measurement of VO_2 max

Assessment of VO_2 maximum is best performed using exercises that activate the large muscle groups, ensuring the intensity and duration of exertions are sufficient to activate the long term/aerobic energy transfer systems (Thompson et al., 2001). There are two important considerations; the mode of exertion and the protocol chosen, e.g. graded versus non graded, (Bruce, Balke) etc. Exercise tests should remain independent of the subjects skill, strength, speed, and body size unless it is a specialised sport's specific testing setting (Thompson et al., 2001).

The protocol ceases once the subject simply refuses to continue exercising. This is a proxy for exhaustion but in practice there are other factors, e.g. motivational,

psychological factors that can influence the attainment of this point. It is important that the operator continues to motivate and urge on the subject especially as the endpoint nears (Riedl et al., 2000).

2.10.4.1 Important factors in measuring VO_2 maximum

i. Mode of exertion

The type of exertion undertaken reflects on VO_2 max attained. The speciality sport of the subject is also an important factor in aerobic capacity. Short duration high intensity exercise such as football or weight lifting use the immediate anaerobic energy transfer system of ATP and PCR. Thus they may exhibit a lower VO_2 max than endurance runners. Treadmill running yields a higher VO_2 max than walking, which is turn is higher than cycling. Cycling yields a VO_2 max of 6.4 to 11% lower than treadmill running. Walking scores were 7% higher than cycling and 5% lower than running tests (Thompson et al., 2001).

ii. Choice of performance test

Protocols may be divided into graded or non-graded. Non-graded exercise protocols involve 5-10 minutes of continuous steady rate exertion. Graded exercise protocols in which there is a gradual stepwise increase in speed and/or gradient. Graded protocols may be continuous with no rest periods between graded steps or discontinuous which involves recovery periods between increments. Both approaches provide similar VO2 max values (Chalmers, 1979). The advantage of the continuous program is that it allows assessment of VO_2 max in approximately 10 to 15 minutes whereas discontinuous systems require 65 minutes on average. Also subjects report local discomfort especially in calves

on graded walking tests at higher elevations and in running tests although in the latter the most common feeling is one of “being winded” (Thompson et al., 2001).

A continuous treadmill running test is often used for increased subject tolerability and ease of standardisation and comparison in the laboratory setting (Thompson et al., 2001). Various exercise protocols exist and the protocol chosen in this study is a Bruce Protocol detailed below. It is standardised and allows easy comparison with other studies.

iii. Treadmill versus track running

There is no measurable difference in the aerobic expenditure between running on a treadmill and running on a track and the VO_2 maximum recorded is equivalent in both setting (Erotokritou-Mulligan, 2009). However, at high velocities, e.g. speed skating, cycling, the impact of air resistance increases the oxygen cost of track exercise (Thompson et al., 2001).

2.10.4.2 Methodology of indirect calorimetry during exercise.

Indirect calorimetry was performed to VO_2 maximum on two separate occasions to all subjects in the double blind study and on one occasion in the longitudinal study. The machinery consists of a Woodway® treadmill (Woodway USA) and a Cortex Metalyser 3B gas analyser® (Cortex Biophysix, Germany). The subject was non-fasting, rested, and had a heart rate detector/polar belt placed around their chest with an electrode placed over the xiphisternum, an appropriately fitted mask over their mouth and nose adjusted to attain an adequate seal and minimise air leaks. The subject performed a maximal Bruce protocol as shown in table 2.1.

Baseline measurements are performed for 30 seconds and then the protocol was commenced. The subject was instructed to exercise for as long as possible and

encouraged throughout the measurement to continue to the point of exhaustion. On-going measurements of VO_2, VCO_2, heart rate, and respiratory rate were made.

Stage	Time (mins)	Speed (Km/hr)	Incline (%)
1	0	2.74	10
2	3	4.02	12
3	6	5.47	14
4	9	6.76	16
5	12	8.05	18
6	15	8.85	20
7	18	9.65	22
8	21	10.46	24
9	24	11.26	26
10	27	12.07	28

Table 2.10 Bruce protocol treadmill test.

The machine was calibrated monthly with a 3-litre syringe volume and known gas concentration analysis. Prior to every measurement calibration was performed with ambient air pressure, and zero adjustment performed to determine ambient air gas concentrations.

2.11 Energy expenditure

2.11.1 Principles

Basal metabolic rate (BMR) or basal energy expenditure (BEE) is defined as the energy expended during complete rest, in a post absorptive state and in a thermo neutral environment (Frayn, 1996). Resting metabolic rate (RMR) is defined as the sum of basal and sleeping rates as well as the metabolic cost of arousal and is equivalent to the sum of the metabolic processes of the active cell mass required to maintain normal regulatory balance and body function at rest (Thompson et al., 2001). BMR measured in a controlled laboratory environment is only slightly less than the RMR which is generally measured 3-4 hours after a light meal with no prior physical exertion. Total energy expenditure is the sum of the energy expenditure in resting metabolic rate (60–75%), postprandial thermogenesis (10%), and physical activity (15-30%) (Thompson et al., 2001).

BMR reflects energy expended in viscera for oxidative phosphorylation. The principle consumers of energy are the liver (27%) and brain tissue (19%), heart (7%), skeletal muscle (18%), kidneys (10%) and other tissues, e.g. fat (19%) (Thompson et al., 2001). The metabolic rate between tissues differs considerably. The brain in an average 65 kg man weighs 1.6 kgs approximately while the skeletal muscle weighs 32kgs and both consume the same amount of oxygen per minute. This proportion differs with age with children's brain tissue consuming 50% of resting energy expenditure. Exertion can change these proportions considerably with skeletal muscle oxygen consumption increasing by up to 100-fold.

Assessment of BMR is best performed in the post absorptive state which is a state of nutrition after absorption of ingested nutrients has occurred and before starvation, e.g. the morning after an overnight fast. During this phase the insulin:

glucagon ratio is low, GH concentrations are high and approximately one third of an individual's energy requirements are fulfilled by hepatic glycolysis and two thirds by hepatic gluconeogenesis. Contributors to the gluconeogenesis include 25% as alanine from skeletal muscle metabolism, 50% from anaerobic glycolysis within red cells and the renal medulla as lactate and 25% as glycerol from adipose tissue (Frayne, 1996).

Post prandially CHO is absorbed from the intestine, insulin concentrations are high and glucose is carried in the portal vein for hepatic storage under the influence of GLUT 2 and insulin induced glycogen synthetase, and to skeletal muscle for storage. However, in the post absorptive state muscle cannot release glycogen into the circulation although it is available for muscle usage itself.

Several factors influence energy expenditure. Women have higher fat content than men and in view of the fact that fat has a lower metabolic activity than lean tissue, women exhibit a BMR 5-10% lower than men of a similar body size (Altman and Dittmer, 1968). BMR declines 2-3% per decade most likely due to the decrease in lean body mass seen with ageing (Bemben et al., 1995). However, physical training can slow down this loss in resting metabolism. Resistance training in men 50-65 years old increased RMR by 8% by increasing fat free mass (Pratley et al., 1994). Similarly an 8 week aerobic training regimen, despite no change in fat free mass, induced a 10% increase in RMR (Poehlman, 1990).

The levels of habitual physical activity are also important. During large muscle exercise, e.g. swimming, an average individual can increase their metabolic rate by 10-fold. Skeletal muscle physical activity is an inefficient energy source with approximately 25% of chemical energy being converted to mechanical work (Roden, 1996). The resting metabolism of individuals in tropical climates is generally 15% higher than those in temperate climates (Thompson et al., 2001). Exertion in hot climates has an additional energy consumption of 5% compared

to thermo neutral controls because of the raised core body temperature, excess work of sweating and circulatory changes seen in the heat (Thompson et al., 2001). Similarly in cold climates, the work of shivering has considerable energy demands with reports of an increase in metabolic rate of up to fivefold (Thompson et al., 2001).

Pregnant women have higher energy expenditure for a given activity because of the added weight carried during the pregnancy. Non-weight bearing exercise, e.g. weight supported bicycle exercise, demonstrates similar energy expenditure to matched non-pregnant individuals, however, weight bearing exercise e.g. jogging has been shown to have added energy expenditure (Knuttgen and Emerson, 1974).

2.11.2 Evaluating Energy Expenditure

Energy expenditure can be estimated by direct or indirect calorimetry, doubly labelled water, or use of predictive equations e.g. Harris-Benedict (Long et al., 1979), or the Fick method which involves pulmonary artery catheterisation and monitoring of haemodynamic variables (Liggett et al., 1987). In this study indirect calorimetry was used for energy expenditure evaluations.

2.11.3 Respiratory quotient (RQ)

This is a ratio used as a guide for estimating the contributions of each of the macronutrients to total energy production. It is only valid during rest and steady-state exercise as it assumes that the metabolic gas exchange at the respiratory border is accurately reflective of metabolic catabolism at a cellular concentration. This assumption is not necessarily the case in non-steady states such as

exhaustive exercise where the respiratory exchange ratio is used (RER). RQ describes the ratio of metabolic gas exchange

$RQ = CO_2$ produced ÷ O_2 consumed

(VCO_2) (VO_2)

The equation gives an indication of the type of substrate being used at the time of measurement. The usual value range in the post absorptive state in humans is 0.7 to 1.0.

Oxidation of one molecule of carbohydrate utilizes 6 molecules of oxygen and produces 6 molecules of carbon dioxide. Thus during oxidation of one molecule of carbohydrate the gas exchange is as follows;

$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$

$RQ = 6CO_2 \div 6O_2$

= 1.00

The RQ for lipids depends on the lipid chosen. By convention in UK palmitic acid is chosen as representative of fat oxidation. Fats contain more hydrogen and carbon relative to oxygen when compared with carbohydrates thus 16 carbon dioxide molecules are produced by oxidation of palmitic acid for every 23 oxygen molecules consumed;

$C_6H_{12}O_2 + 23O_2 = 16CO_2 + 16H_2O$

$RQ = 16CO_2 + 23\ O_2$

= 0.696

This is generally interpreted to by a value of 0.7 for fat oxidation.

The metabolism of proteins is not simply oxidation to carbon dioxide. Proteins undergo hepatic deamination, removal of nitrogen and sulphur components via urine, sweat, and faeces, and only then is the remaining keto-fragment oxidised to carbon dioxide and water.

$$C_{72}H_{112}N_2O_{22}S + 77O_2 \longrightarrow 63CO_2 + 38H_2O + SO_3 + 9CO(NH_2)_2$$

$$RQ = 63CO_2 \div 77O_2$$

$$= 0.818$$

The kidneys excrete 1gm of urinary nitrogen for every 5.57 of protein excreted which is the currently accepted value (modern value). Jungas et al reported that the true value was 5.57 as opposed to the previously accepted value of 6.25 g (classic value) of protein metabolised by demonstrating a greater contribution of amino acids to hepatic gluconeogenesis than previously recognised (Jungas, 1992). Urinary nitrogen excretion is generally accepted as a measure of protein turnover. A positive balance is indicative of overall protein anabolism, and a negative balance indicative of overall protein catabolism. An average adult excretes approximately 12 to 14 g of urea nitrogen per day but in cases of pharmacological intervention, e.g. rhGH administration, this cannot be assumed to be the case and a timed urinary collection must be performed.

In life the RQ measured by respiratory gas exchange represents oxidation of all 3 macronutrients in differing proportions and it is generally accepted that an RQ of

0.82 represents metabolism of a mixture of 40% carbohydrates, and 60% fat. However, if more accuracy is required there are published tables for non protein RQ, i.e. carbohydrate and fat oxidation, from which more precise proportions can be obtained (Zuntz, 1901). In practice the contribution of protein metabolism to the overall energy metabolised is small and not taking protein into account introduces only a 0.5% error.

2.11.4 Indirect calorimetry

Indirect calorimetry is technically demanding, machinery is expensive, and there are inherent assumptions but it is generally regarded as the gold standard for assessment of energy expenditure (Flancbaum et al., 1999). It involves the measurement of rates of respiratory gas exchange (oxygen consumption, carbon dioxide production) and urinary nitrogen excretion, to calculate macronutrient oxidation rates (Roden, 1996, Tappy, 1998). There are two types of indirect calorimetry. Closed circuit calorimetry involves the subject breathing 100% oxygen via a sealed tubing device and exhales into this tubing (Thompson et al., 2001). In this study open circuit method was used in which subjects breathed ambient air of constant composition, 20.93% oxygen, 0.03% carbon dioxide, 79.04% nitrogen as well as a small quantity of inert gases. The volumes and constituents of expired gases are quantified using a gas analyser and compared to the inspired values (Thompson et al., 2001).

During this study energy expenditure was assessed in the resting fasting state and at 1 and 2 hours post glucose load using a GEM Europa® computerised indirect calorimeter with expired gas collection and sampling collection via a plastic hood and sample line device.

2.11.4.1 Assumptions

There are a number of inherent assumptions involved in indirect calorimetric measurements;

All the oxygen that is consumed and all carbon dioxide produced is utilised in the oxidation of three macronutrients.

Each of the macronutrients involved oxidises with a known quantity of oxygen to produce a known quantity of carbon dioxide and water (Tappy, 1998).

No other energy nutrients are oxidised except the three main macronutrients, e.g. ethanol (Livesey and Elia, 1988).

Energy production and expenditure are equal upon measurement in the steady state and no energy is lost or gained, e.g. as heat.

The oxygen and carbon dioxide concentrations measured at the respiratory borders are equal to the true rates of oxygen utilisation and carbon dioxide production (Tappy, 1998).

Measurements are of net and not actual substrate oxidation rates. It corresponds to the portion of ingested carbohydrate used for oxidation and does not take into account carbohydrates stored as glycogen.

It does not take in account endogenous production of fuel e.g. glycerol or amino acids converted to glucose, or de novo lipogenesis (Tappy, 1998).

Blood urea nitrogen is all excreted via the kidneys, when in fact nitrogen is lost through the bowel and sweat glands also.

The values for protein metabolism have a modest effect on the VO_2 and VCO_2 and hence an empirical value of urinary nitrogen excretion of 0.01g/min assuming 1g urinary nitrogen arises from the oxidation of 6.25gms of protein.

Subjects metabolise 1g glucose, or 1g triglyceride (whereas in U.S.A. calculations are based on the 1 fatty acid molecule). In our study the substrate chosen as the best representative of the form being utilised in the body was the triglyceride Palmitoyl-Sterol-Oleoyl-Glycerol.

2.11.4.2 Methodology of indirect calorimetry

A gas exchange measurement flow-through ventilatory canopy indirect calorimeter was used in our study (GEM Europa®). The equipment consists of a bedside unit, hood and computer. Before commencing measurement the references gases were turned on 20 minutes with outlet pressures of 0.5 – 1.0 millibars. The subject is placed on the bed supine, rested for 30 minutes pre testing, fasting for 5 hours, non-stressed, having no infection, and instructed to lie still and relax with no talking or motion permitted. A ventilatory hood is placed over their head and the airflow chamber sealed by tucking in the surrounding material.

The calorimeter is calibrated and fraction of end tidal CO_2 ($FeCO_2$) airflow rate optimised over 20 seconds to 0.5% to ensure a comfortable concentration of CO_2 in the hood system for the subject and the measurements are allowed to equilibrate for approximately 15 minutes or until a stable measurements reached on the data acquisition sample histogram. The $FeCO_2$ is the percentage of expired air that is CO_2 (typically 2.5 to 6%). Measurement continues for another 10 to 15 minutes. This procedure is repeated at 1-hour post OGTT and at 2 hours post OGTT. Please see figure 2.8.

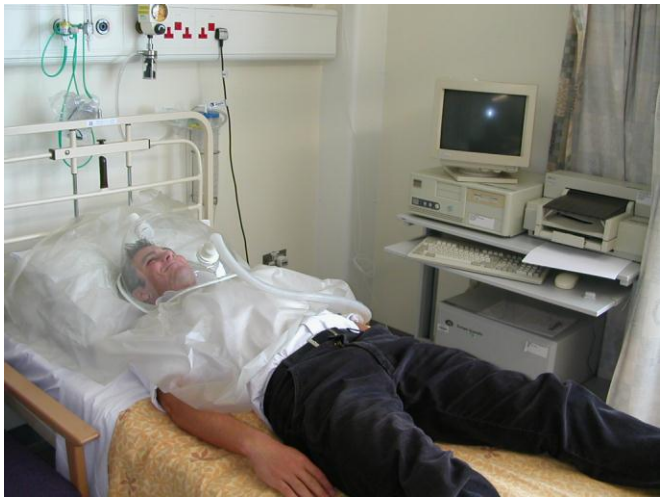


Figure 2.8 Subject undergoing indirect calorimetry.

2.11.4.3 Sources of error

Despite the technical complexity of the device, the output data reflect the accuracy of the measuring device and regular and appropriate validation, calibration and maintenance is critical.

Technical considerations – no air leak, validated and calibrated, isothermal environment.

Subject considerations – rested with no physical activity for 6 hours prior to assessment, be relaxed and stress free, fasting for the initial basal readings and consuming only the glucose load provided during the measurement, infection free, and for the GEM Europa® the subject must lie motionless as subject movement can induce a 20-40% change in values obtained.

Choice of predictive equation must be appropriate and consistent.

2.11.4.4 Validation of the calorimeter

A known quantity of ethanol was burned in the hooded system every 2 weeks with the production of carbon dioxide according to standard equations. Values must be within 5%, approximately 300kcal. Autocalibration of the machine was performed prior to each test to check system set up using defined gases.

2.12 Insulin Resistance

2.12.1 Principles

Insulin resistance is defined as a suboptimal response to insulin mediated glucose uptake in tissues (Ferrannini et al., 1987) or an inappropriately high level of insulin required to maintain metabolic homeostasis (Doherty et al., 1997) and is characterised by the reduction in insulin sensitivity and responsiveness of tissues to the metabolic actions of insulin. The effects of GH on insulin sensitivity were described in chapter 1.

The ideal methodology for measuring insulin sensitivity would have the following characteristics (Hermansen, 1974).

Achieve insulin levels of sufficient magnitude to stimulate glucose metabolism, allow detection of small changes glucose uptake and inhibit glucose production.

Allow distinguishing between hepatic and peripheral glucose metabolism.

Measure steady state conditions

Be physiologically sound

Achieve hyperglycaemia not dramatically outside physiological limits

The hyperinsulaemic euglycaemic clamp (HEC) is regarded as the gold standard for measurement of insulin sensitivity but is expensive, and is operator and subject intensive. In this study oral glucose tolerance testing (OGTT) with 5 minute blood sampling and measurement of glucose, insulin, adiponectin, NEFA and calculation of Quantitative insulin sensitivity check index (QUICKI),HOMA, insulin to glucose ratio (I:G) were chosen in preference to HEC because of the practical time constraints involved in the already lengthy day 1 and 28 visit.

2.12.1.1 Oral glucose tolerance testing (OGTT)

The OGTT involves administration of 75g (U.K.) or 100gms (U.S.A.) of glucose orally and samples taken at regular intervals e.g. 0, 60, 120 minutes or at more frequent 5 minute sampling intervals. Diagnosis of normal and impaired glucose tolerance and diabetes is performed by OGTT according to World Health Organisation guidelines (Alberti and Zimmet, 1998). It is easier and cheaper to administer than either a euglycaemic hyperinsulinaemic clamp or intravenous glucose tolerance test (IVGTT) and as such is used more frequently in clinical and epidemiological practice.

The greater the degree of peripheral insulin resistance the greater the rise in plasma glucose (Nishiyama et al., 1988). However, use of the oral route activates gastric factors that alter insulin secretion and peripheral glucose utilisation (Cagnacci et al., 1999). Suppression of hepatic glucose output is less complete than with a HEC, thus results reflect hepatic glucose production as well as peripheral glucose utilisation (Soonthornpun et al., 2003).

There are a number of inherent assumptions in the use of OGTT

glucose uptake occurs only in insulin dependent tissues in the post absorptive state (Nishiyama et al., 1988).

endogenous glucose production is entirely hepatic in origin (Nishiyama et al., 1988).

peripheral insulin sensitivity is equal to hepatic insulin sensitivity (Matthews et al., 1999).

Application of the specific models of mathematical analysis to oral or intravenous GTT equations detailed below provides assessments of insulin sensitivity (American Diabetes Association, 1997). Radikova reviewed these mathematical models compared to the HEC as gold standard and determined that the optimum sampling times for OGTT are 0, 30, 60, 120 minutes post glucose ingestion. Use of these models in the OGTT is useful in epidemiological studies to determine risk of diabetes in a normal and impaired glucose tolerance population and to assess the degree of insulin sensitivity in non-diabetic populations with other risk factors (Radikova, 2003).

The product equations, HOMA and QUICKI,) provide valid identification and discrimination of individuals over a wide range of weights and glucose tolerances from normal glucose tolerance to frank diabetes (Lanzi, 1999). The lower, the product the more sensitive the tissues (Nishiyama et al., 1988)..

HOMA (Matthews et al., 1985)= 22.5/(fasting insulin x fasting glucose)

The HOMA index correlates well with hepatic insulin sensitivity (0.88 ($p<0.0001$)) (Matthews et al., 1985) and basal hepatic glucose production 0.59 ($p<0.0005$)

(Stumvoll et al., 2000). It is a useful epidemiological index where only fasting glucose and insulin values are available or obtainable (Stumvoll and Gerich, 2001).

HOMA 2 (computer model) was used in this study as it is more appropriate for modern insulin assays and allows an assessment of beta cell function (%B) and insulin sensitivity (%S) where 100% is normal (Wallace et al., 2004).

$$\text{HOMA } \beta = \frac{20 \times \text{insulin}}{\text{Glucose} - 3.5}$$

$$\text{HOMA IR} = \frac{\text{glucose} \times \text{insulin}}{22}^{.5} \%$$

Quantitative insulin sensitivity check index (QUICKI) has been shown to correlate better with the HEC method than HOMA, or fasting insulin or the minimal model in a group of obese, non obese and diabetic individuals (Katz et al., 2000).

$$\text{QUICKI (Katz et al., 2000)} = \frac{1}{\log(\text{fasting insulin}) \times \log(\text{fasting glucose})}$$

Glucose/insulin (G/I) or insulin/ glucose (I/G) ratio are useful in situations of normal glucose tolerance but as fasting glucose concentrations rise it has been

shown to be less reliable (Bastard et al., 2003). Use of plasma glucose: insulin ratio provides poor correlation with HEC, but the product equations (HOMA,QUICKI) correlated well and were superior to fasting insulin alone (Katz et al., 2000, Nishiyama et al., 1988).

2.13 Sample handling

Venesection was performed in the antecubital fossa by the candidate or other trained personnel. All blood samples for IGF-I, P-III-NP, NEFA and adiponectin were collected in serum separator tubes (SST) tubes, glucose into fluoride oxalate tubes, and insulin into lithium heparin tubes (Becton Dickinson Vacutainer tube ®). Clotted samples were centrifuged at 3000rpm for 15 minutes; the serum was extracted and placed in individual labelled aliquot bottles for immediate freezing at –80°C. Fluoride and lithium heparin samples were centrifuged at 3000rpm for 15 minutes, and plasma extracted. Heparin and fluoride were centrifuged immediately while clotted samples was stored at room temperature and allowed to clot for 15-20 minutes before being centrifuged at 3000rpm for 15 minutes.

2.14 Assays

2.14.1 Performance of assays

IGF-I and P-III-NP assays were performed in The Drug Control Centre, King's College London by the candidate and Christiaan Bartlett under the direction of Professor David Cowan. Glucose and adiponectin assays were performed by the candidate. Adiponectin and glucose assays were performed by the candidate. NEFA analysis was kindly performed by Chris Glen in the Institute of

Human Nutrition in the University of Southampton, and insulin assays by Southampton NHS Trust Endocrinology Laboratory.

2.14.2 Glucose Assay

Glucose assays were performed in the Wellcome Trust Clinical Research Facility using theYSI 2300 STAT Blood Glucose Analyser (YSI Bioanalytics Inc.). The plasma sample was aspirated into a buffer filled chamber and exposed to an internal platinum probe mechanism with a three-layer membrane system. The substrate diffuses through the outer protective membrane, and comes into contact with the middle layer membrane that contains immobilized glucose oxidase, where it is oxidised to release hydrogen peroxide H_2O_2. The steady state is achieved when the rate of production of H_2O_2 is equal to the rate at which it leaves this layer. The electron flow rate is a linear relationship between the electron flow rate and the steady state H_2O_2 concentration and thus the glucose concentration. The platinum electrode has an anodic potential and oxides the substrate. The concentration is calculated by differences in current measured in nano amps from baseline current levels.

Reaction 1 (glucose); β-D-glucose + O_2 $\xrightarrow{GO_X}$ Glucono-δ-lactone + H_2O_2

Reaction 2; $H_2O_2 \xrightarrow{\text{Pt anode}} 2H^+ + O_2 + 2e^-$

The inner layer has a cellulose acetate membrane that protects the probe surface from high molecular weight compounds that could potentially act as reducing agents, or damage the platinum surface, e.g. protein or detergents. It can, however, be penetrated low molecular weight agents and hydrogen peroxide. As the sensitivity of the sensors may be affect by temperature-induced

changes, there is a temperature probe within the sample chamber and mathematical corrections are made to results obtained based on the differences in temperature between the sample and the chamber fluid.

2.14.2.1 Calibration

Baseline stability must be assessed prior to measurement. An auto-calibration was performed and if the baseline current was unstable, the chamber was flushed with buffer until the current was stabilised. After each calibration and every 5-sample measurements, the final baseline current was compared to the initial current and flushing performed until stabilisation occurred. Calibration was performed against a known concentration of glucose 180mg/dL (10mmol) ± 1mg/dL. Repeated test sampling is performed 3 times using a 10mmol and 25mmol solution and values within 2% are acceptable.

2.14.3 Insulin Assay

Insulin analysis was performed using an in-house dissociation enhanced lanthanide fluoroimmunoassay (DELFIA). Antibodies and antigens are labelled with non fluorescent chelates e.g. europium, and acidification and addition of the DELFIA enhancement solution induces release of the label ion, chelation with 2-naphthoyl-trifluoroacetone, surrounded by trioctylphosphine oxide and development of fluorescence which is then measured.

2.14.4 Adiponectin Assay

Adiponectin analysis was performed by the candidate using a quantitative sandwich ELISA (Quantikine® Human adiponectin/Acrp30 Immunoassay, R&D Systems, Minneapolis, USA). The sample is added to wells coated with recombinant monoclonal antibody to human adiponectin and a complex formed with the adiponectin in the sample. A further enzyme linked monoclonal adiponectin antibody binds to this complex and a coloured substrate solution added and optical density was measured using a spectrophotometer (TitertekMultiskan Plus). Intra-assay precision provided by the manufacturer at 19.8, 69.9, 143 ng/ml is 2.5%, 3.4%, and 43.7% and inter-assay precision at 20.5, 74.4, 157 ng/ml is 6.8%, 5.8%, 6.9% respectively.

2.14.5 Non esterified fatty acid assay

Non-esterified fatty acid analysis was performed using the Konelab 20 Auto-analyser. NEFA concentrations were measured using a Wako NEFA C ASC-ACOD kit (Alpha Laboratories, Eastleigh, UK).NEFAs undergo a series of reactions, resulting in the production of hydrogen peroxide, which in the presence of peroxidase, produces a change in colour of quinoneimine dye. The intensity of the red pigment produced is proportional to the FA levels in the sample. During venesection any diluted heparin used to maintain line patency was flushed adequately to ensure removal prior to collection of the sample. The volunteer was asked about the ingestion of any possible interfering supplements such as vitamin C.

Nineteen quality control samples with an expected range of 1.09-1.47mmols (mean 1.28 mmols) for NEFA and 0.95-1.16mmols (mean 1.06mmols) for TAG

were analysed during the procedure. Mean values of 1.317±0.02mmols and a coefficient of variation of 1.57% was obtained for NEFA and a mean of 1.15±0.02mmols and a coefficient of variation of 1.7% was obtained for TAG. Within run CV of 3 repeated assays is ≤2.7%.

2.14.6 Urinary nitrogen assessment

Urinary nitrogen concentrations were assessed using the Kjeldahl method. The Kjeldahl method for determination of urinary nitrogen is a three-stage method, involving digestion and distillation and the collection of dissolved ammonia, followed by titration and neutralization by a known amount of hydrochloric acid. The sample was mixed with a digestion mixture of H_2SO_4, a catalyst and salt that was added to increase the boiling point of the mixture. All nitrogen-containing compounds were converted to NH_4^+. An alkali was added and the NH_4^+ was converted to NH_3which was steam distilled into boric acid and titrated with standard HCL. The indicator solution used was methyl purple which was green at pH 5.4, grey at pH 5.1 and purple at pH 4.8.

2.14.6.1 Assumptions

There are three inherent assumptions in the Kjeldahl method. First, recovery of protein is complete or at least is constant. Secondly biological proteins contain 16% nitrogen by weight, so the nitrogen (N) content is multiplied by 6.25. However, this N content depends on the type of protein fraction. It can range from 5.9 to 12.5 with higher factors from protein species that are lipid or carbohydrate conjugates. Non-protein moieties are generally disregarded but even allowing for this the factor can range from 5.69 to 6.52 in normal serum. Thirdly the protein distribution for plasma is similar to that of urine or

cerebrospinal fluid. This is in fact not true and the use of 6.25 as a correction factor is not ideal in these specimens.

2.14.6.2 Calibration

A blank sample was run on all reagents. It contains no sample and the measurement value obtained should be zero. Measurement was performed using known standard solutions to assess accuracy.

2.14.6.3 Calculations

The following equation was then used to calculate urinary nitrogen in grams per litre:

$$\text{Nitrogen (g/L)} = \frac{(\text{test titration} - \text{mean of blanks}) \times 0.1 \times 14.008}{\text{Volume urine used (0.5 mls)}}$$

Protein (g/L) = nitrogen (g/L) x 6.25

The standard is representative of healthy urinary nitrogen levels and had a mean of 5.7±0.39grams/litre and a coefficient of variation of 6.9%.

2.15.7 IGF-I assay

IGF-I concentration was determined using the DSL IGF-I immunoradiometric assay (IRMA) (Diagnostic Systems Laboratories, Webster Texas). It has a minimum detection level (sensitivity) of 0.8ng/ml corrected to 1:30 dilution and intra assay variation of 3.4%, 1.5%, 3.7% at 9.4, 53.8, 255.9 ng/ml respectively.

The DSL IRMA is a non-extraction assay with the addition of acid to dissociate IGF-I from its binding proteins and subsequent flooding with IGF-II to prevent reattachment. This step allows competing of IGF-I and IGF-II and because of the

higher concentration of IGF-II there is more binding of IGF-II to IGF-BPs. This assay utilises 2 region-restricted monoclonal antibodies; antibody to the C-terminal amino acids 62 -70 for capture, and radiolabel led antibody to the amino acid sequences 1-23, and 42-61 for detection purposes. A sandwich complex is formed with an avidin-coated bead, IGF-I and the antibodies, incubated and gamma counted.

2.14.7.1 Issues with IGF-I assays

The IGF-I data in this study were compared to that of the GH-2000 project which used the hydrochloric acid–ethanol extraction Nichols RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) (Dall et al., 2000)In the interval between the GH-2000 and GH-2004 project, Nichols ceased trading and thus the GH2004 data were analysed using the DSL-5600 IRMA. For the analyses in chapter 3, some of the GH-2000 samples were reassayed using the DSL assay and a conversion factor was determined. The conversion factor is discussed in chapter 3.

In addition to this there are some underlying technical issues with the measurement of IGF-I.

i. Elimination of Binding Proteins

The concentration of serum IGFBPs is high and they are known to compete with antibody or receptors for tracer binding and thus reduce the availability of IGF-I binding sites for antibody complex formation as the binding affinity of binding proteins are similar to that of primary antibody used in commercial immunoassay kits (Mohan and Baylink, 1995). It is imperative to dissociate and subsequently

eliminate from solution as much binding protein as possible prior to the radioligand assay. This is dissociation step can be achieved by a number of means. The most common separation step is acidification to a pH of <3.4 to induce dissociation of IGF-I from IGFBP and subsequent removal of these IGFBP by physical or functional extraction techniques.

Physical extraction techniques include size exclusion chromatography in acidic conditions, acid-ethanol extraction (AE) or acid-ethanol cryoprecipitation (AEC) or a combination of these (Mohan and Baylink, 1995), while functional extraction involves addition of excess cold IGF-II. Size exclusion chromatography is time consuming and expensive and dependent on the column used as excessively small dimensions allow fragments of binding proteins to mix with free IGF-I but offers excellent extraction of IGFBP compared to other methods (Deyssig, 1993), and use of Bio-Gel P-10 columns allows IGF-I recovery rates of up to 90%.

AE is a technically simpler technique but up to 30% of IGF-I can be lost with IGFBPs as a co-precipitant (Deyssig, 1993) but a combination of AE and cryoprecipitation improves IGF-I recovery to 95% (Blum and Breier, 1994); improved recovery is also seen with the use of truncated IGF-I as radioligand (Bang and Hall, 1990). Formic acid extraction techniques give higher IGF-I compared to other techniques (Crawford et al., 1992). All these techniques remove IGFBP equally effectively but only 40% removal of lower molecular weight IGFBPs but these molecules do not interfere with the assay measurements on AE or AEC (Crawford et al., 1992). Addition of IGF-II to the sample mixture after dissociation of IGF-I and IGFBPs using acidification allows IGF-II to block the binding sites on the binding proteins (Deyssig, 1993).

ii. Standards

Standardisation of antibody, standards and calibrants used in laboratory practice is important to allow cross comparison of research results and methodology between laboratories (Sjogren et al., 2001). Studies have demonstrated better consistency of interlaboratory data with use of polyclonal antibodies due to the wider binding site possibilities. Monoclonal antibodies are more specific to epitophs and can result in lower detection rates.

iii. Choice of assay

An IRMA was chosen as it is technically easier to perform and does not require overnight incubation which is necessary for many RIA assays (Homayoun et al., 1996). There is a high correlation in IGF-I concentrations between assays and variations in binding protein concentrations does not affect either assay (Homayoun et al., 1996).

2.14.8 P-III-NP Assay

P-III-NP concentration was analysed using the RIA-gnost immunoradiometric assay manufactured by CIS RIA-gnost which is an in-vitro 2 sandwich assay. A solid phase monoclonal mouse antibody, ^{125}I labelled antibodies and P-III-NP in serum are complexed and the tracer counts measured using a gamma scintillation counter. The lower detection limit is 0.1 units of P-III-NP/ml and upper limit of 14 units of P-III-NP/ml. The intra assay variability (manufacturer data) at 0.8, 1.5, 4.0 units/ml concentration is 2.9%. 2.9% and 4.0% respectively, with inter assay coefficient of the variation of 11.3%, 7.8% and 9.3% at concentrations of 0.25, 1.5, and 5.6 units/ml (Schering, 2002).

Chapter 3

The response of IGF-I and P-III-NP to supraphysiological GH administration in non-Caucasian athletes and the validation of the GH marker method as a test for GH abuse

3.1 Introduction

IGF-I and P-III-NP have been shown to be both sensitive and specific markers for detection of GH abuse among athletes. The GH2000 team developed equations for the detection of GH abuse allowing a specificity of approximately 1 in 10,000, but questions have been raised about the validity of the equations outside the Caucasian population in their studies as the vast majority of the subjects in the GH-2000 studies were White Europeans (Wallace et al., 2000). While the sensitivity of the test is also important for its detection rate and thus usefulness, it is considered more important to avoid false positives than miss occasional true positives. This study aimed to determine if there are ethnic differences in the effects of short term supraphysiological GH administration on IGF-I and P-III-NP concentrations, and the validity of the GH 2000 equations for the use of these markers to detect GH abuse. For the purposes of this chapter, dosing regimens were divided into high dose (0.2 IU/kg/day), low dose (0.1 IU/kg/day) and placebo. The study data were compared with the GH2000 data which were previously collected under similar study conditions in four European centres on Caucasian subjects. Apart from the difference in ethnicity of the subjects, the study designs of the GH-2000 and GH-2004 projects were similar.

3.2 Methods

3.2.1 Subjects

The recruitment and inclusion and exclusion criteria for the subjects in this study are described in chapter 2

3.2.2 Procedure

The overall study protocol was outlined in chapter 2. Blood samples for IGF-I and P-III-NP serum sampling were obtained on day 0, 7, 14, 21, 28, 30, 45, 84.

3.2.3 Statistical analysis

Statistical analysis for this part of the study was performed using the SAS software (SAS Institute, Inc., Cary, NC, USA). All analysis was performed on the log-transformed values of IGF-I and P-III-NP. The concentrations of both biomarkers and the GH-2000 scores at each visit day were assessed against the clean observation values (baseline and placebo-treated samples). The analysis was carried out using a mixed effect model with subject defined as a random effect. This model specification allows the analysis to take into account that individual responses might vary because of inherent individual differences. The mixed model was defined with each time point as a within-subject factor, and treatment, ethnicity and age as between-subject factors.

The key feature of a mixed model is that there is at least one factor (the subject in this case) which is considered to be selected at random from the relevant population, and at a least one factor considered to be fixed (e.g. time point).

The GH-2000 study showed marked sexual dimorphism in response to GH, and therefore, the genders were analysed and presented separately. The analysis was performed separately for each treatment arm following an adjustment for the reciprocal of age of each subject. Results are expressed as the mean± SEM.

3.2.3.1 Adjusting for IGF-I assay differences

In the GH-2000 studies, serum IGF-I was determined by a hydrochloric acid–ethanol extraction RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Initially, it was planned that identical assays would be used for the GH-2004 studies but in 2005, Nichols Institute Diagnostics ceased trading and an alternative IGF-I assay was therefore needed. In order to compare the results of the current study with the previous GH-2000 studies, the values of serum IGF-I measured in this study were converted to the scales used by the GH-2000 group. To adjust for assay differences between the GH-2000 and GH-2004 studies, 73 GH-2000 samples were analysed by the Nichols RIA and the DSL-5600 IRMA as previously described. A conversion factor was estimated to convert measured concentrations from the DSL-5600 scales to the measurement scales of the Nichols RIA assay.

GH-2000 RIA=0:660 X DSL - 5600 IRMA

No adjustment for P-III-NP was needed because no significant adjustments had been made to the assay.

3.2.3.2 The GH-2000 detection method

The previously published GH-2000 discriminant function formulae are as follows (Dall et al., 2000):

Male score = -6.586+2.905xlog(P-III-NP)+2.100xlog(IGF-I)-101.737/age

Female score =-8.459+2.454xlog(P-III-NP) +2.195xlog(IGF-I)-73.666/age

These discriminant formulae were derived from amateur athletes participating in the GH-2000 double-blind placebo-controlled GH administration study and are calibrated against the GH-2000 elite athlete population (Dall et al., 2000, Healy, 2005, Wallace, 2000). They are defined such that the mean GH-2000 score is 0 and SD is 1 in elite athletes. A cut-off point for these GH-detection formulae has not been agreed by the WADA. It has been suggested that a possible cut-off point should be at the value of 3.7, equivalent to a false positive rate of ~1 in 10,000 tests.

The GH2000 study analysed IGF-I and P-III-NP concentrations on elite athletes resting and post competition and this was modeled as part of that study with various correction factors e.g. log age, reciprocal of age. Reciprocal of age produced the best fit for the data and this has been confirmed in several subsequent datasets (Erotokritou-Mulligan, 2009).

3.2.3.3 Comparison with the GH-2000 study

In order to compare the response to GH with the white European amateur athletes in the GH-2000 study, assay adjusted IGF-I, P-III-NP and GH-2000 score before and after GH or placebo administration as well as the maximal change in these variables were compared by independent sample t-test.

The details of the previous GH-2000 double-blind placebo-controlled GH administration study are given elsewhere (Dall et al., 2000, Wallace, 2000). In brief, the design was similar to the one adopted in the current study. In total, 102 recreational athletes self-administered placebo or 0.1 IU/kg per day (low dose) or 0.2 IU/kg per day (high dose) GH under double-blind placebo-controlled conditions for 28 days. Blood samples were obtained at baseline and on days 21 and 28 of treatment. IGF-I was measured by the Nichols RIA using a monoclonal antibody after acid–ethanol extraction, and P-III-NP was measured by the CIS Biointernational assay. Despite encouragement from the GH-2004 team, there was evidence that some volunteers may not have completed the treatment or visit schedule as per the protocol (i.e. marker levels fell in some before day 28). We therefore considered that it is appropriate to examine the peak response in relation to baseline values. In order to improve the power of the comparison between ethnicities, the low- and high-dose groups were combined. When these groups were analysed separately, this did not affect the results.

3.3 Results

3.3.1 Baseline characteristics

Fourteen subjects (10 males and 4 females) received the low-dose GH, 16 subjects (11 males and 5 females) received the high-dose GH and 15 subjects (10 males and 5 females) received placebo (please see table 3.1).

	High Dose	Low Dose	Placebo	p value
Men (n)				
n	11	10	10	
Age	23.3 ± 0.52	22.6 ± 0.52	24.6 ± 0.52	p=0.42
Ethnicity				
Indo-Asian*	6	5	6	
Afro-Caribbean	4	4	3	
Oriental	1	1	1	
BMI (kg/m^2)	24.2 ± 0.58	23.4 ± 0.58	25.9 ± 0.58	0.34
IGF-I (µg/L)	545 ± 25.0	578 ± 25.0	554 ± 25.0	0.91
P-III-NP (U/ml)	0.36 ± 0.02	0.56 ± 0.02	0.40 ± 0.02	0.24
GH-2000 score	-1.7 ± 0.17	-0.9 ± 0.17	-1.2 ± 0.17	0.41
Women				
n	5	4	4	
Age	21.9 ± 0.28	22.1 ± 0.28	23.3 ± 0.28	0.43
BMI (kg/m^2)	22.4 ± 0.34	26.7 ± 0.34	23.1 ± 0.34	0.18
Ethnicity				
Asian	1	2	1	0.6
Afro-Caribbean	4	2	3	0.41
Oriental	0	0	1	0.86
IGF-I (µg/L)	457 ± 19	470 ± 19.4	555 ± 19.4	
P-III-NP (U/ml)	0.34 ± 0.01	0.42 ± 0.01	0.32 ± 0.01	
GH-2000 score	-1.5 ± 0.10	-1.2 ± 0.10	-1.6 ± 0.10	

Table 3.1 Baseline characteristics of all subjects by GH allocation.

*14 of the men were from the Indian Subcontinent and 3 were Arabic. All Asian women were from the Indian Subcontinent.

The participants in the three study arms were well matched for age and body composition. Baseline IGF-I and P-III-NP concentrations were similar in each

group. There was no difference in mean baseline GH-2000 score between the groups for either gender. There was no significant correlation between IGF-I and P-III-NP levels at baseline (P=0.65). The GH-2000 scores are below zero in all groups reflecting the lower average IGF-I and P-III-NP in amateur athletes compared with the elite athletes against whom the GH-2000 score was calibrated. This was also found in the original GH-2000 study (Dall et al., 2000).

Although the numbers of subjects within any one ethnic group were small, particularly for the women and Oriental men, there was no difference in the mean baseline IGF-I, P-III-NP or GH-2000 score between the three ethnic groups.

3.3.2 Effect of GH on IGF-I

IGF-I concentrations rose in response to both low- and high-dose GH in both men and women (please see figure 3.1). In men, mean IGF-I concentrations were significantly higher in both GH-treated groups throughout the treatment period (day 28 high-dose GH $p<0.0001$, low-dose GH $P=0.041$ versus placebo). Mean IGF-I remained higher in the high-dose GH group than in placebo for up to 7 days after discontinuation of GH ($p=0.003$). In women, mean IGF-I concentrations were significantly higher in the high-GH group throughout the treatment period compared with placebo (day 28 high-dose GH $p=0.0019$). In the low-dose group, mean IGF-I levels were significantly higher than placebo at the visit on day 21 ($p=0.03$) but not on day 28 ($p=0.09$). Mean IGF-I concentrations were not different from placebo in the washout period in the high-dose group. There was no change in IGF-I concentrations in the placebo group throughout the study period ($p=0.48$ for men and $p=0.23$ for women).

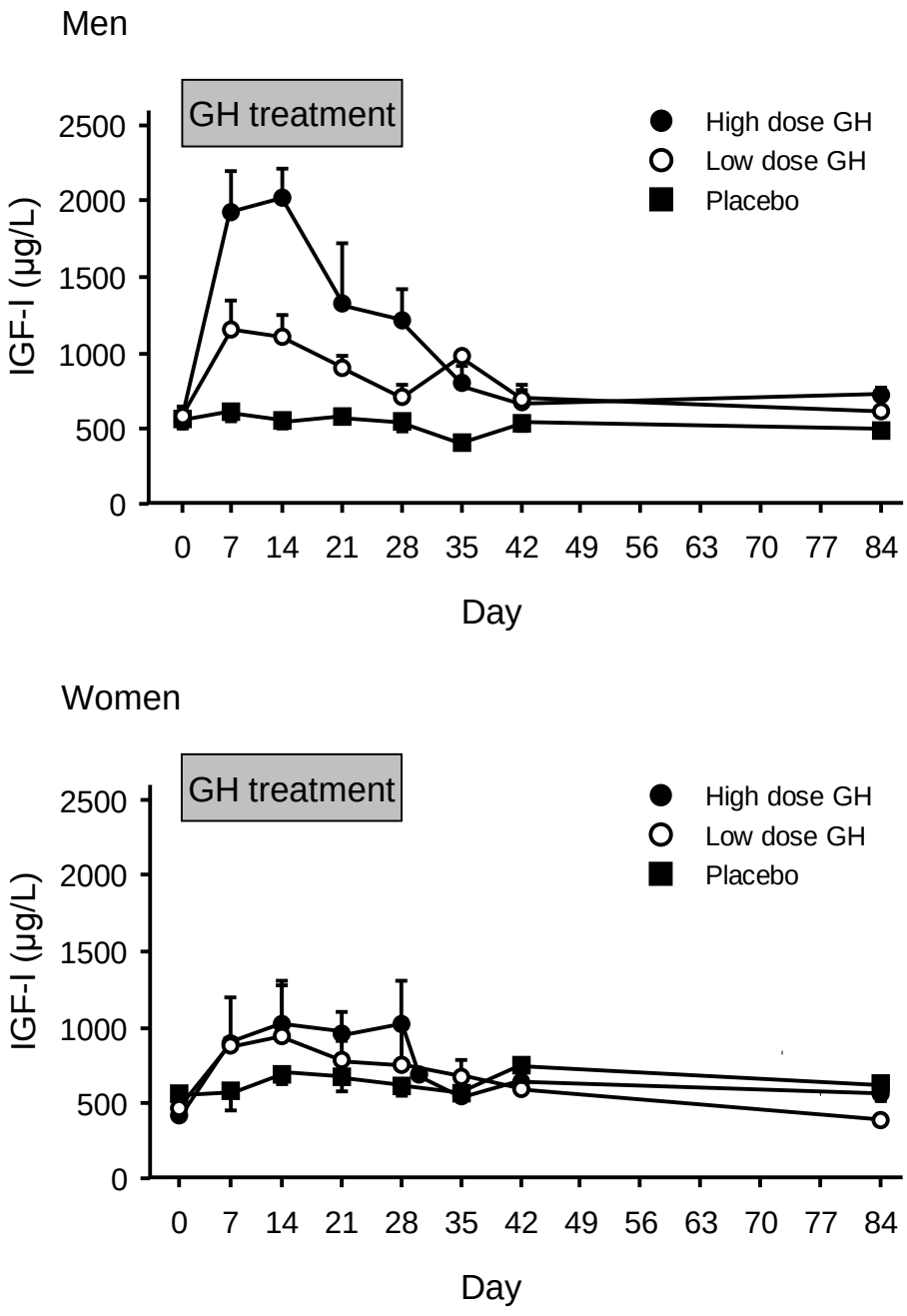


Figure 3.1: Change in serum IGF-I in response to GH 0.2 mg/kg/ day (high dose), 0.1 mg/kg/day (low dose) or placebo. GH was self-administered for 28 days by subcutaneous injection. The subjects were then followed for 56 days during the wash-out period. a) men, b) women. Data are mean ± SEM.

3.3.3 Effect of GH on P-III-NP.

P-III-NP concentrations rose in response to both low- and high-dose GH in both men and women (please see figure 3.2). In men, mean P-III-NP concentrations were significantly higher in both GH-treated groups throughout the treatment period (day 28: high-dose and low-dose: GH $p<0.0001$ versus placebo). In the high-dose GH group, mean P-III-NP remained higher than placebo for up to 56 days after the end of the discontinuation of GH ($p=0.01$ on day 84). In the low-dose GH group, mean P-III-NP remained higher than placebo for up to 14 days after the end of the discontinuation of GH ($p=0.006$) on day 42).

In women, mean P-III-NP concentrations were significantly higher in the high GH group from day 14 of the treatment period compared with placebo (day 28) high-dose GH $p=0.0001$). There was no difference in P-III-NP between the high-dose GH group and placebo during the washout period. There was no difference in P-III-NP between the low-dose GH group and placebo. There was no change in P-III-NP concentrations in the placebo group throughout the study period.

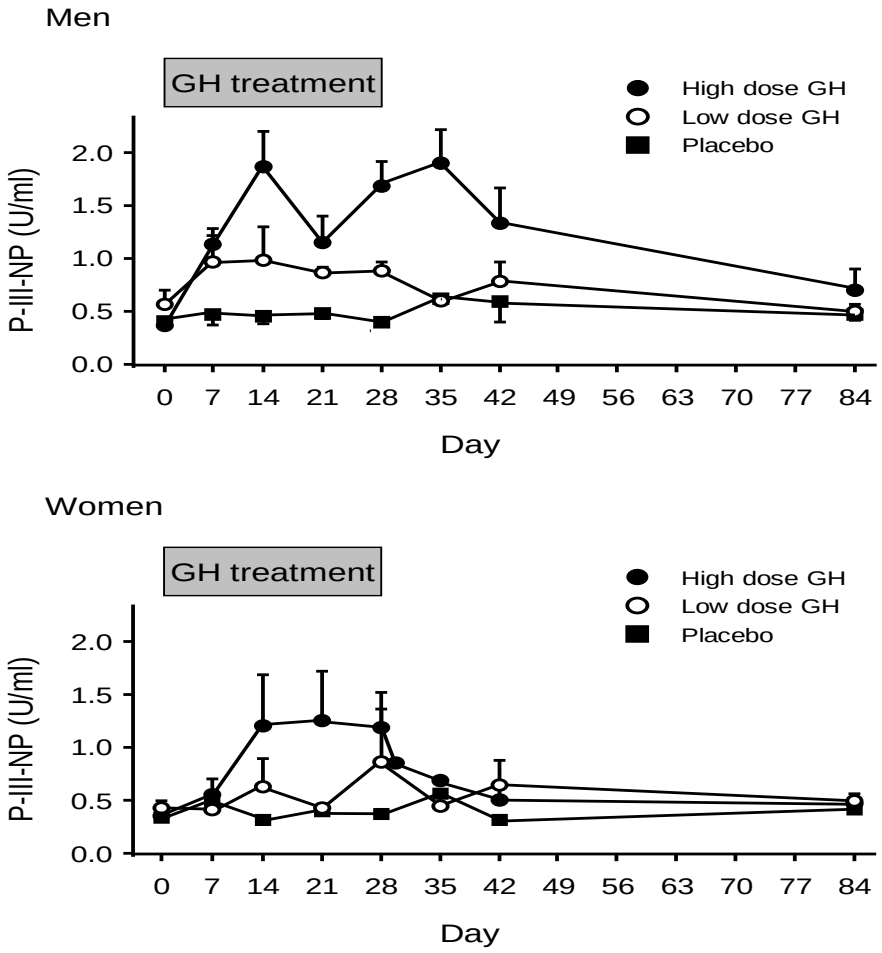


Figure 3.2 Change in serum P-III-NP in response to GH 0.2 mg/kg/ day (high dose), 0.1 mg/kg/day (low dose) or placebo. GH was self-administered for 28 days by subcutaneous injection. The subjects were then followed for 56 days during the wash-out period. a) men, b) women. Data are mean ± SEM.

3.3.4 Effect of GH on GH-2000 score.

The GH-2000 score increased in response to low- and high-dose GH in both men and women (please see figure 3.3). In men, the mean GH-2000 score was significantly higher in GH-treated subjects throughout the treatment period (low- and high-dose GH versus placebo $p<0.0001$ on visit day 28). Compared with the placebo group, the mean score remained significantly higher in the high-dose GH

group throughout the washout period (p=0.01 on day 84) and for 14 days in the low-dose GH group (p=0.002 on day 42). In women treated with high-dose GH, the mean GH-2000 score was significantly higher than placebo throughout the treatment period but not in the washout phase (p<0.0005 versus placebo on visit day 28). There was no significant difference in GH-2000 score between the low-dose GH group and placebo group throughout the study. There was no change in GH-2000 score in the placebo-treated group throughout the treatment period (men p=0.62; women p=0.49).

3.3.5 Effect of ethnicity within the GH-2004 study.

While the numbers were limited, particularly for women and for the Oriental men, there were no trends suggesting any differences between the ethnic groups in peak IGF-I and P-III-NP concentrations, or the GH-2000 score after GH administration.

3.3.6 Comparison with the GH-2000 study.

The subjects in the GH-2000 study were slightly older than the GH-2004 subjects (men 25.7±0.6 vs 23.5 ±0.6 years, p=0.018; women 25.6±0.6 vs 22.5 ±0.5 years, p=0.006). In keeping with the younger age of the subjects in the GH-2004 study, the mean assay adjusted baseline and placebo-treated IGF-I concentrations were higher in the GH-2004 study in the men (p=0.001; table 3.2).

	IGF-I (µg/L) Placebo and Baseline	Peak IGF-I (µg/L) Post-GH	Delta Maximal change in IGF-I from baseline	P-III-NP (U/ml) Placebo and Baseline	Peak P-III-NP (U/ml) Post-GH	Delta Maximal change in P-III-NP from baseline	GH-2000 score Placebo and Baseline	Peak GH-2000 score Post-GH	Delta Maximal change in GH-2000 score from baseline
Men									
GH-2000 study	297 ± 10	871 ± 40	548 ± 191	0.53 ± 0.02	1.71 ± 0.11	1.15 ± 0.52	-0.7 ± 0.1	4.9 ± 0.2	5.1 ± 1.5
GH-2004 study	361 ± 18	888 ± 88	518 ± 382	0.43 ± 0.04	1.49 ± 0.17	1.00 ± 0.73	-1.3 ± 0.2	3.4 ± 0.4	4.7 ± 2.2
P value	p=0.001	p=0.85	p=0.72	p=0.01	p=0.26	p=0.41	p=0.005	p=0.01	p=0.41
Women									
GH-2000 study	320 ± 12	647 ± 40	348 ± 181	0.50 ± 0.01	1.03 ± 0.08	0.54 ± 0.39	-0.6 ± 0.1	2.6 ± 0.3	3.4 ± 1.6
GH-2004 study	335 ± 23	701 ± 103	377 ± 304	0.37 ± 0.02	1.06 ± 0.29	0.73 ± 0.95	-1.4 ± 0.2	1.7 ± 0.8	3.2 ± 2.5
P value	p=0.57	p=0.64	p=0.74	p=0.001	p=0.92	p=0.40	p=0.004	p=0.29	p=0.88

Table 3.2 Baseline, delta and peak IGF-I, P-III-NP and GH-2000 scores in the men and women who participated in the GH-2000 and GH-2004 double blind placebo controlled GH administration study.

There was no difference in the assay adjusted baseline and placebo-treated IGF-I concentrations in the women (p=0.57). By contrast, the baseline and placebo-treated P-III-NP concentrations were lower in the GH-2004 study in both men (p=0.01) and women (p=0.001). The baseline and placebo-treated GH-2000 score were lower in the GH-2004 study (men p=0.005, women p=0.004). Following GH administration, there was no difference in the peak IGF-I or P-III-NP between the studies (see figure 3.4, 3.5 and table 3.2).

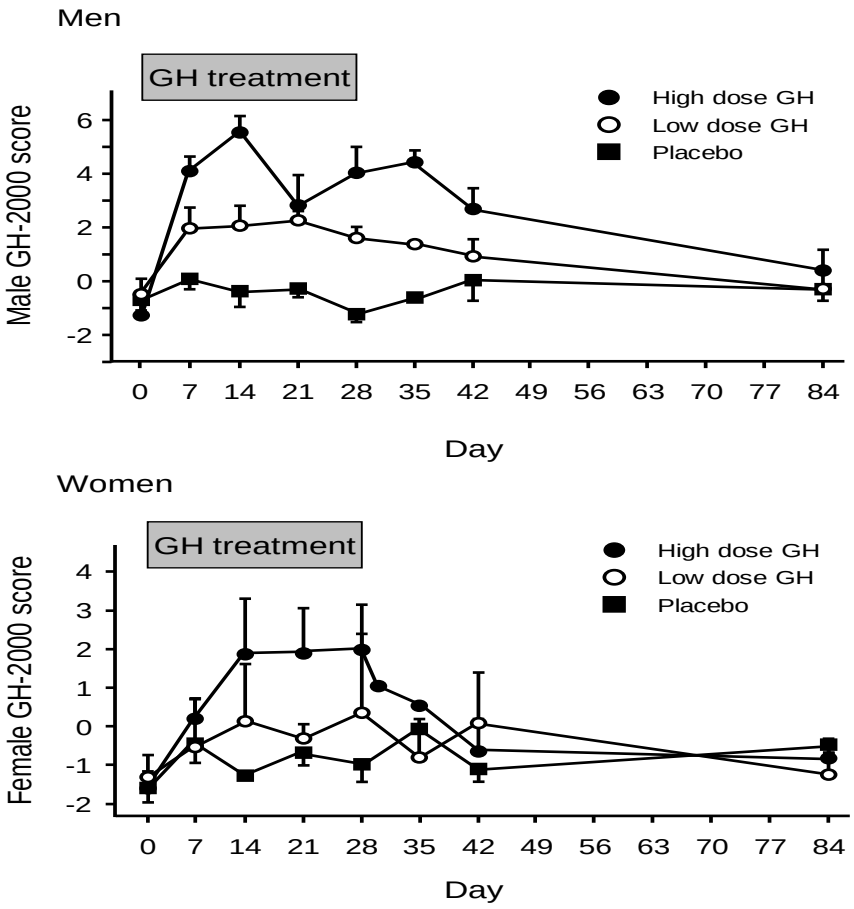


Figure 3.3 Change in GH-2000 score in response to GH 0.2 Units/kg per day (high dose), 0.1 Units/kg per day (low dose) or placebo. GH was self-administered for 28 days by subcutaneous injection. The subjects were then followed for 56 days during the washout period. (A) men and (B) women. Data are mean±S.E.M. The fall in mean GH-2000 score in high-dose male group from days 14 to 21 reflects the non-compliance of two men whose scores remained low for the remainder of the study.

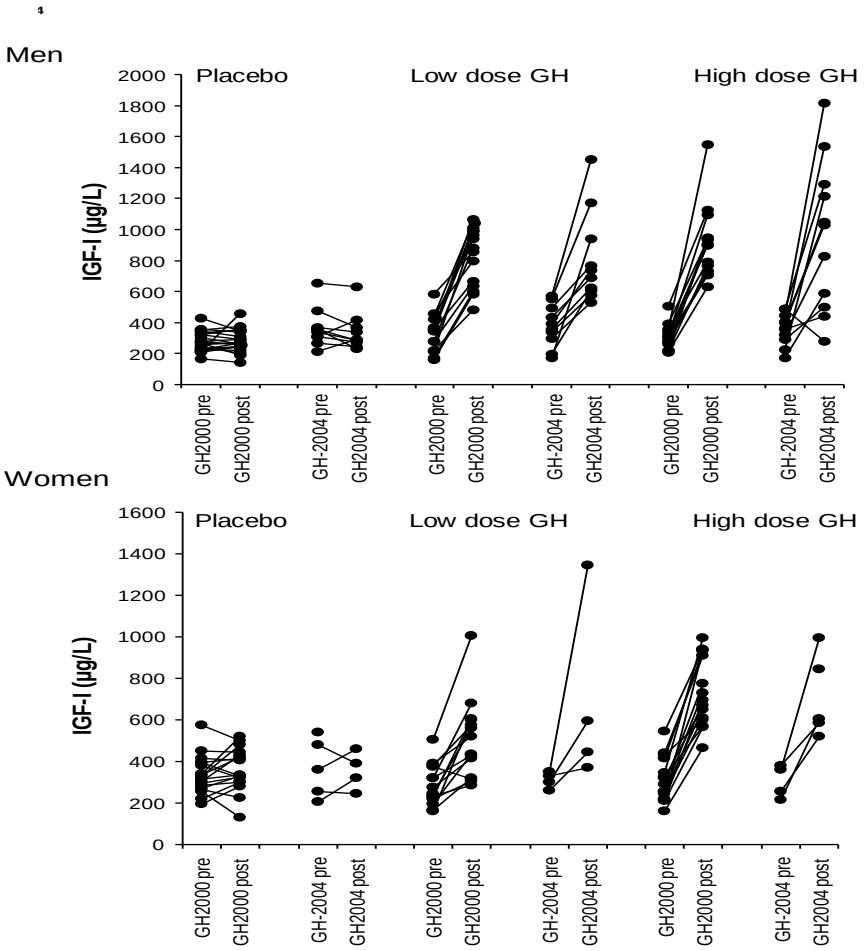


Figure 3.4 Change in serum IGF-I from baseline to peak IGF-I in the individual subjects who had received GH 0.2 mg/kg/ day (high dose), 0.1 mg/kg/day (low dose) or placebo. These data are compared with the previous GH-2000 project which utilised an identical protocol

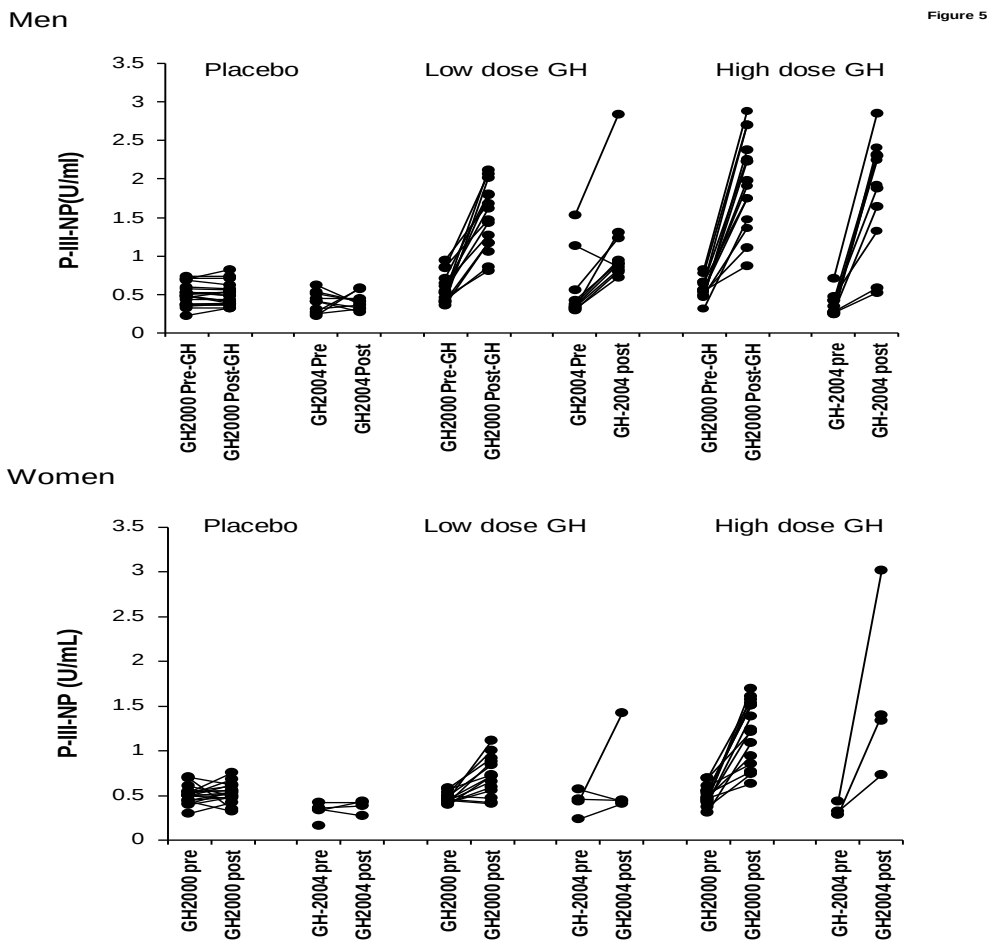


Figure 3.5 Change in P-III-NP from baseline to peak P-III-NP in the individual subjects who had received GH 0.2 mg/kg/ day (high dose), 0.1 mg/kg/day (low dose) or placebo. These data are compared with the previous GH-2000 project which utilised an identical protocol.

There was one women in the high dose GH group and a man and women in the low dose group with a P-III-NP concentration that was higher than that expected and remains an outlier. There was no reported injury or other reason found for these rises.

The peak post-GH GH-2000 score was lower in the men of the GH-2004 study but there was no difference in women (see figure 3.6). There was no difference between studies in the maximal change in IGF-I, P-III-NP and GH-2000 score in response (calculated as difference between peak and baseline) to GH in either

men or women. There was no significant effect of ethnicity in response to GH even when both treatment groups were combined.

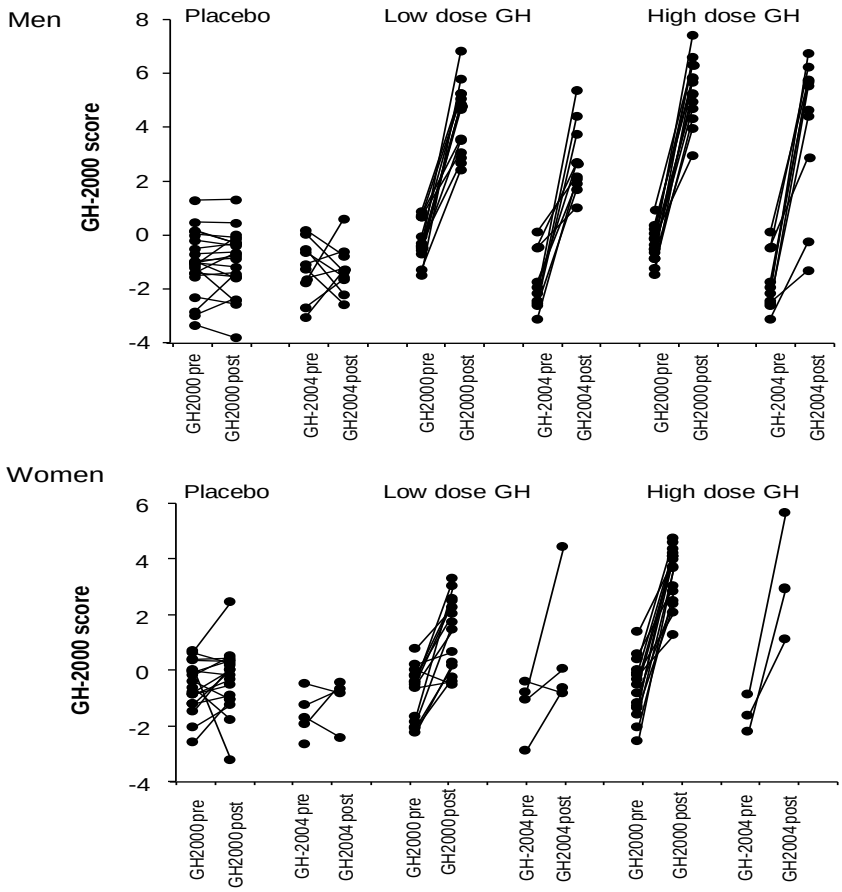
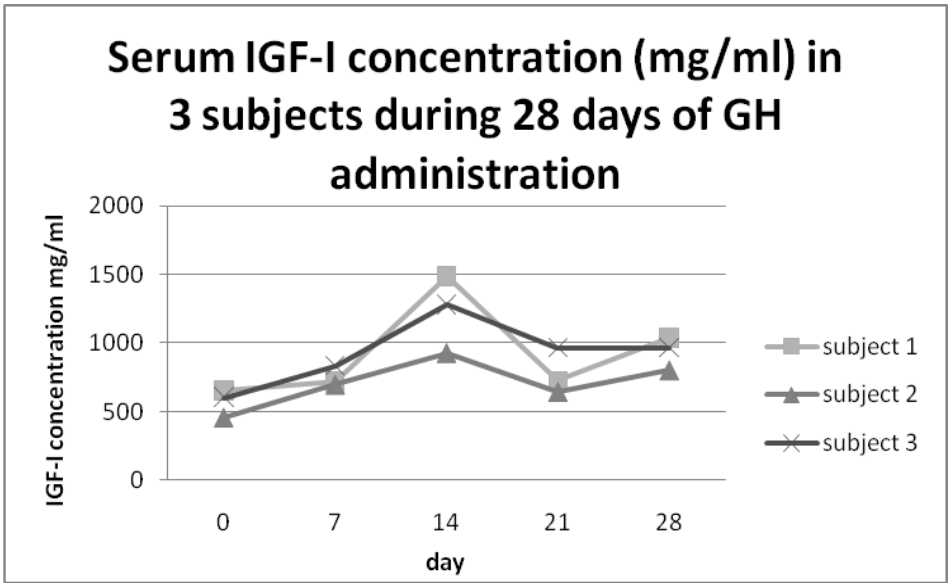


Figure 3.6 Change in GH-2000 score from baseline to peak GH-2000 score in the individual subjects who had received GH 0.2 IU/kg/ day (high dose), 0.1 IU/kg/day (low dose) or placebo. These data are compared with the previous GH-2000 project which utilised an identical protocol

Some subjects who were receiving GH had a drop in IGF-I at day 21 which was unexpected and figure 3.7 shows IGF-I and P-III-NP concentrations over the 84 day period in 3 such subjects. This resulted most likely from non-compliance.



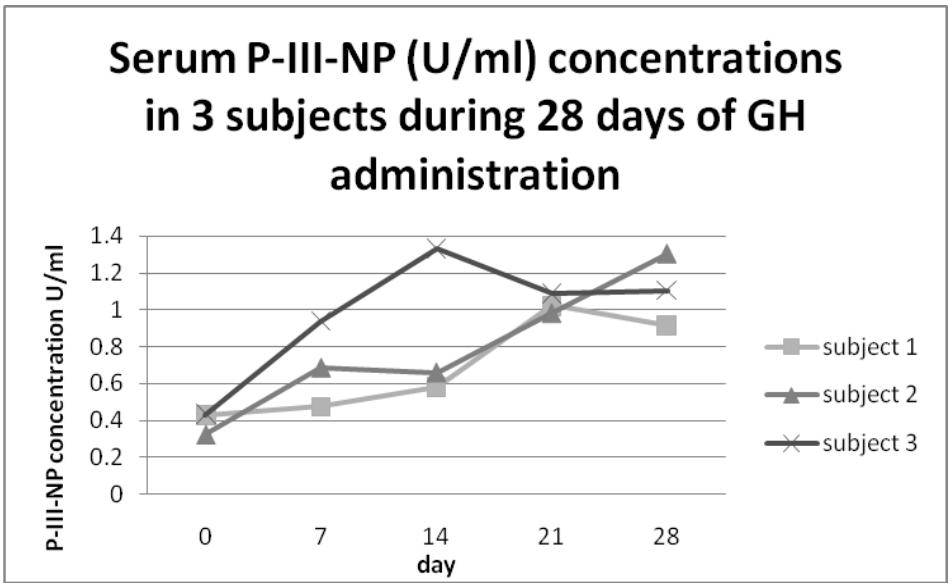


Figure 3.7 IGF-I and P-III-NP concentrations in 3 subjects receiving GH showing a drop in IGF-I at day14 and 21, suggesting poor compliance.

3.4 Discussion

The study has confirmed that serum IGF-I and P-III-NP concentration, and GH-2000 score increase in response to GH administration in a dose-dependent manner in healthy male and female amateur athletes of non-white European ethnic origin. The gender differences observed in the GH-2000 study were also seen in the current study (Powrie, 2007, Dall et al., 2000). Although there were baseline differences in IGF-I and P-III-NP between the GH-2000 and GH-2004 studies, there was no measurable difference in the peak IGF-I and P-III-NP or maximal change in these markers following GH administration. The overall study numbers were low but based on the data from this study ethnicity should not have a major impact on the performance of the GH-2000 detection method.

This is critical to the implementation of the GH-2000 test for the detection of GH abuse in professional sportsmen and women, and addresses the concerns raised by the IOC expert panel in Rome. Previous studies of non-athletic populations have shown that IGF-I concentrations tend to be slightly higher (~10%) in Caucasians of Northern European ancestry (DeLellis, 2003, McGreevy), although this may reflect differences in body composition (Bagg, 2006). Two studies have examined IGF-I concentrations in elite athletes (Nelson, 2006, Erotokritou-Mulligan, 2009). An Australian study of 699 males and 404 female elite athletes found no significant difference in IGF-I concentrations between African, Asian, Oceanian and Caucasian athletes. The same study also reported that P-III-NP was ~8.5% higher in Asians compared with Caucasians but overall the contribution of ethnicity to the variation of IGF-I and P-III-NP was <2% (Nelson, 2006). The second study, which was undertaken by the GH-2004 project and included 242 male and 62 female elite athletes from 50 different nationalities,

found small differences in IGF-I and P-III-NP concentrations between athletes of different ethnicities but almost all the observations were below the upper 99% prediction limits derived from white European athletes (Erotokritou-Mulligan, 2009).

In keeping with these findings, the baseline and placebo-treated P-III-NP values were lower in the GH-2004 study compared with the white European subjects in the GH-2000 study. By contrast, the baseline and placebo-treated IGF-I concentrations were slightly higher in the male GH-2004 subjects, which may reflect their younger age (Toogood, 2003). Consequently, the baseline and placebo-treated GH-2000 score was lower in the GH-2004 study. Although this may reflect the ethnic difference between studies.

Notwithstanding genetic homogeneity within ethnic groupings, there have been physical differences between ethnicities reported in the literature but the relative contributions of physiological and sociocultural factors remain controversial. Afro-Caribbean men have been shown to have a higher percentage of type II muscle fibres than Caucasian men (Ama et al., 1986). Type II muscle fibres concentrations have been shown to be high in power athletes (Komi et al., 1977) Caucasians have <60% skeletal muscle creatinine kinase activity compared to Afro-Caribbean's which confer advantage to the latter in short distance events (Ama et al., 1986).

These findings of lower baseline GH2000 scores in our study may also reflect differences in the levels of training between the studies. Although the entry criteria were similar for both studies, recruitment was more challenging for the GH-2004 because this was restricted to non-white European subjects. Consequently, the subjects in the GH-2004 were more difficult to recruit, and may have been less well trained. This same difficulty meant that fewer women and Oriental men were recruited than intended, which reduced the power to detect changes between women of different ethnicities and in Oriental men.

Despite these small baseline differences in marker concentrations, there was no measurable difference in the peak IGF-I and P-III-NP or maximal change in these markers following GH administration, indicating that people of different ethnicities respond to GH in a similar manner. This is important for the implementation of the GH-2000 methodology as an anti-doping test. For practical reasons, it is not easy to assess accurately 'ethnicity' in an anti-doping setting as many athletes have parents or grandparents from differing ethnic backgrounds. Furthermore, if a test was dependent on ethnicity, it may be possible for a defence lawyer to argue that there had been insufficient validation of the test if their client had a rare ethnic background. In both the GH-2000 and GH-2004 studies, the double-blind rhGH administration studies were undertaken in amateur athletes for ethical reasons. During the statistical analysis of the GH-2000 study, it was found that IGF-I, P-III-NP and GH-2000 score were significantly higher in professional athletes than in amateur athletes (Healy, 2005). Since the methodology will only be used in elite athletes, this required an adjustment of the cut-off point in the GH-2000 score to accommodate the higher baseline values seen in elite athletes (Powrie, 2007). The GH-2000 score was defined such that the mean of the elite athlete population is 0 and each S.D. is 1. In keeping with this difference between amateur and professional athletes, the baseline GH-2000 scores were again lower in both men and women in the current GH-2004 study.

Following GH administration, the GH-2000 score rose significantly in both men and women, to a similar extent to that observed in the original GH-2000 study that included a large majority of white European people. The post-GH GH-2000 score was lower in the men in the current study, possibly reflecting the lower baseline score. It is conceivable that this may affect the performance of the test, but in reality this study is unsuitable for any formal estimation of 'sensitivity'. We cannot estimate the sensitivity of the test in the anti-doping arena where the

doses are thought to be five- to tenfold higher than those used in this study and regimens of GH administration are unknown. Higher doses and longer periods of administration are likely to result in further increases in IGF-I and P-III-NP, particularly in women who are less sensitive to the actions of GH. Nevertheless, a high proportion of men taking the higher dose of GH were detected, in whom compliance was thought to be relatively good. For the same reasons, it is not possible to measure 'window of opportunity' accurately, but it is interesting to note that one of the men who had received high-dose GH had a score above 3.7 56 days after discontinuing GH.

The GH-2000 test relies on immunoassays that measure IGF-I and P-III-NP and, according to the WADA rules, two assays measuring each analyte and recognising different epitopes are needed. Ideally, as recommended by GH-2000 in 1999, the IOC and WADA should own the assays to retain the control of the assay performance and to prevent problems arising when manufacturers of the commercial assays make changes that affect the cut-off level for the GH-2000 score (Holt, 2004a). Following the Rome meeting, the Institute for Bioanalytics (IBA, Branford, CT, USA) was successful in obtaining funding from the US anti-doping agency (USADA) to develop two 'in-house' IGF-I and P-III-NP immunoassays but this work was never completed. As in-house assays were not available, the GH-2004 project initially planned to use the same commercial assays as the GH-2000 project. In the interim, however, and in keeping with the concerns raised to the IOC in 1999, the Nichols IGF-I RIA was withdrawn from the market (Dall et al., 2000). An alternative commercial assay was chosen but in order to interpret the results from the DSL IRMA used in this study, it was necessary to convert the DSL IGF-I values to the scales used in the GH-2000 study. The decision to use the GH-2000 measurement scale as the 'gold standard' was made to maintain consistency across the substantial information previously published by the GH-2000 group. The relationship between the DSL and Nichols assay has been studied by Krebs et al. (Holt, 2004b), who found that

the DSL assay had a higher concordance with the Nichols assay than four other commercially available IGF-I immunoassays (IDS OCTEIA IGF-I, DRG IGF-I 600 ELISA, Mediagnost IGF-I ELISA and Siemens Immulite 2500 IGF-I). There was the anticipated spread of data round the fitted line, which adds a degree of measurement uncertainty that must be taken into account when calculating the appropriate cut-offs for the test. The 95th percentile of the dispersion of residuals was 56.9 mg/l, and the correlation coefficient was 0.864.

Consistent with our analysis, the median of the DSL IGF-I results was 28.6% higher than Nichols values. In the Bland–Altman analysis, the mean difference was 30.8 mg/l, and the scattering of results appeared homogeneous across the measurement range. The need to use different assays will have reduced the precision with which we can estimate differences in mean response to GH between the white European recreational athletes of the GH-2000 study and non-Caucasian athletes of the current study. It is reassuring, however, that the results obtained were comparable to the original GH-2000 study. Nevertheless, before the test can be finally implemented, WADA has asked that further validation of another set of commercial assays in a population of elite athletes be undertaken.

This study has a number of limitations; it did not examine the ethnic differences in responses over the entire treatment and washout periods because the difference in the timing of the samples between the GH-2000 and GH-2004 studies precluded area under the curve analysis; however, this is less relevant to anti-doping than peak response. The small numbers in each individual ethnic group meant that the power to detect differences between the three ethnicities studied was limited. Not all the individuals attended for all their scheduled follow-up visits; this was particularly apparent on day 35 which limited our ability to ascertain how quickly the markers and scores fell once rhGH was discontinued. In this study, the samples from one individual were not all assayed in the same assay run in order to maintain blinding in the laboratory as this reflects current

anti-doping procedures; however, this approach may have reduced the power to detect differences between individuals because of inter-assay variability. Larger studies involving more amateur athletes would be needed to prove conclusively that there are no differences in the response to GH between different ethnic groups.

In conclusion, this study has shown that there is no evidence that non-Caucasian athletes respond differently to GH than white European athletes, at least in terms of the peak and maximal change in IGF-I and P-III-NP. While the study cannot exclude small differences between ethnic groups, these data indicate that ethnicity should not have a major impact on the performance of the GH-2000 detection method.

Chapter 4

Study of the effects of 28 days of supraphysiological GH administration on body composition and markers of physical fitness

4.1 Introduction

Growth hormone has been shown to have anabolic properties. GH deficiency is associated with loss of muscle mass and strength and exercise capacity while restoration of GH concentrations to normal levels leads to restoration of muscle mass, and exercise capacity. Chronic GH excess states such as acromegaly have not been shown to enhance strength and in fact lead to reduced muscle strength (Nagulesparen, 1976).

Growth hormone (GH) administration has also been shown to be lipolytic, reduces total body fat mass, most notably visceral fat mass as well as reducing whole body protein turnover (Salomon, 1989). Reductions in body fat and increased muscle mass and tensile strength could potentially confer a performance advantage in weight bearing sports (Gibney, 2007). The performance enhancing effect of changes in extracellular water content remain controversial (Gibney, 2007).

This study assessed the effect of 28 days of GH administration on markers of physical performance; body composition measured by total body weight, anthropometry (skinfold thickness and circumference measurement), air displacement plethysmography, bioelectrical impedance analysis, blood pressure, and indirect calorimetric assessment of VO_2 maximum during a Bruce

protocol treadmill running test in young healthy non-obese non-Caucasian individuals.

4.2 Methods

4.2.1 Subjects

The recruitment and inclusion and exclusion criteria for the subjects in this study are described in chapter 2. Additional data is included in this chapter referring to the fitness level of participants. Subjects were required to participate in sporting activity with a minimum of 30 minutes duration, with a minimum frequency of twice per week.

A breakdown of the sporting activities recorded are listed in the table 4.1. Many volunteers participated in multiple activities.

Participation in sporting activity	GH	Placebo
	N	n
Rugby	1	3
Squash	2	0
Swimming	2	0
Gym (cardiovascular and resistance)	11	3
Tennis	3	1
Basketball	5	5
Football	8	3
Volleyball	0	2
Athletics	3	1
Hockey	2	0
Badminton	1	0
Cricket	2	0
Fencing	0	1
Boxing	2	0
Martial arts	1	1

Table 4.1 Baseline data for sporting activities undertaken by subjects in each group, for a minimum of 30 minutes duration, at least twice per week for 1 year.

Most volunteers were playing at club level (20 in GH group and 13 in placebo group) while some played in local competitive events (4 in GH and 3 in placebo group) and 1 competed at regional level in the GH group and one at national. The average competitive level was 5.7 and 4.0 respectively in each group.

Level of competition	GH	Placebo
Club (level 6)	20	13
Local (level 5)	4	3
Regional (level 4)	1	0
National (level 3)	1	0
International (level 2)	0	0
Olympic (level 1)	0	0
unreported	5	1
Mean level of competition	5.7	4.0

Table 4.2 Current competitive level of volunteers in each group. Club competition was given a value of 6, local (5), regional (4), national (3), international (2) and Olympic (1).

4.2.2 Procedure

The study protocol is described in chapter 2.

4.2.3 Statistical analysis

The statistical analysis is described in chapter 2. Because of the small study numbers, the subjects were divided into 2 groups only: those receiving GH which included subjects receiving low and high dose GH, and those receiving placebo.

4.3 Results

4.3.1 Baseline characteristics

The baseline data were that of the patient group described in chapter 3. There was no significant difference in any measure of body composition or exercise capacity between the groups at baseline. Please see table 4.3.

	GH		placebo	
	men n=14	women n=9	men n=9	women n=3
	mean±SEM	mean±SEM	mean±SEM	mean±SEM
BMI	23.2 ± 0.8	24.8 ± 1.5	25.3 ± 1.3	24.3 ± 1.6
% body fat:				
anthropometry	14.8 ± 0.9	27.1 ± 1.6	18.5 ± 2	29.1 ± 1.8
bioelectrical impedance analysis	12.4 ± 0.8	26.5 ± 2.2	15.1 ± 2.3	27.1 ± 3.8
BODPOD	11.6 ± 1.9	22.2 ± 3	15.5 ± 2	22 ± 1.9
% lean	86.5 ± 1.1	77.2 ± 2.8	82.6 ± 2.6	74.0 ± 4.4
% water	61.1 ± 1.1	52.9 ± 2.3	58 ± 2.2	50.5 ± 3.8
systolic blood pressure	131 ± 2	111 ± 6	124 ± 5	111 ± 13
diastolic blood pressure	67 ± 2	69 ± 3	65 ± 3	66 ± 4
time on treadmill(minutes)	13.64 ± 0.43	10.13 ± 0.74	13.98 ± 0.86	10.82 ± 0.55
VO_2 maximum (L/min)	2.44 ± 0.16	1.45 ± 0.17	3.42 ± 0.44	1.97 ±0.25
spec VO_2 maximum (ml/kg/min)	34.27 ± 7.22	28.16 ± 4.65	44.89 ± 5.43	36.45 ± 5.21

Table 4.3 Baseline characteristics of subjects.

4.3.2 Body Weight

There was a significant increase in mean body weight in men receiving GH (p=0.01), but there was no change in weight in any other group. The weight changes from baseline in the subject groups are shown in the table 4.4.

Weight (kgs)	GH		placebo	
	men n=14	women n=9	men n=9	women n=3
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
day 0	72 ± 3.4	65.7 ± 3.9	81.7 ± 4.0	69.1 ± 6.2
day 28	73.1 ± 3.2	65.2 ± 3.8	82.1 ± 4.0	68.6 ± 5.8
	p=0.01	p=0.47	p=0.2	p=0.59

Table 4.4. Total body weight data (kgs) before and after GH administration (day 0 and 28 respectively) in all groups, and the statistical significance values for the change (paired t tests).

There was no significant difference when comparing variables between the GH and placebo group.

4.3.3 Anthropometry

4.3.3.1 Waist, Hip and Midarm Circumference.

In men treated with GH there was a significant rise in hip circumference (p= 0.04). No change in waist or mid-arm circumference was observed. There were no changes in any of the measurements in any of the other groups. The data for waist, hip and midarm circumference are shown in table 4.5, and 4.6. The significance values for the comparison between day 0 and day 28 in each group by paired ttests are given in table 4.7

Circumference measurements and waist to hip ratio		GH	
		men	women
		n=14	n=9
		mean ± SEM	mean ± SEM
waist (cm)	day 0	79 ± 2.1	77.6 ± 3.2
	day 28	79.1 ± 1.7	76.8 ± 2.9
hip (cm)	day 0	96.3 ± 2.7	96.3 ± 2.2
	day 28	98.6 ± 2.9	99.2 ± 3.1
midarm (cm)	day 0	33.9 ± 2.3	30.6 ± 2.3
	day 28	32.3 ± 1.0	32.9 ± 4.6
waist to hip ratio	day 0	0.84 ± 0.04	0.8 ± 0.03
	day 28	0.79 ± 0.01	0.78 ± 0.02

Table 4.5 Waist, hip, midarm circumference measurements and waist to hip ratio before and after treatment (day 0 and 28) in the group receiving GH.

Circumference measurements and waist to hip ratio		Placebo	
		Men	women
		n=9	n=3
		mean ± SEM	mean ± SEM
waist (cm)	day 0	87.5 ± 3.4	83.7 ± 6.2
	day 28	86.7 ± 3.2	79.0 ± 2.5
hip (cm)	day 0	103.9 ± 3.2	101.4 ± 3.1
	day 28	103.08 ± 3.1	100.4 ± 1.6
midarm (cm)	day 0	36.6 ± 3.6	25.4 ± 0.6
	day 28	33.2 ± 1.0	27.6 ± 1.3
waist to hip ratio	day 0	0.84 ± 0.01	0.82 ± 0.04
	day 28	0.84 ± 0.02	0.78 ± 0.02

Table 4.6 Waist, hip, midarm circumference measurements and waist to hip ratio before and after treatment (day 0 and 28) in the group receiving placebo.

Circumference measurements and waist to hip ratio		p values comparing day 0 and day 28 (paired ttest)			
		GH		Placebo	
		men	women	men	women
		n=14	n=9	n=9	n=3
waist (cm)		0.94	0.28	0.21	0.37
hip (cm)		0.04	0.53	0.77	0.7
midarm (cm)		0.57	0.46	0.36	0.37
waist to hip ratio		0.28	0.25	0.89	0.45

Table 4.7 Significance values using unpaired ttests comparing day 0 and day 28 (before and after treatment) for waist, hip, midarm circumference measurements and waist to hip ratio in all groups.

When comparing treatment and placebo groups, the baseline and day 28 waist circumference in men receiving placebo was significantly greater than those receiving GH (day 0;p=0.04, day 28;p=0.03). There was no difference between any other groups.

4.3.3.1.1 Waist to Hip Ratio

There was no significant change in waist to hip ratio in any group from day 0 to day 28. When comparing treatment and placebo groups there was a significant difference in waist-to-hip ratio on day 28 (p=0.03) in the men on GH not seen in the placebo group and not present at baseline on day 0 (p=0.98). There was no significant difference in women on day 0 or 28 (p=0.72, 0.82 respectively). This data is presented in table 4.5, 4.6.

4.3.3.2 Total body fat

There were 3 measures of body fat undertaken in this study (anthropometry, bio electrical impedance analysis and air displacement plethysmography (BODPOD®)). There was no statistically significant correlation on regression analyses between body fat calculated by anthropometry and any other measure of body fat. Please see table 4.8 and 4.9.

Regression analyses

Statistical data correlating body fat calculated by anthropometry(day 28) (log transformed) with other variables (linear regression analysis) in subjects on GH						
dependent variable : log transformed IGF-I day 28	Men			women		
	N	p	r2	n	p	r2
BMI day 28 (log)	14	0.363	0.363	9	0.33	0.515
fat by bioelectrical impedance analysis	14	0.46	0.075	9	0.65	0.605
Air displacement plethysmography	14	0.71	0.025	9	0.28	0.623

Table 4.8. Regression analysis correlation data between total body fat on day 28 calculated by anthropometry and that of BMI, fat calculated by bioelectrical impedance analyses and air displacement plethysmography. Subjects receiving GH.

Statistical data correlating body fat calculated by anthropometry(day 28) (log transformed) with other variables (linear regression analysis) in subjects on placebo						
dependent variable := log transformed IGF-I day 28	men			women		
	n	p	r2	n	p	r2
BMI day 28 (log)	14	0.48	0.533	3	none available	0.077
fat by bioelectrical impedance analysis	14	0.25	0.882	1	none available	0
Air displacement plethysmography	14	0.4	0.914	1	none available	0.642

Table 4.9. Regression analysis correlation data between total body fat on day 28 calculated by anthropometry and that of BMI, fat calculated by bioelectrical impedance analyses and air displacement plethysmography. Subjects receiving placebo.

Statistical data correlating IGF-I (day 28) (log transformed) with other variables (linear regression analysis) in subjects on GH						
dependent variable : log transformed IGF-I day 28	men			women		
	n	P	r2	n	p	r2
BMI day 28 (log)	14	0.635	0.021	9	0.438	0.156
fat by bioelectrical impedance analysis	14	0.591	0.025	9	0.769	0.021
Air displacement plethysmography	14	0.395	0.081	9	0.963	0.002
adiponectin	14	0.153	0.177	9	0.674	0.027

Table 4.10. Regression analysis correlation data in subjects on GH between IGF-I (log transformed) on day 28 calculated by anthropometry and that of BMI, fat calculated by bioelectrical impedance analyses and air displacement plethysmography.

Statistical data correlating IGF-I (day 28) (log transformed) with other variables (linear regression analysis) in subjects on placebo						
dependent variable : log transformed IGF-I day 28	men			women		
	n	p	r2	n	p	r2
BMI day 28 (log)	14	0.896	0.003	3	none available	none available
fat by bioelectrical impedance analysis	14	0.755	0.015	1	none available	none available
Air displacement plethysmography	14	none available	0	1	none available	none available

Table 4.11. Regression analysis correlation data in subjects on placebo between IGF-I (log transformed) on day 28 calculated by anthropometry and that of BMI, fat calculated by bioelectrical impedance analyses and air displacement plethysmography

4.3.3.2.1 Total body fat measured by anthropometry

There was no significant change in any group following treatment, or between GH and placebo groups in any of the skinfold measurements or in total body fat calculated by anthropometry.

In men treated with GH, there was no significant change with treatment (day 0 and day 28) in biceps skinfold (p= 0.64), triceps skinfold (p= 0.61), subscapular skinfold (p= 0.33), suprailiac skinfold (p=0.37). In women treated with GH, there was no significant change with treatment (day 0 and day 28) in biceps skinfold (p= 0.46), triceps skinfold (p= 0.16), subscapular skinfold (p= 0.86), suprailiac skinfold (p=0.85).

Men receiving GH had a total body fat percentage calculated from anthropometry on day 0 of 14.8±0.9%, and on day 28 of 15.1±0.9% (p=0.41). Women treated with GH had a total body fat calculated by anthropometry on day 0 of 27.1±1.6%, and on day 28 of 27.8±1.7% (p=0.55), men on placebo on day 0 of 18.5±2.0% and on day 28 18.3±2.1 (p=0.71), women on placebo on day 0 of 29.1±1.8% and on day 28 of 30.5±0.7% (p=0.68).

4.3.3.3.2 Total body fat measured by bioelectrical impedance analysis

No significant change was seen in total body fat measured by bioelectrical impedance analyses from day 0 to day 28 in any group. Please see table 4.12.

total body fat calculated by bioelectrical impedance analysis					
		GH		placebo	
		men	Women	men	Women
		n=14	n=9	n=9	n=3
		mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
	day 0	12.4 ± 0.8	26.5 ± 2.2	15.1 ± 2.3	27.1 ± 3.8
	day 28	11.7 ± 1.0	24.2 ± 2.0	16.5 ± 2.7	25.8 ± 2.6
		p=0.47	p=0.0.07	p=0.62	p=0.33

Table 4.12 Total body fat calculated by bioelectrical impedance analysis before and after treatment (day 0 and 28) in all groups, and the statistical significance data for the change (paired t tests).

When comparing changes in the treatment group to the placebo group there was a statistically significant difference in the men on GH and the men on placebo (p=0.05).

4.3.3.3.3 Total body fat measured by air displacement plethysmography.

Air displacement plethysmography was assessed using a BODPOD®. There was no significant change in fat measured in men receiving GH from 11.6±1.9% to 14.3±1.7% (p=0.17) but a significant rise in total body fat in women receiving GH measured by BODPOD® from 22.3 ± 2.0% on day 0 and 27.4 ± 3.2% (p=0.04). There was no significant change when comparing the GH to the placebo group on day 0 or 28 for men (p=0.7), or women (p=0.67).

4.3.4 Percentage body water and lean tissue.

The percentage of body composition which is non fat or “lean” tissue was also assessed by bioelectrical impedance analysis. There were no significant changes in lean mass or body water in any group (comparing day 0 and day 28). Please see table 4.13.

total body fat calculated by bioelectrical impedance analysis					
		GH		placebo	
		men	Women	men	Women
		n=14	n=9	n=9	n=3
		mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
% lean	day 0	86.5 ± 1.1	77.2 ± 2.8	82.6 ± 2.6	74.0 ± 4.4
	day 28	87.5 ± 1.3	70.1 ± 7.2	84.4 ± 3.0	74.1 (n=1)
		p=0.33	p=0.4	p=0.69	none available
% water	day 0	61.1 ± 1.1	52.9 ± 2.3	58.0 ± 2.2	50.5 ± 3.8
	day 28	62.2 ± 1.5	54.2 ± 1.8	61.3 ± 3.0	51.0 ± 2.5
		p=0.36	p=0.32	p=30	p=0.36

Table 4.13 Percentage body weight that consists of lean tissue and water calculated by bioelectrical impedance analysis before and after treatment (day 0 and 28) in all groups, and the statistical significance data for the change (paired t tests).

When combining the genders and performing paired ttests the results are shown in table 4.14. There is a statistically significant change in air displacement plethysmography only.

Statistical significance testing for measures of body fat and insulin resistance combining male and female gender groups		p value	n
GH	total body fat measured by anthropometry	0.28	23
	BMI	0.91	23
	Bioelectrical impedance	0.16	23
	Air displacement plethysmography	0.03	23
Placebo	total body fat measured by anthropometry	0.43	12
	BMI	0.68	12
	Bioelectrical impedance	0.86	12
	Air displacement plethysmography	0.35	23

Table 4.14. Statistical significance data (paired ttests) for parameters of insulin resistance using male and female genders combined. All parameters are log transformed and compare day 0 (pre-treatment) and day 28 data (post treatment).

4.3.5 Blood pressure

There was no significant difference in systolic blood pressure (p= 0.59) or diastolic blood pressure (p= 0.38) between the groups at baseline. There was a significant fall in mean systolic blood pressure in men treated with GH (pre GH 131 ± 2 mmHg, post-GH 118 ± 5 mmHg, p=0.03). There was no change in diastolic blood pressure in men treated with GH, and no change in either systolic or diastolic blood pressure in women treated with GH or any placebo group.

Mean systolic and diastolic blood pressure readings (mmHg) are shown in the table 4.15.

No treatment effect was found when analysed by ANOVA.

blood pressure measurements (mmHg)					
		GH		placebo	
		men	women	Men	women
		n=14	n=9	n=9	n=3
		mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
systolic BP	day 0	131 ± 2	111 ± 6	124 ± 5	111 ± 13
	day 28	118 ± 5	115 ±5	124 ± 5	116 ± 5
		p=0.03	p=0.65	p=0.69	p=0.05
diastolic BP	day0	67 ± 2	69 ± 3	65 ± 3	66 ± 4
	day 28	67 ± 3	71 ± 3	68 ± 4	62 ± 4
		p=0.97	p=0.87	p=0.11	p=0.81

Table 4.15 Mean systolic and diastolic blood pressure recordings (mmHg) pre and post treatment (day 0 and 28 respectively) in all groups and the statistical significance data for the change from day 0 to 28 (paired t tests).

4.3.5.1 Correlation data by regression analysis

There was a correlation on regression analysis between systolic and diastolic blood pressure in men receiving GH on day 28 and both IGF-I (p=0.02, 0.005 respectively) and P-III-NP (p=0.003 and 0.002 respectively). There was no correlation with systolic or diastolic blood pressure in any other group (table 4.16, 4.17).

Regression analyses	GH group					
dependent variable = log transformed IGF-I day 28	men			women		
	n	p	r^2	n	p	r^2
Systolic BP	14	0.02	0.016	9	0.93	0.001
Diastolic BP	14	0.005	0.004	9	0.81	0.058
dependent variable = log transformed P-III-NP day 28						
Systolic BP	14	0.003	0.055	9	0.99	0.023
Diastolic BP	14	0.002	0.003	9	0.82	0.183

Table 4.16 Regression analysis correlation data between blood pressure and IGF-I and P-III-NP on day 28 in subjects receiving GH.

Regression analyses	Placebo groups					
	men			women		
	n	p	r^2	N	p	r^2
Systolic BP	9	0.86	0.009	1	none available	none available
Diastolic BP	9	0.21	0	1	none available	none available
dependent variable = log transformed P-III-NP day 28						
Systolic BP	9	0.2	0.02	1	none available	none available
Diastolic BP	9	0.28	0.021	1	none available	none available

Table 4.17 Regression analysis correlation data between blood pressure and IGF-I and P-III-NP on day 28 in subjects receiving placebo.

4.3.6 Exercise capacity

4.3.6.1 VO_2 maximum

Subjects underwent full Bruce protocol treadmill running test with assessment of VO_2 maximum. Data are shown in figure 4.1, as well as the time to exhaustion (minutes) running on the treadmill.

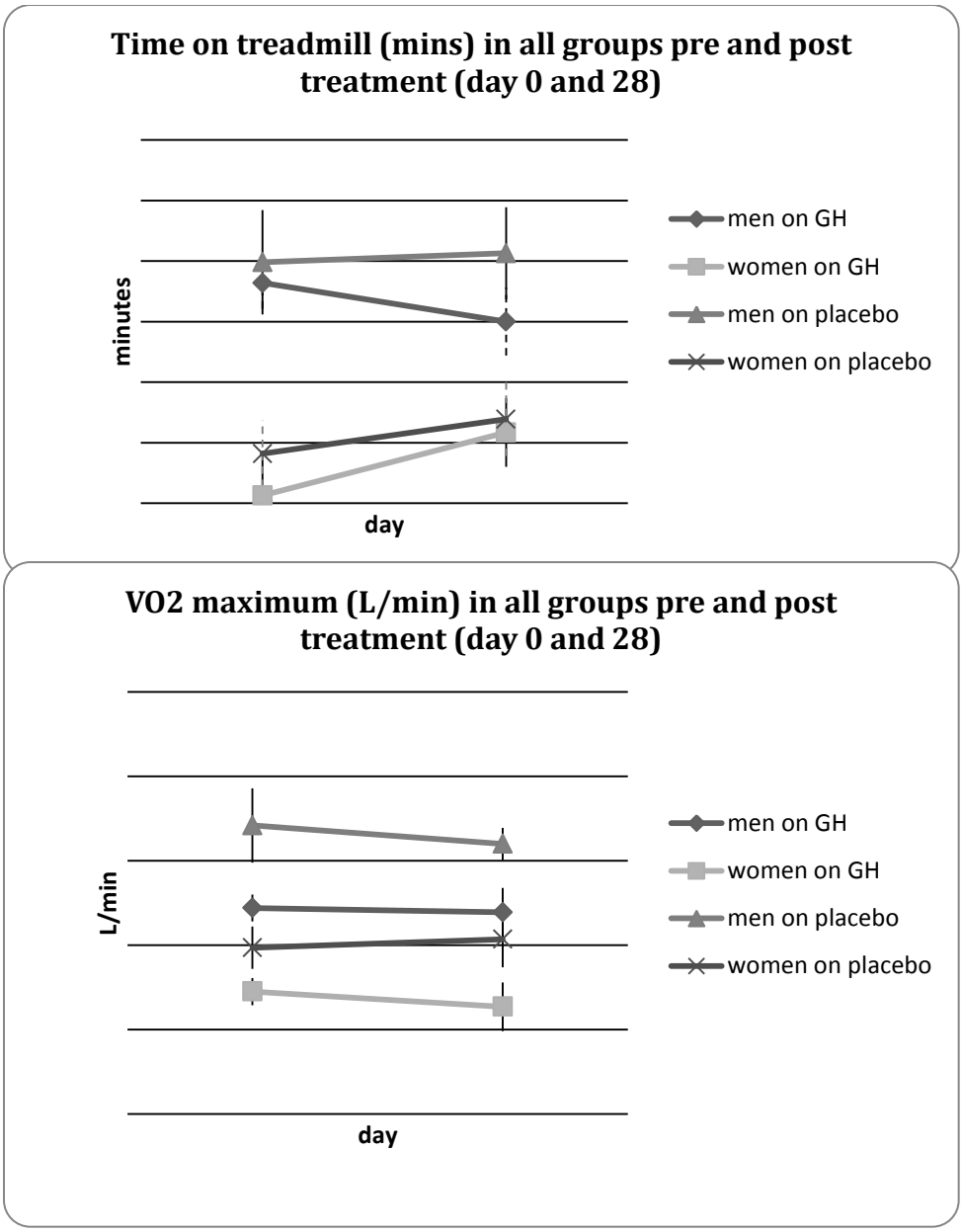


Figure 4.1 Mean time to exhaustion and VO_2 maximum values for day 0 and day 28 in all groups during a treadmill running test (Bruce protocol).

There was no significant change in the length of time before subjects were exhausted and ceased running on the treadmill, i.e. length of time to exhaustion. Similarly there were no significant changes in VO_2 maximum in any group.

4.4 Discussion

Despite the results of previous studies, there was only limited evidence from this study to suggest that GH leads to changes in body fat content and distribution. Only men treated with GH had a rise in hip circumference and a corresponding fall in waist to hip ratio. There was no reduction in waist circumference was seen to provide evidence of a redistribution of body fat from central to peripheral depots. Such redistribution of body fat and improved muscle definition could be potentially beneficial for certain sporting disciplines e.g. body building.

4.4.1 Body composition

4.4.1.1 GH deficiency and effects of therapy with GH

GH deficient individuals have increased central body fat which reduces following GH therapy but this is not consistently reproduced in GH replete adults. Redistribution of fat occurs with rhGH administration in the non-growth hormone deficient individual but to a less impressive extent (Liu, 2008).

4.4.1.2 Acromegaly

In active acromegaly, individuals have a lower visceral and subcutaneous fat mass, and an increase in total body water, lean body mass and bone mineral content (Freda, 2008, Tominaga, 1998, O'Sullivan, 1994, Sucunza, 2008). This body composition changes are reversed after successful therapy (Tominaga, 1998).Body weight falls initially following a reduction in body water and lean body mass but returns to baseline at 3 months of therapy with an increase in fat mass (Bengtsson, 1989, Tominaga, 1998, Bengtsson, 1990). It is postulated that the changes in fat mass are caused by GH induced lipolysis and inhibition of lipogenesis and increased energy expenditure both of which reduced with successful treatment (Rau, 1991, Goodman, 1983, Hu, 1994, Bengtsson, 1989). Chronic GH excess of acromegaly is not associated with improved muscle strength despite hypertrophied muscles but there may be a window during which

muscles have increased strength prior to the development of weakness and fatigability of acromegaly and illness (Widdowson, 2009).

4.4.1.3 GH therapy in GH replete individuals

Use of rhGH in obese adults without an underlying endocrinopathy leads to a reduction in waist to hip ratio, total body fat mass, percentage body fat and visceral adiposity, and percentage of body weight lost as fat. Subjects have an increase in lean body mass with no change in body weight, BMI, resting energy expenditure, or blood pressure. A meta-analysis by Mekala et al found a positive correlation between GH dose, and lean body mass, and between serum IGF-I and fasting glucose concentration (Mekala, 2009)

Replacement of GH in GHD has been shown to increase lean body mass by 2 to 5.5 kgs, and increase skeletal muscle mass (Jorgensen et al., 1989, Salomon, 1989, Jorgensen, 1994b, Whitehead, 1992). Total body fat mass can be reduced by 4 to 6 kgs (Snel, 1995, Salomon, 1989, Bengtsson, 1993).

There was a rise in the percentage body fat in women receiving GH when measured by air displacement pleythysmography. This was not replicated by any other measurement method. There was no change in body fat percentage in any other group by any method of calculation. There were considerable technical difficulties with the BODPOD® during the course of the study. A considerable number of readings had to be excluded from analyses because they were so far from the physiological mean (e.g. body fat percentage in women of 2%). Also the machine was not available because of on-going repairs, only 17 men and 12 women were included compared to 22 and 12 respectively for bioelectrical impedance analysis, and 23 and 12 respectively for anthropometry. One subject had to be omitted from bioelectrical impedance analysis because of recurrent

“error” recording on the instrument. The laboratory environment was inconsistent with air leakages from doorways and unreliable temperature regulation in the study room. The findings are therefore of questionable reliability. It is likely that the duration of therapy and dose regimes used were too small to show changes by the measures and analyses used.

Loss of body fat could be beneficial for sporting performance insofar as lipolysis provides substrate for exercising muscle, a leaner body profile provides a more efficient metabolic machine with less carriage of fat. A lean physique and enhanced muscle definition is important in body building.

4.4.2 Body weight

This study also reported a rise in body weight in men treated with GH. This is likely to be due to increased body water although this was not found when the subjects were measured by bioelectrical impedance analysis. Water retention is a known side effect of GH administration. 2 subjects reported ankle oedema, both of which were receiving GH treatment. Bioelectrical impedance analysis provides an assessment of body water and did not report an increase in total body water. However it would seem like the most likely possibility despite not reaching clinical significance on bioelectrical impedance analysis and has implications for interpreting the body fat changes. Bioelectrical impedance analysis reports body fat changes based on analyses of body water and extrapolating body fat content. Thus a rise in body water may be interpreted as a fall in body fat. There is no consensus as to the effect of body water on sporting performance (Gibney, 2007). It is less likely to result from increased muscle mass as there was no change in urinary nitrogen studies (see chapter 5), no rise in midarm circumference or in basal metabolic rate to indicate higher muscle mass (chapter 5).

Extracellular body water increases within 3 to 5 days of commencing therapy and increased plasma volume after 3 weeks and 3 months of GH therapy (Møller et al., 1996, Christ et al., 1997). It is postulated to occur through raised glomerular filtration rates, up-regulation of Na-K-ATPase activity and effects of IGF-I on the renin-angiotensin system, as well as other neurohumoral stimuli such as atrial natiuretic peptide (Holt, 2004c). Raised levels of growth hormone induce volume overload in the heart and in turn induce GH receptor gene expression. Isotope studies have shown a reduction in whole body and particularly extracellular water and plasma volume in GH deficiency (Møller et al., 1996, Amato and D., 1993, Rosen, 1993) which improves with treatment.

4.4.3 Blood pressure

There was a significant fall in this study in systolic blood pressure in men following treatment with GH which is statistically correlated with changes in IGF-I and P-III-NP. There was also a correlation between diastolic BP on day 28 and IGF-I and P-III-NP in men receiving GH although no statistically significant change was detected in diastolic BP in this study. Our findings concur with studies of the effects of GH on blood pressure in people with GHDA from the literature. Men in the KIMS (Pharmacia, Upjohn International Metabolic Database) database, as detailed in chapter 2, showed a reduction in diastolic blood pressure following treatment with GH (Monson JP, 2003). GH replacement in GHD individuals leads to reduced blood pressure after 9 months' therapy (Binnerts et al., 1992, Carroll et al., 1998). There have been studies showing reduce diastolic blood pressure, and in the KIMS database there was a reduced systolic BP in men and women <65 years and in men >65 years but not in women >65 years (Monson, 2000, Bengtsson et al., 1999, Fideleff, 2008).

The precise mechanism of this fall in BP associated with GH use is unknown, but may reflect biochemical changes induced by IGF-I. Some authors suggest the fall in blood pressure seen with GH therapy may be due to nitric oxide production induced directly by IGF-I (Nyström, 2005). GH has been shown to be an increase endothelial nitric oxide synthase in vitro, and increase endothelial nitric oxide synthase gene expression (Ho, 1996, Wickman, 2002, Thum, 2003, Roden, 1996) and NO formation secondary to IGF-I in vivo (Thum, 2007).

Familiarisation with operation personnel may also have been significant in this study but the lack of effect in the placebo group in this study would suggest otherwise (Böger, 1996).

In acromegaly the prevalence of hypertension is 37.5% by World Health Organisation criteria which is higher than in the general population and the exact mechanism is unknown (Ohtsuka H, 1995). It is thought to occur as a result of a combination of factors including genetic factors, plasma volume expansion and the anti-natiuretic action of GH, sympathetic nervous system activation, hyperinsulaemia, and as well as the vascular growth factor properties of IGF-I.

4.4.4 Exercise capacity

There was no change in VO_2 maximum or time to exhaustion on the treadmill running test in this study. Assessment of aerobic capacity with maximal oxygen uptake is commonly used as a measure of physical work capacity (Mitchell, 1958, Taylor, 1955). Deficiency of growth hormone is known to be associated with impaired physical functioning secondary to reduced skeletal muscle mass, isometric muscle performance, and aerobic capacity (Woodhouse, 2006). GHD

adults have been shown to have reduced VO_2 maximum by 18 to 28% compared to matched normal adults, and levels can be similar to those with congestive cardiac failure (Whitehead, 1992, Jones, 1988, Jones, 1985)(Kiilavuori, 1996). This is thought to be due to reduced skeletal muscle mass and metabolic changes within skeletal muscle, reduced cardiac capacity (Carroll et al., 1998, Longobardi, 1998, Colao, 2000, Colao et al., 2001) and lower red cell volume (Amato and D., 1993, Christ et al., 1997, Cummings, 2003, Cuneo, 1991).

Reports of the effect of GH replacement on the aerobic capacity of GHD adults are conflicting which may reflect the differing duration of treatment and modes of exercising, e.g. upright cycling as this supports body weight whereas treadmill running does not (Nass and C.J., 1995, Whitehead, 1992, Woodhouse, 1999, Degerblad et al., 1990, Cuneo, 1991). However, not all trials show improvements in VO_2 maximum with treatment (Rodriguez-Arnao, 1999). This was apparent when reported in litres per minute or millilitres per kilogram per minute (Nass and C.J., 1995, Woodhouse, 1999). 6 months GH therapy showed no change in VO_2 maximum measured during treadmill running but a significant improvement after a further 6, 12, and 18 month open follow up (Beshyah, 1995). There was no change in aerobic capacity walking, upright or recumbent cycling after 6 months of therapy (Rodriguez-Arnao, 1999, Whitehead, 1992, Nass and C.J., 1995).

Previous studies have shown no change in recumbent cycling or treadmill walking compared to a rise in VO_2 maximum with upright cycling (Woodhouse, 1999, Nass and C.J., 1995). Cross over trials report benefits in aerobic capacity beyond the treatment period (Woodhouse, 1999, Whitehead, 1992). Peripheral oxygen uptake changes are minimal with exercise so increases in VO_2 maximum would likely occur as a result of increased cardiac output and there are reports of increased VO_2 per heartbeat, with little increase in maximal heart rate so it would be likely that changes reflect increased stroke volume. (Whitehead, 1992, Nass and C.J., 1995, Woodhouse, 2006). Cardiac performance during exercise has

been shown to improve with GH replacement in GHD (Longobardi, 1998, Colao, 2000, Colao and A.M., 1999). There is little consensus as to whether the increased stroke volume is caused by improved cardiac inotropism or increased plasma volume secondary to fluid retention (Woodhouse, 2006).

Adults with acromegaly report fatigue, impaired physical performance and submaximal aerobic capacity which are improved by treatment (Thomas, 2002). They have reduced VO_2 maximum compared to normal matched controls by 60 and 85% (Canadian Association of Sport Sciences, 1987, Longobardi, 1998). This may be due to reduced muscle mass and cardiac dysfunction (Colao, 2004, Legrand, 1994, Colao et al., 2001). They have been shown to have diminished diastolic filling capacity and impaired ejection fraction at rest and occur during exercise (Colao and A.M., 1999, Fazio, 1994). Successful treatment with somatostatin analogues improves VO_2 maximum and cardiac performance (Padayatty, 1996, Thomas, 2002).

4.4.5 Effect of ethnicity

Subjects in this study were of non Caucasian ethnicity and therefore have a different body fat distribution, insulin sensitivity and metabolic profile associated with insulin resistance (IR) syndromes when compared to Caucasians. African American individuals have higher percentage body fat for a given age and gender adjusted BMI and also have a higher incidence of IR, adverse blood pressure and atherogenic lipid profile compared to Caucasians (Falkner et al., 1995).

4.4.6 Criticism of the study

The largely negative findings for body composition changes in this study may be reflective of the short duration of dosing. Most studies in which changes in body fat and VO_2 maximum have been reported have been of longer duration of administration (>6 months). The replacement of GH for 6 months in men and women lead to an 8.8% increase in the muscle mass and 14.4% loss in fat mass (Salomon, 1989). 9 months GH administration has been shown to reduce central adiposity substantially in obese adults (Marcus and Reaven, 1997). A further criticism of the study is the small study numbers and the relatively crude measures of body fat used in the study. The issues with BOD POD® analyses in this study were discussed earlier. Use of bioelectrical impedance analysis to measure body fat is not ideal in conjunction with administration of a substance which changes body water content. Isotope labelling and radiographic imaging would perhaps have provided better evidence of changes.

4.4.7 Summary

In summary there was a rise in body weight, and a redistribution of central fat depots (waist) to peripheral sites (hips) and a fall in systolic blood pressure in men receiving GH with no change in aerobic capacity providing some evidence of the effects of GH in men but none evident in women. The rise in body water leading to improved cardiac preload and stroke volume and thus blood volume for carriage of substrate, rise in hip circumference may confer metabolic health and performance advantage, reduced systemic vascular resistance allowing a pool of blood for exercising muscle. There was no change in exercise capacity (VO_2 maximum).

Chapter 5

Effects of 28 days of supraphysiological GH administration on measures of substrate utilisation and NEFA.

5.1 Introduction

Administration of growth hormone (GH) has been shown to affect metabolic rates and utilisation of protein, carbohydrates (CHO) and fat. GH infusion increases basal metabolic rate (BMR) and lowers respiratory quotient (RQ), protein energy expenditure, forearm glucose uptake and overall sensitivity to insulin. Total body lipid oxidation is increased and fat cells become more sensitive to lipolysis by circulating catecholamines in the presence of GH (Jorgensen et al., 1993). Serum concentrations of free fatty acids reach peak serum concentrations after 2 to 3 hours following a GH pulse (Kreitschmann-Andermahr, 2010).

GH replacement in GHD individuals may improve physical performance by inducing a rise in metabolic rate, a reduction of carbohydrate oxidation rates leading to increased glycogen stores, a rise in fat oxidation providing substrate for exercising muscles, and a fall in urinary nitrogen excretion through protein anabolism. In this study substrate utilisation is assessed using indirect calorimetry and urinary nitrogen excretion (UN) to determine carbohydrate, fat and protein usage, as well as serum non esterified fatty acids concentrations (NEFA) and metabolic rates at baseline (fasting), and 1 and 2 hours post glucose administration during OGTT.

5.2 Methods

5.2.1 Subjects

The recruitment and inclusion and exclusion criteria for the study subjects in this were those outlined in chapter 2 and 3.

5.2.2 Procedure

The study protocol is described in chapter 2.

5.2.3 Statistical analysis

The statistical analysis is described in chapter 2.

5.3 Results

5.3.1 Baseline data

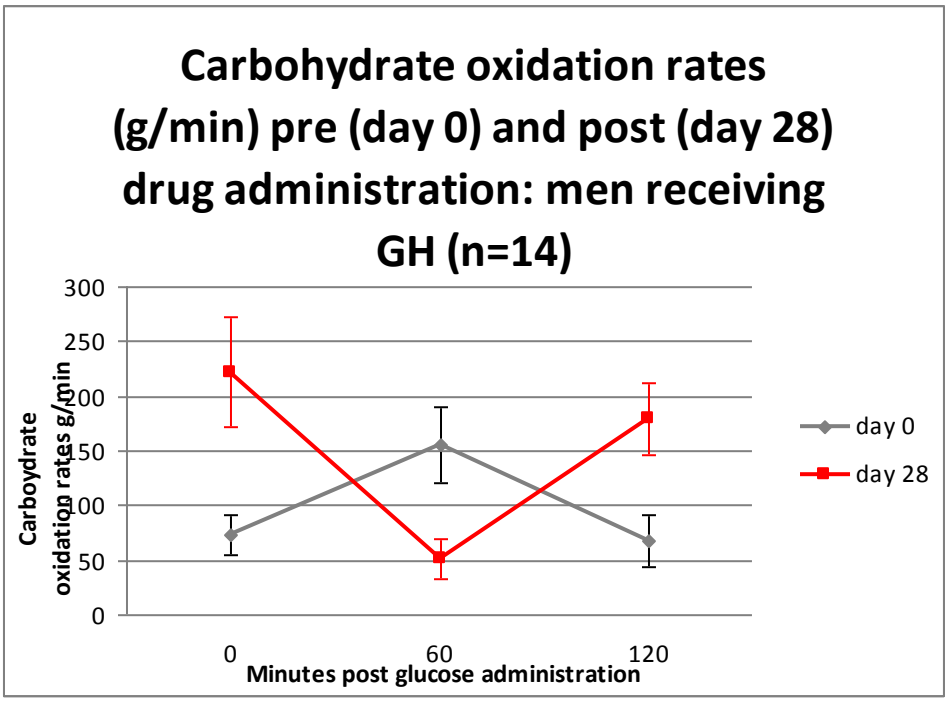
Baseline data for BMI, carbohydrate and fat utilisation (g/L) at time 0, 60 and 120minutes post glucose administration, as well as serum NEFA (μmol/L) at time 0 is shown in table 5.1.Values for substrate utilisation <0 were rounded up to 0 for the purposes of analyses.

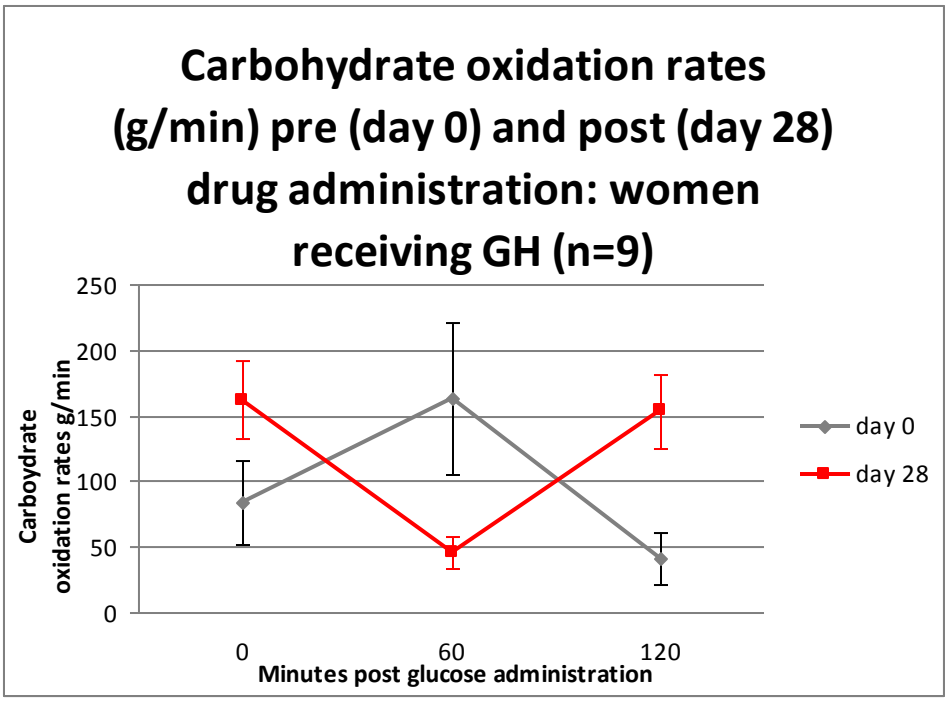
Baseline data on day 0					
		GH		placebo	
		men	women	men	women
	Time (minutes post glucose administration during OGTT)	mean±SEM	mean±SEM	mean±SEM	mean±SEM
N		14	14	13	14
BMI		23.2 ± 0.8	24.8 ± 1.5	25.3 ± 1.3	24.3 ± 1.6
Carbohydrate utilisation g/L	0	73 ± 19	84 ± 32	126 ± 81	18 ± 18
	60	155 ± 35	163 ± 58	97 ± 35	151 ± 76
	120	68 ± 24	41 ± 20	126 ± 33	-5 ± 5
fat utilisation g/L	0	51 ± 18	73 ± 21	52 ± 37	80 ± 45
	60	179 ± 33	30 ± 3	59 ± 15	81 ± 39
	120	222 ± 50	28 ± 15	54 ± 15	40 ± 40
NEFA concentrations(µmol/L)	0	430 ± 63	602 ± 139	406 ± 66	645 ± 119

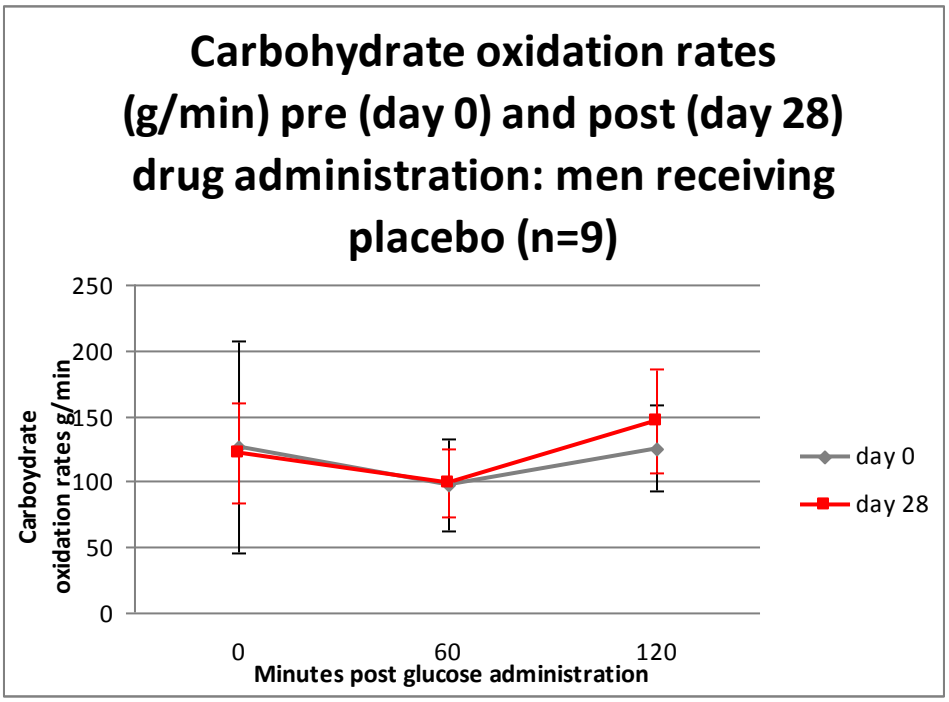
Table 5.1 Baseline data for all groups. Table shows BMI, carbohydrate and fat utilisation at 0, 60 and 120 minutes post glucose administration and serum NEFA concentration at baseline on day 0 (pre drug administration).

5.3.2 Carbohydrate utilisation

Carbohydrate utilisation rates from day 0 to 28 are shown in the figure 5.1. At all time points the measured UN value was used, unless unavailable, in which case the standard value for UN excretion (UN=0.008333) was used.




Carbohydrate oxidation rates (g/min) pre (day 0) and post (day 28) drug administration: women receiving GH (n=9)
250
200
150
100
50
0
Carboydrate oxidation rates g/min
0
60
120
Minutes post glucose administration
day 0
day 28


Carbohydrate oxidation rates (g/min) pre (day 0) and post (day 28) drug administration: men receiving placebo (n=9)
250
200
150
100
50
0
Carboydrate oxidation rates g/min
0
60
120
Minutes post glucose administration
day 0
day 28

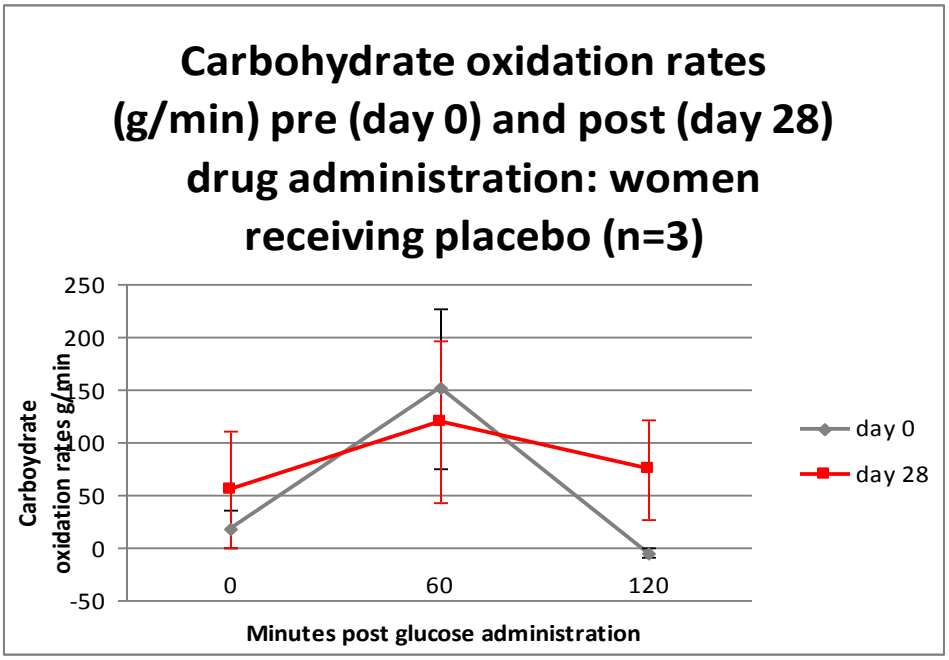


Figure 5.1 Mean carbohydrate oxidation rates (g/min) before the OGTT, at 60 minutes and 120 minutes post glucose administration in all groups pre and post treatment (day 0 and day 28 respectively).

There was no significant change in carbohydrate utilisation rates in the fasting or post glucose administration period in any group. The mean differences between day 0 and day 28 as well as the statistical significance values for the changes are shown in table 5.2.

Mean difference in carbohydrate utilisation between day 0 and day 28 (g/min)				
	GH		placebo	
	Men	women	men	**women**
	n=14	n=9	n=9	**n=3**
minutes	mean ± SEM	mean ± SEM	mean ± SEM	**mean ± SEM**
0	-24 ± 38	-46 ± 61	-10 ± 84	**139 ± 36**
	p=0.41	p=0.29	p=0.58	
60	28 ± 59	9 ± 65	50 ± 40	**-77 ± 47**
	p=0.82	p=0.18	p=0.09	**p=0.33**
120	128 ± 51	0 ± 32	-41 ± 62	**60 ± 60**
	p=0.1	p=0.22	p=0.83	

Table 5.2 Mean difference in carbohydrate oxidation rates (g/min) between day 0 and day 28 and the statistical significance values for the changes in all groups.

5.3.2 Fat oxidation

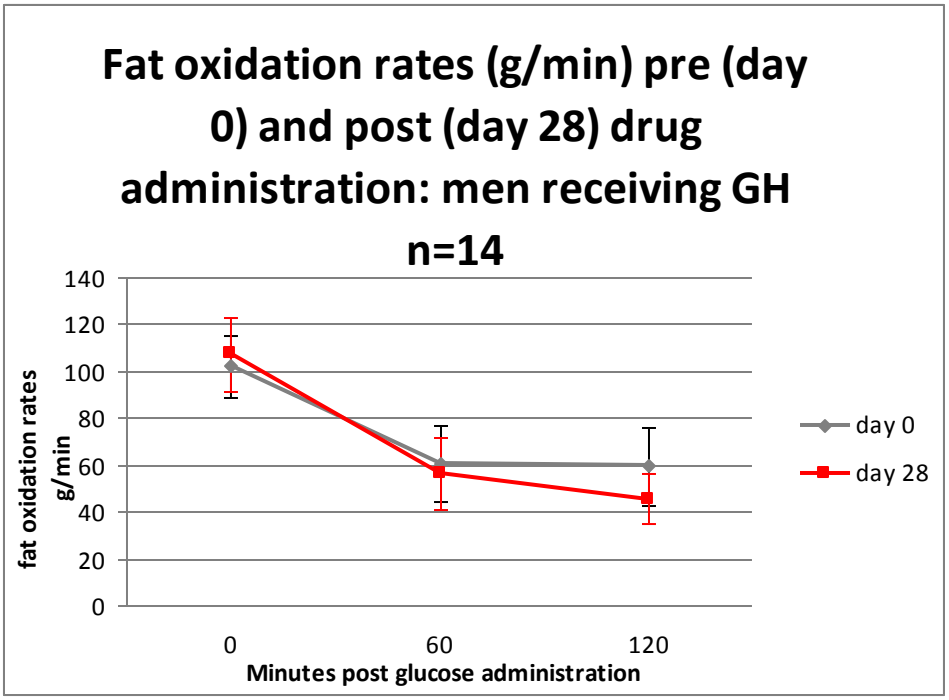
There was a significant fall in fat oxidation rates 120 minutes post glucose administration in men receiving GH: from 59.39±16.96 g/min to 45.67±10.77 g/min (p=0.01). There was a fall in fat oxidation rates in women receiving GH at 60 minutes post glucose administration from 28.01±15.14 g/min to 19.84±9.84 g/min (p<0.01).

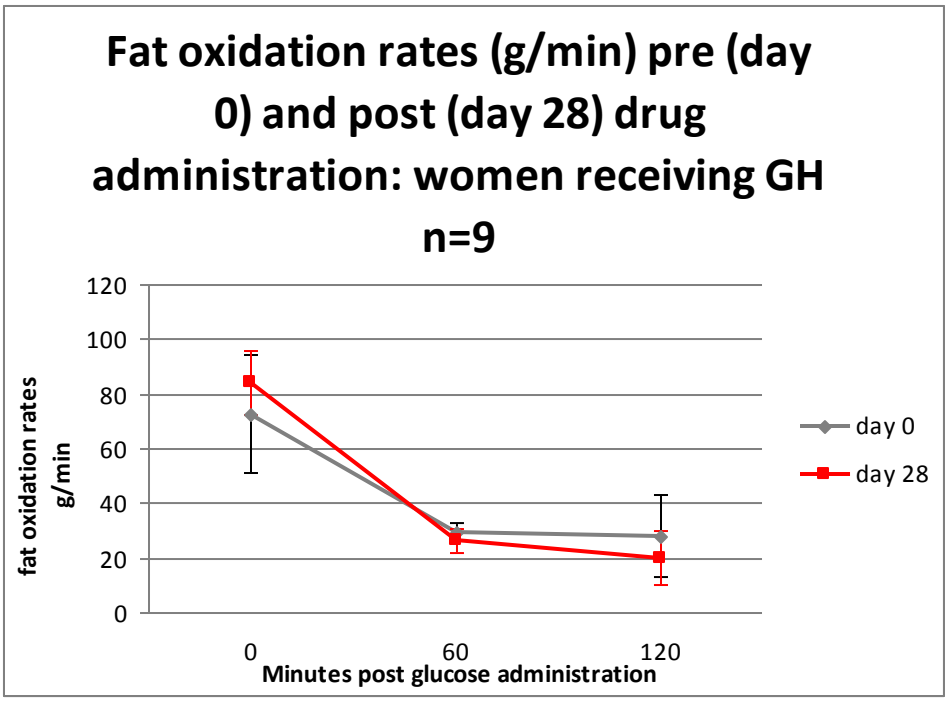
There was a rise in men receiving placebo at baseline (t=0) from 51.94±37.42 to 62.52±14.6 g/min (p=0.05), and at 120 minutes from 54.35±15.31 g/min to 56.39±20.61g/min (p=<0.01).

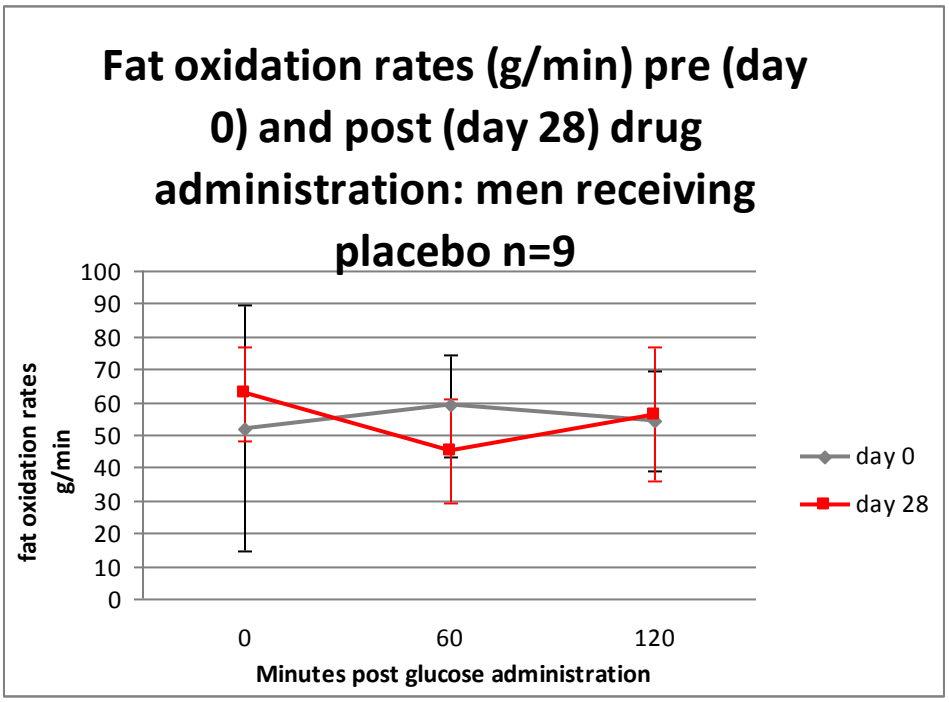
There was no significant change in any other group.

There was no significant change in fat oxidation in any group when comparing GH and placebo groups.

Data for fat oxidation rates (g/min) are shown in figure 5.2, and mean differences in table 5.3.




Fat oxidation rates (g/min) pre (day 0) and post (day 28) drug administration: women receiving GH n=9
fat oxidation rates g/min
120
100
80
60
40
20
0
0
60
120
Minutes post glucose administration
day 0
day 28


Fat oxidation rates (g/min) pre (day 0) and post (day 28) drug administration: men receiving placebo n=9
fat oxidation rates g/min
100
90
80
70
60
50
40
30
20
10
0
0
60
120
Minutes post glucose administration
day 0
day 28

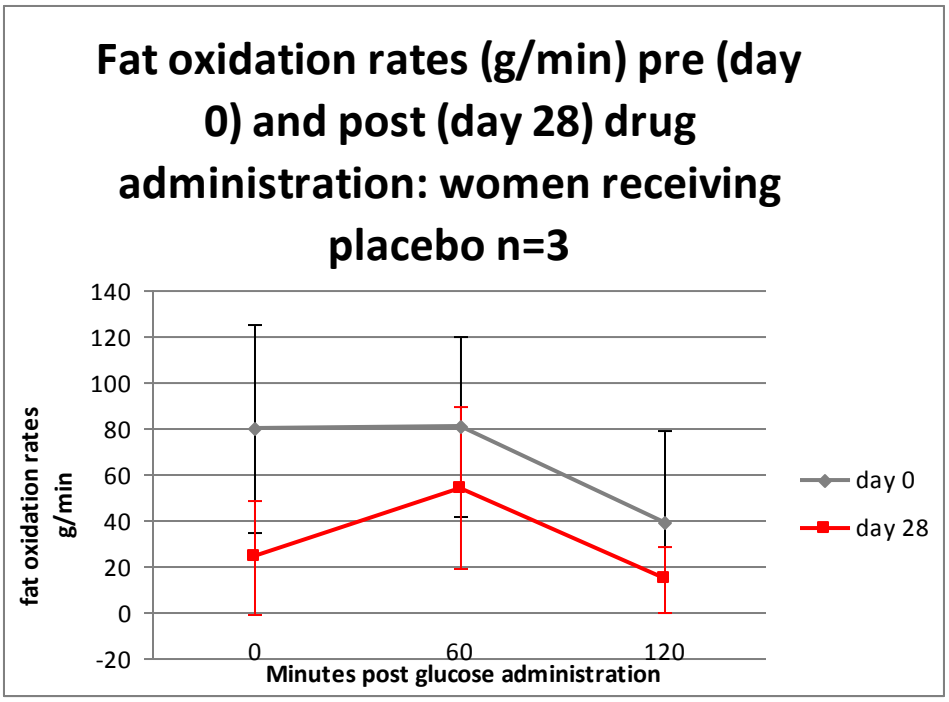


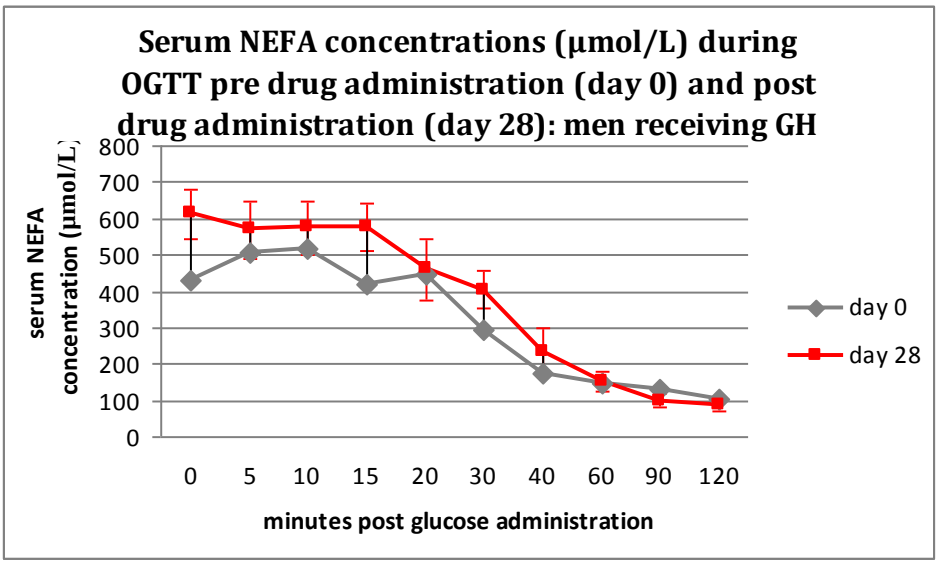
Figure 5.2 Mean fat oxidation rates (g/min) at baseline, 60 minutes and 120 minutes post glucose administration on day 0 and 28.

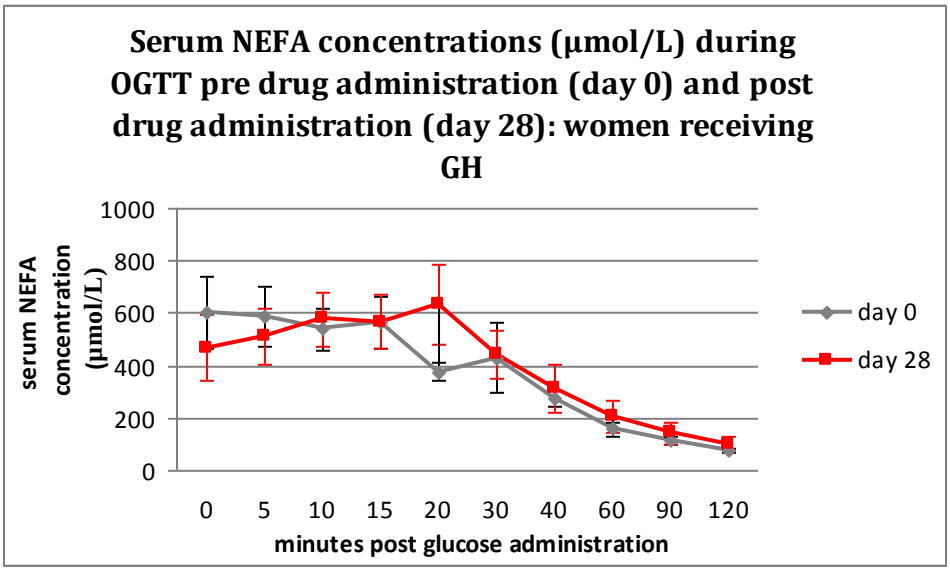
Mean difference in fat utilisation between day 0 and day 28 (g/min)				
	GH		placebo	
	men	women	men	women
	N=14	n=9	n=9	n=3
minutes	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
0	5.06±22.37	14.47±28.13	10.57±39.76	-56.22±68.07
60	60.52±16.01	29.62±3.14	58.84±15.37	80.97±39.25
120	4.18±27.05	24.12±14.82	67.79±22.74	60 ± 60

Table 5.3 Mean difference in fat oxidation rates (g/min) between day 0 and day 28 and the statistical significance values for the changes in all groups.

5.3.3 NEFA during OGTT

Following treatment with GH there was a significant rise in baseline serum NEFA concentrations (μmol/L) in men (p=0.04) (day 0: 429 ± 62 μmol/L, day 28; 613 ± 66μmol/L). There was no change in women receiving GH (p=0.13) or in the placebo groups. Mean serum NEFA concentrations (μmol/L) during OGTT on day 0 and day 28 are shown in figure 5.3.





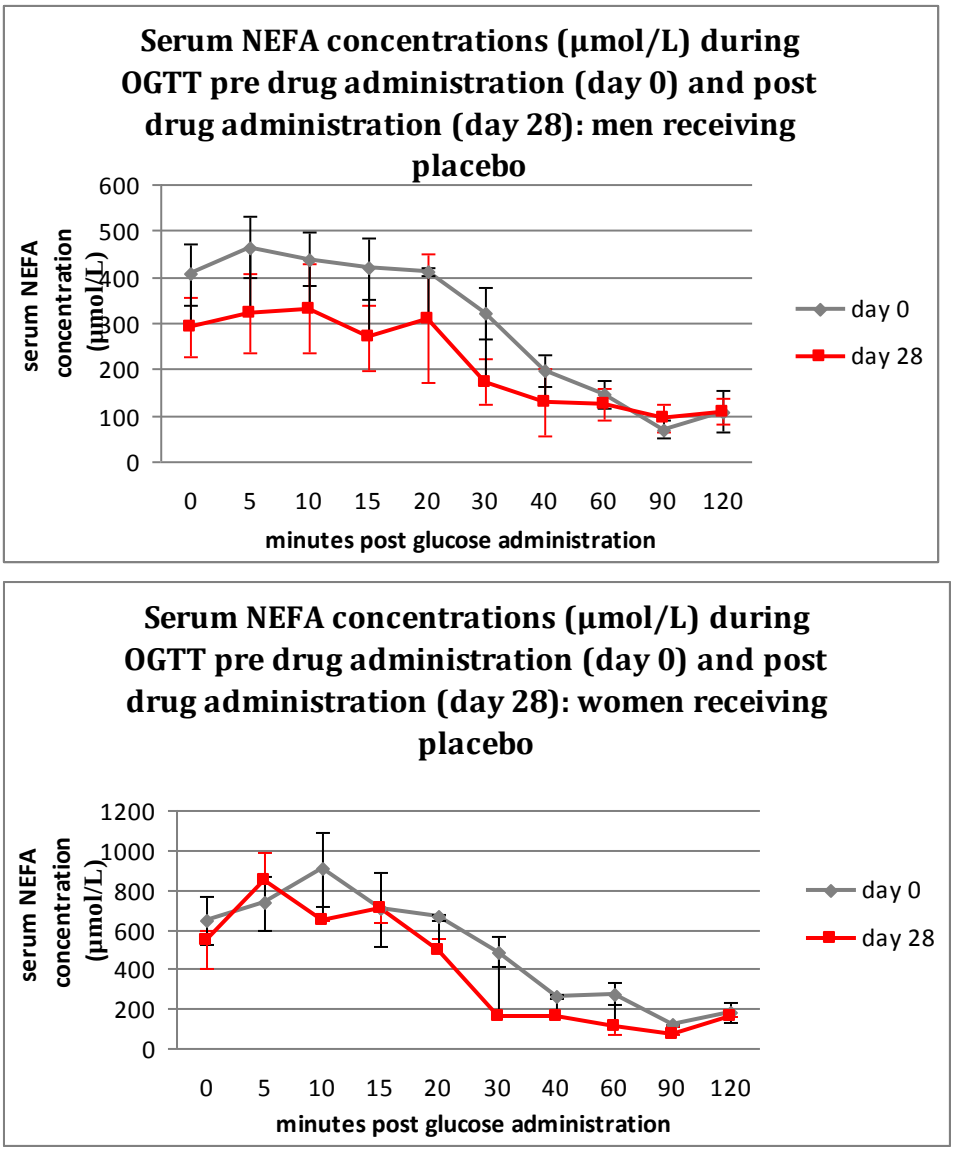


Figure 5.3 Mean NEFA concentrations (µmol/L) during OGTT on day 0 and day 28 in all groups.

There is a significant treatment effect when comparing the baseline NEFA in the GH and placebo groups on day 28 ($p<0.01$).

A tabulation of the mean differences between 0, 60 and 120 minutes post glucose administration (OGTT) and the respective p values for the change is seen in table 5.4.

Mean difference and statistical significance data for NEFA concentration change from day 0 to 28 (µmol/L)					
		time points (minutes post glucose)	Mean difference between day 0 and 28	SEM	p value
GH	men	0	181	76	0.04
		60	-23	30	0.3
		120	-6	25	0.8
	women	0	-232	111	0.08
		60	-1	48	0.88
		120	-5	14	0.73

Table 5.4 Mean differences in serum NEFA concentration (µmol/L) pre and post treatment (day 0 and 28 respectively) at time 0, 30 and 120 minutes during OGTT and the statistical significance data for the change (paired t test).

5.3.3.1 NEFA; area under curve (AUC)

There was no significant change in NEFA AUC in any group: men receiving GH (p= 0.32), and receiving placebo (p= 0.84), women receiving GH (p= 0.58), and placebo (p=0.06).

5.3.3.2 Correlation data

There is a correlation between the change in AUC NEFA on day 28 and the serum insulin area under the curve (AUC) for insulin in men receiving GH (p=0.01)

There is no correlation between AUC NEFA on day 28 and any other variable on linear regression analyses see table 5.5 and 5.6.

Statistical data correlating AUC NEFA (day 28) with other variables (linear regression analysis) in subjects receiving GH.							
		GH					
		men			women		
		n	p value	R square	n	p value	R square
BMI		10	1.00	0.00	7	0.57	0.01
total body fat							
	anthropometry	10	0.65	0.02	7	0.49	0.03
	Bioelectrical impedance	10	0.30	0.11	7	0.37	0.06
	BODPOD®	10	0.97	0.00	7	0.54	0.02
Adiponectin		10	0.60	0.03	1	0.31	0.17
HOMA		10	0.38	0.10	7	0.42	0.05
AUC glucose		10	0.79	0.01	7	0.25	0.40
AUC insulin		7	0.01	0.54	7	0.35	0.01
fasting glucose		10	0.52	0.04	7	0.07	0.19
fasting insulin		7	0.43	0.08	7	0.41	0.01

Table 5.5 Regression Statistical data correlating NEFA with other variables (linear regression) for subjects receiving GH.

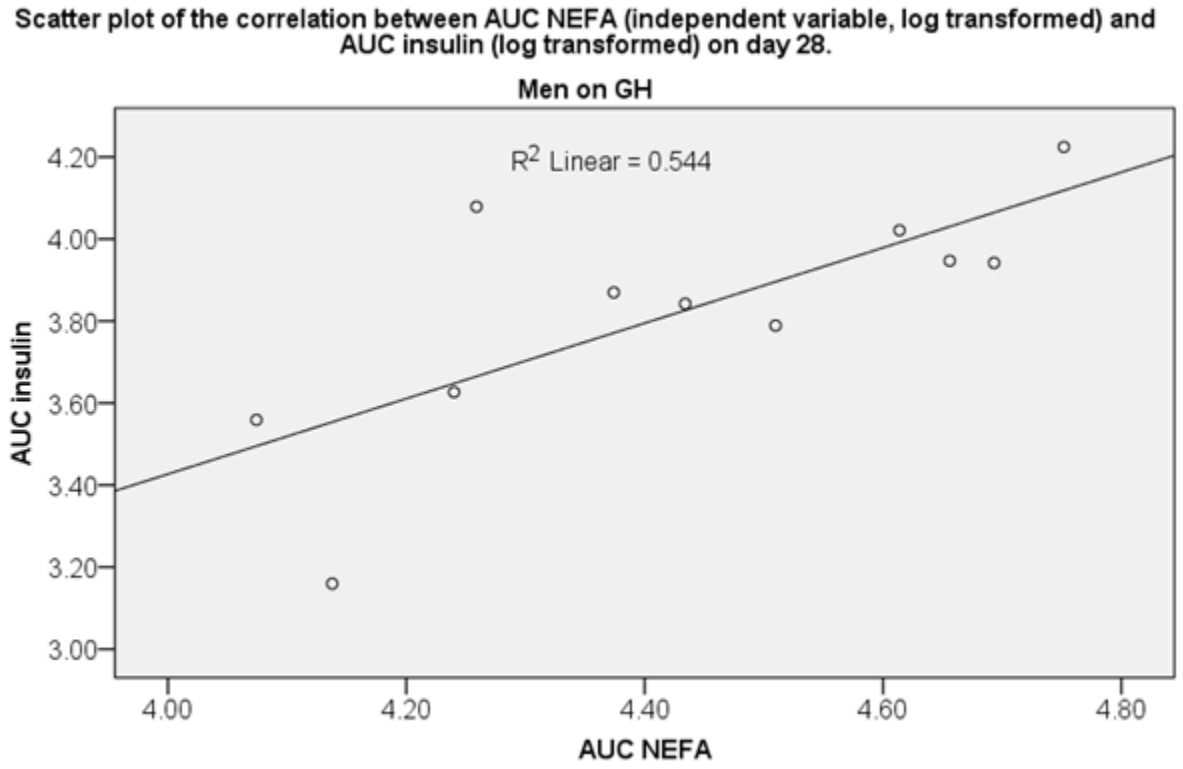


Figure 5.4 Correlation graph for regression analysis using AUC NEFA (log transformed, independent variable) and AUC insulin (log transformed, dependent variable) on day 28 during OGTT.

Statistical data correlating AUC NEFA (day 28) with other variables in subjects on placebo					
			placebo		
			men		
			n	p value	R square
BMI			7	0.81	0.06
total body fat					
		anthropometry	7	0.91	0.08
		bioelectrical impedance	7	0.59	0.16
		BODPOD®	7	0.79	0.08
HOMA			7	0.64	0.13
AUC glucose			7	0.13	0.26
AUC insulin			7	0.84	0.17
fasting glucose			7	0.32	0.45
fasting insulin			7	0.63	0.18

Table 5.6 Regression Statistical data correlating AUC NEFA with other variables (linear regression) for subjects receiving placebo. There is no data available for women on placebo as n=1.

There is no statistically significant correlation between fat oxidation on day 28 at any time point and AUC NEFA on day 28. There is a correlation between IGF-I concentrations on day 28 and fasting fat oxidation at (time 0) on day 28 in women receiving GH ($p=0.034$) but not in any other group at any other time point.

5.3.4 Metabolic rate assessment

There was no significant change in metabolic rate at any time point after GH administration in either men or women. Please see table 5.7.

Metabolic rate (kcal/day)									
		men				women			
		GH		placebo		GH		Placebo	
	time (mins)	n	mean ± SEM	n	mean ± SEM	n	mean ± SEM	n	mean ± SEM
day 0	0	14	1725 ± 109	7	1763 ± 139	4	1710 ± 155	8	1447 ± 133
	60	12	1883 ± 122	8	1918 ± 97	4	1609 ± 74	7	1598 ± 288
	120	11	1876 ± 127	8	1765 ± 74	3	1526 ± 138	5	1320 ± 179
day 28	0	14	1612 ± 140	8	1637 ± 56	3	1394 ± 74	8	1233 ± 92
	60	14	1765 ± 106	8	1700 ± 87	3	1539 ± 231	8	1295 ± 129
	120	14	1734 ± 102	8	2211 ± 564	2	1382 ± 143	8	1215 ± 118

Table 5.7 Mean metabolic rates (kcal/day) in all groups at baseline, 60 and 90 minutes post glucose administration during OGTT on day 0 and day 28.

5.3.5 Urinary nitrogen excretion

There was a significant fall in urinary nitrogen (after OGTT) in women after 28 days of treatment of GH ($p=0.02$) but no change in men on GH ($p=0.28$) or in the placebo groups. Please see table 5.8.

Urinary nitrogen (mg/min)		GH				placebo			
		men		Women		men		women	
		n	mean ± SEM	n	Mean ± SEM	n	mean ± SEM	n	mean ± SEM
day 0	Pre OGTT	0		0		0		0	
	Post OGTT	11	19 ± 4	5	11 ± 2	8	13 ± 2	2	5 ± 2
day 28	pre OGTT	1	6 ± 0	2	7 ± 2	2	5 ± 3	1	17
	post OGTT	11	13 ± 3	7	8 ± 2	7	18 ± 3	2	4 ± 1

Table 5.8 Urinary nitrogen (mg/min) data before and after OGTT in all volunteers.

5.3 Discussion

There was evidence of an increase in lipolysis in men receiving GH in this study seen in higher baseline NEFA concentrations on day 28 compared to day 0. There was a fall in fat oxidation rates in men on GH and women on GH (120 and 60 minutes post glucose administration respectively), and a rise in fat oxidation rates in men receiving placebo at 0 and 120 minutes. There was a fall in urinary nitrogen excretion on day 28 in women but not in men receiving GH. There was no change in metabolic rates in either the basal or post OGTT in any group after drug administration. There is evidence that changes AUC insulin in men on GH is correlated with changes in AUC NEFA post treatment by regression analysis.

An increase in NEFA but fall in fat oxidation by indirect calorimetry could be interpreted as small and early changes (NEFA), but the study GH exposure was of too short a duration to induce changes in overall body fat oxidation/utilisation especially without changes in overall body fat percentage. Changes seen in the placebo group are difficult to account for and with a crude methodology and large standard errors they may be spurious results.

The rise in the rate of lipolysis is consistent with upregulation of hormone sensitive lipase and inhibition of lipoprotein lipase by GH, and increased circulating NEFA allowing competitive inhibition of glucose usage at tissue sites and rise in some markers of insulin resistance (chapter 6). GH is known to lead to increases in free fatty acids (FFA) and induce hepatic and peripheral insulin resistance via lipolysis (Norrelund et al., 2009),(Moller, 1989, Fowelin et al., 1991, Ho et al., 1996). GH stimulates lipolysis by activating adenylyl cyclase, cAMP dependent protein kinase and then phosphorylation leading to upregulation of hormone sensitive lipase and resulting in increased circulating free fatty acids which act as a substrate for exercising muscle. GH induced inhibition of lipoprotein lipase leads to an increase in circulating NEFA and glycerol for storage in adipose tissue as triacylglycerol. These FFA released by GH contribute to the acute increase in insulin resistance by competing with glucose in the fatty acid/glucose cycle the liver contribute to the acute increase in insulin resistance and resulting increase in hepatic glucose output (Randal PJ, 1963).

An alternative mechanism to the glucose fatty acid cycle proposed by Randle has been suggested by a number of authors. Two studies propose that FFA lower intracellular glucose-6-phosphate levels, glucose levels and subsequently glycogen synthesis (Roden, 1996, Dresner et al., 1999). The exact mechanism of FFA influencing glucose metabolism is complex and likely to be directly and indirectly accomplished via changes in glucoregulatory hormones, indeed insulin itself has an indirect action via lowering of FFA on glucose metabolism (Mittelman, 1997, Festen, 2007, Rebrin, 1995).

GH also alters the effect of other lipolytic agents e.g. catecholamines on adipocytes to indirectly induce lipolysis (Marcus and M., 1994, Beauville and J.P., 1192, Harant and J.P., 1994). In the post absorptive resting state fatty acids are the predominant substrate fuel source. Exercise induces a GH surge leading to a release of fatty acids (Hunter, 1965). As the intensity of exercise increases up to 65% of VO_2 maximum (Wolfe, 1990) the proportion of energy provided by fatty

acids increases up to 50% of energy expenditure thereafter glucose oxidation becomes more important (Jeukendrup, 2002).

Pulses of GH in both GHD and normal individuals lead to an increase plasma FFA and glycerol, and enhances forearm muscle uptake and oxidation as well as FFA release from adipose tissue (Fineberg, 1974, Moller et al., 1990, Laursen, 1994). Exercise induces a GH rise which leads to a 3 fold rise in lipolysis rates, and increased FFA uptake into skeletal muscle. In GHD individuals discontinuation of GH administration had no effect at rest but lipolysis rates during exercise were reduced as were FFA uptake rates in skeletal muscle (Gibney et al., 2003). Kanaley et al demonstrated the importance of this GH burst by administering IV GH to a GHD population who were on long term replacement GH and demonstrated no change in lipolysis rates at rest but with 45 minutes exercise at lactate threshold found increased fatty acid turnover (Kanaley, 2004).

The importance of substrate utilisation in exercise in GHD is two-fold. Firstly changes in substrate utilisation in GHD are worse in conditions of exertion than in the resting state and improve with replacement of GH. Also the GH burst associated with exertion induces changes in substrate utilisation in normal and GHD individuals that could potentially confer a performance advantage and certainly facilitate more effective exercise. Replacement GH in GHD individuals is particularly important in exercise conditions and leads to increased FFA availability by lipid oxidation. It has less marked effects in the resting state (Gibney, 2007). The effects on exercise induced changes in glucose utilisation are less marked.

Supraphysiological GH administration to resting GH replete individuals increases lipolysis, fat oxidation and FFA. It also induces hyperinsulaemia, and reduces skeletal muscle glucose uptake (Giustina, 1998, Moller et al., 1990, Bak, 1991,

Copeland and Nair, 1994, Jorgensen, 1994a, Møller and K.G., 1993). There is evidence for increased rates of lipolysis in both the post prandial and post absorptive states during exercise but it is not dependent on the circulating GH during or after exercise (Lange et al., 2002, Healy, 2006). The effects of GH on CHO metabolism vary according to the duration of exposure (Davidson, 1987).

The subjects in this study were involved in regular exercise training and it is known that regular exercise induces metabolic changes prior to changes in lean body mass and body fat. Therefore there is a possible role for up regulation of the GH pulses and GH responses to exercise in general. GH secretion rates over a 24 hour period and IGF-I correlate positively with habitual physical activity levels and VO_2 maximum (Eliakim and D.M., 1996, Kelly and Gwinn, 1990, Poehlman, 1990, Weltman and Evans, 1994). Raised IGF-I concentrations become apparent within 2 weeks of commencing regular training and are above baseline for 6 months if training continues (Roelen et al., 1997, Koziris, 1999), and IGF-I and IGFBP3 are also raised by training (Roelen et al., 1997, Poehlman, 1990). The post exercise anabolic effect of GH may be due to changes in rates of lipolysis. Increasing fatty acid availability leads to reduced leucine oxidation (Tessari, 1986) and fat oxidation correlates negatively with oxidative protein loss (Solini, 1997) also administration of acipimox, which is antilipolytic, attenuates the protein sparing effect of GH during fasting (Norrelund, 2003). One study demonstrated a rise in rates of muscle protein synthesis after 4 months of endurance training(Short, 2004). Another demonstrated a rise in mixed muscle protein synthesis after endurance exercise (Sheffield-Moore, 2004).

In previous studies GH has been shown to be carbohydrate and glucose sparing in favour of lipid metabolism (Jorgensen et al., 1993). However, this was not seen in our study. Short term administration of GH has been shown before to

increase lipid (Mjaaland, 1993, Zenobi, 1992, Rizza, 1982) and carbohydrate oxidation and reduce glucose disposal (Rizza, 1982). Longer term administration induces rises in IGF-I and the insulin-like effects are antagonistic to those of GH (Zenobi et al., 1992, Boulware et al., 1992). Ikkos found no difference in substrate utilisation between normal and acromegalic subjects whereas others found increases in lipid oxidation rates (Ikkos et al., 1959). O'Sullivan et al found higher basal glucose levels and higher glucose and insulin response to OGTT in acromegalic patients compared to controls with greater insulin resistance in the acromegalic group.

In individuals with acromegaly there has been positive correlation between IGF-I levels and basal and post absorptive CHO oxidation rates and this correlation was seen only in the post absorptive state in normal subjects (Ho, 1996). It is also possible that changes in body fat with acromegaly account for some of these changes. Recombinant IGF-I administration studies increases CHO oxidation and glucose disposal rates (Boulware et al., 1992).

There was a significant fall in urinary nitrogen excretion in women on GH as a measure of whole body protein synthesis. GH stimulates nitrogen retention (Ikkos et al., 1959) and normal subjects demonstrate a reduction in urinary nitrogen excretion after acute GH infusion with a reduction in whole body urea synthesis and protein oxidation (Nygren et al., 2002). This has also been shown to occur in obese adults and children with hypopituitarism (Moller et al., 1990). GH is known to suppress hepatic ureagenesis (Wolthers et al., 1997) but the effect in GHDA is short lived and dose dependent likely to be reflective of circulating IGF-I and GH levels (Lucidi et al., 1998). There is a possibility that in this study the period of rising anabolism and falling UN was missed because samples were only collected at day 28. There are two issues with UN measurement in the protocol for the study. During the course of the study the error of not collecting urine for urinary nitrogen analysis on day 0 so each subject could act as his/her own control became evident and the protocol was amended to include collections but

some volunteers had already completed the study by that stage. Using a standard value for UN=0.008333 g/L is not appropriate in conditions where a substance is administered that alters UN excretion rates, i.e. GH. The urinary nitrogen measurement is also subject to variation due to the protein intake of volunteers on the day preceding the measurement (Lariviere, 1990). In this study this was recorded on day 0 but not on day 28.

A 40 hour fast increases urinary nitrogen excretion by 50% in GHDA which normalises if GH is replaced (Norrelund et al., 2009) thought to be due to an inhibition of muscle protein breakdown. Volunteers in this study were fasting for 8 hours prior to the OGTT but not 40 hours and urinary nitrogen was assessed post OGTT. In the post absorptive state GH has been shown to increase whole body protein turnover (Garibotto et al., 1997, Horber et al., 1996, Healy et al., 2003). Other studies have not shown this effect when looking specifically at muscle protein synthesis (Copeland and Nair, 1994, Yarasheski, 1992). This effect may be dose dependent as Lucida et al reported an increase in skeletal muscle protein synthesis with 3.3 μg/kg/day but not at 2μg/kg/day (Lucidi et al., 1998).

There is no consensus information in the literature of the effect of GH on protein metabolism. This may be reflective of the duration of exposure as occurs with carbohydrate metabolism (Davidson, 1987). In non athletic men there was an increase in whole body protein synthesis with no change in protein breakdown after 1 week of .01U/kg (Horber, 1990) and a lower dose for 12 weeks showed increased whole body protein synthesis but not muscle synthesis (Yarasheski, 1993), whereas Fryburg et al found increased muscle protein synthesis but not whole body protein synthesis after an 8 hour GH infusion (Fryburg, 1993). The baseline training of the muscle also seems to be important as there was no

change in whole body protein synthesis after 12 days of GH administration (Yarasheski, 1993).

Exercise induces a breakdown in whole body and muscle protein and GH administration leads to conservation of whole body protein which increased further post exertion. After 1 week and after 4 weeks of supraphysiological GH leucine oxidation reduced in resting and post exercise states and there was an increase whole body protein synthesis (Healy et al., 2003). It is known that leucine oxidation rates differ during rest and exertion and GH administration increases up to 2-fold with exercise but is reduced after GH administration by 50% after exertion but only 29% in the resting state (Rennie, 1981, Bowtell, 2000, Bowtell, 1998, Knapik, 1991). Exercise is also known to increase lean body mass and total body weight. Further increases with GH administration may be due to the effects of GH on water retention (Hoffman et al., 1996, Crist, 1988) as there was no change in skinfold anthropometry after 6 weeks of GH. Fazio et al (Fazio et al., 2000)) demonstrated increased stroke and cardiac index and a reduction in systemic vascular resistance in early acromegaly (<5 yrs). However a longer duration of illness is associated with more marked loss of protein, poor remodelling, leading to impairments in muscle strength and performance , reduced aerobic capacity (VO2 max) and cardiac performance (Nagulesparen, 1976, Thomas, 2002, Battezzati, 2003). Histologically acromegaly leads to a dose dependent hypertrophy in type 1 fibres associated with atrophy of type 2 fibres (Nagulesparen, 1976). There thus appears to be early anabolism followed by a period of poor protein remodelling (Gibney, 2007).

Fryburg et al report GH administration acutely induces protein synthesis (Fryburg et al., 1991). GH deficiency leads to reduced lean body mass of which skeletal muscle is reduced by 8% (Carroll et al., 1998, Salomon, 1989, de Boer, 1995) associated with reduced isomeric muscle strength and in some studies by isokinetic strength (Degerblad et al., 1990, Degerblad and Knutsson, 2007,

Johannsson, 1997, Janssen, 1999). Studies have yet to conclude whether this reduced strength is as direct result of reduced muscle mass or whether there is some intrinsic weakness associated as a contributing factor also. Muscle anabolism occurs within a short period of commencement of replacement (weeks). Muscle strength changes require longer term administration periods. 9 muscle groups were studied by Cuneo et al after 6 months replacement and only one showed increased strength (Cuneo, 1991). At baseline GHD subjects had reduced isometric and isokinetic muscle strength and local muscle endurance. After a 2 year administration period isometric and isokinetic muscle strength increased and local muscle endurance reduced and persisted at a 5 year follow up (Brill, 2002).

Similar to this study there is evidence in the literature of the effect of GH administration studies on whole body protein turnover and they have shown increased amino acid uptake (Copeland and Nair, 1994). However skeletal muscle protein turnover has been shown to be unchanged by GH (Nygren et al., 2002, Copeland and Nair, 1994) despite whole body increases and forearm musculature was shown to have increases protein synthesis and amino acid uptake in some studies (Fryburg et al., 1991, Fryburg, 1993, Yarasheski, 1992) This forearm effect is only short lived. Thus extra muscular sites may be the site(s) of changes in protein synthesis. Site specific phenylalanine and leucine uptake studies support this hypothesis (Copeland and Nair, 1994). Acute infusion of GH in individuals with reduced hepatic protein synthesis undergoing laparoscopic cholecystectomy was shown to improve the protein synthesis (Barle, 1999).

The effects of IGF-I itself on protein metabolism are controversial. IGF-I infusion has been reported to inhibit proteolysis and increase protein synthesis during concomitant amino acid infusion (Healy et al., 2003, Turkalj et al., 1992, Laager

et al., 1993). IGF-I activity may be dependent on lipid availability similar to GH as antilipolytic agents reduced fasting IGF-I and addition of GH did not reverse this (Norrelund, 2003). Similarly insulin inhibits proteolysis (Fukagawa et al., 1985). Heiling et al reported that insulin and glucose infusion induced a reduction in whole body proteolysis. This is mediated by insulin and not changes in glucose levels per se (Heiling et al., 1993). In contrast some studies reported protein sparing effects of glucose infusion both in the fasting (Cahill et al., 1970) and hyperglycaemic states (Felig et al., 1977).

There was no significant change in metabolic rate any group. GH raises resting energy expenditure (REE) and BMR by increasing lean body mass, increasing levels of tri-iodothyronine, and higher circulating non-esterified fatty acid levels. Muscle is the most metabolically active tissue in the body thus the total body muscle mass has considerable influence on REE. There was no fall in UN in men treated with GH in this study and no rise in midarm circumference to suggest a rise in muscle mass and thus effect REE. Women treated with GH had a fall in UN suggesting anabolism but no change in body weight so it is unlikely to be of a magnitude necessary to alter REE. GH is known to increase metabolic rates but it is likely that the period of administration was too short to allow detectable changes.

Basal energy expenditure has been shown to be increased in acromegaly (Ho, 1996). Higher rates of CHO oxidation are seen in the basal state and after a glucose load and greater suppression of lipid oxidation rates by glucose load in the acromegaly group (Ho, 1996). These changes correlated positively with IGF-I levels and not insulin or glucose levels and groups were matched for body fat, BMI and age.

The small study numbers and short duration of treatment coupled with the crude measurement technique of indirect calorimetry make interpretation difficult. The changes are small, largely insignificant and have wide standard errors.

In summary this study demonstrated some metabolic changes with GH administration. There was a rise in baseline NEFA in men after treatment with GH when comparing pre and post treatment NEFA as well as when comparing treatment and placebo groups. There was also a fall in urinary nitrogen excretion in women after treatment with GH but no changes in basal or post glucose metabolic rates, or overall body fat or carbohydrate metabolism measured by indirect calorimetry. There is some evidence of a correlation between serum NEFA and hyperinsulaemia (AUC insulin) after GH treatment in men.

Chapter 6

Study of the effects of 28 days of supraphysiological GH administration on markers of insulin resistance

6.1 Introduction

Growth hormone (GH) administration has been shown to induce a transient state of increased insulin resistance in adult GH deficiency (GHDA) (Svensson et al., 2002). Increased plasma glucose concentration post GH administration is a result of both increased hepatic glucose output and reduced peripheral uptake of glucose. Increased hepatic gluconeogenesis and glycolysis lead to increased non-oxidative glucose turnover and hepatic glucose output. This hyperglycaemia increases substrate availability to the exercising muscle and circulating hyperinsulinaemia may allow glycogen storage and thus potentially improved performance.

The aim of this study is to determine if 28 days of supraphysiological rhGH administration in GH replete young individuals leads to hyperglycaemia and/or hyperinsulinaemia, and a change in insulin sensitivity and if this change is mediated by changes in adiponectin or NEFA concentrations.

6.2 Methods

6.2.1 Subjects

The recruitment and inclusion and exclusion criteria for the study subjects in this were those outlined in chapter 2 and 3.

6.2.2 Procedure

The study protocol is described in chapter 2.

6.2.4 Statistical analysis

Statistical analysis is described in chapter 2.

6.3 Results

6.3.1 Baseline characteristics

There was no difference in any characteristics between the groups at baseline (table 6.1). 12 volunteers in the GH group and 7 in the placebo group had a family history of diabetes in a first degree relative, and 2 in the GH group and 3 in the placebo group had a family history of obesity.

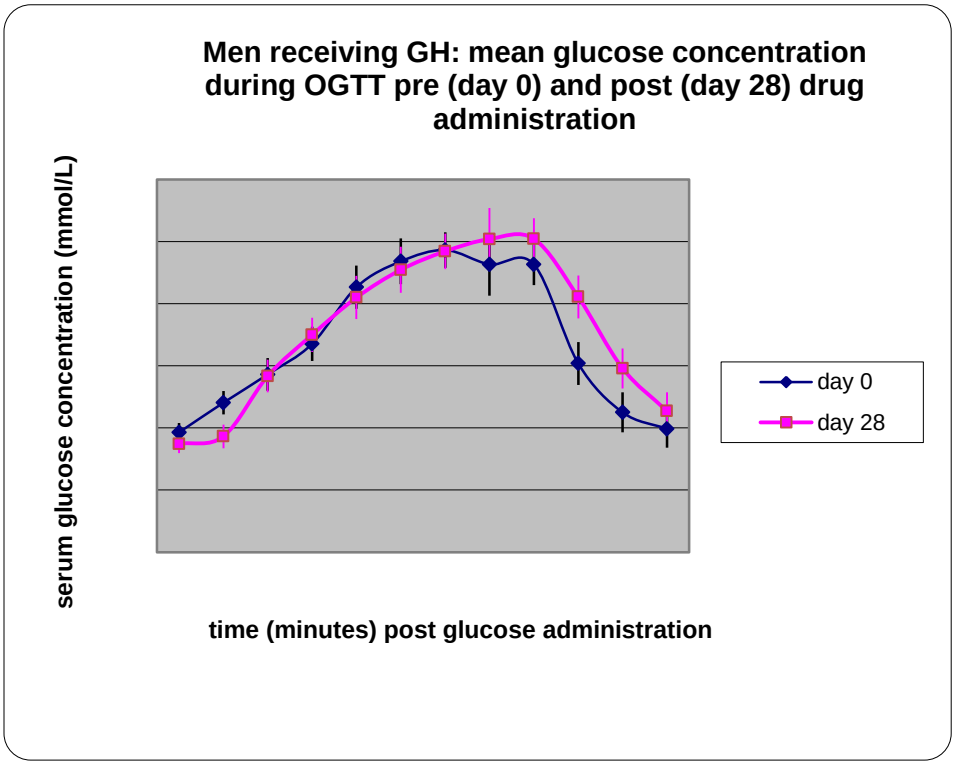
Baseline characteristics	GH		placebo	
	men n=14	women n=9	men n=9	women n=3
	mean±SEM	mean±SEM	mean±SEM	mean±SEM
BMI	23.2 ± 0.8	24.8 ± 1.5	25.3 ± 1.3	24.3 ± 1.6
fasting glucose (mmol/L)	4.93 ± 0.15	4.51 ± 0.24	4.08 ± 0.11	4.38 ± 0.16
fasting insulin (mU/L)	6.97 ± 1.06	6.56 ± 1.44	10.77 ± 4.99	9.33 ± 6.08
fasting insulin to glucose ratio	1.47 ± 0.23	1.26 ± 0.26	2.15 ± 0.91	2.04 ± 1.26
QUICKI	1.36 ± 0.47	2.59 ± 0.39	1.97 ± 0.29	-4.52 ± 6.12
HOMA2	0.92 ± 0.12	0.71 ± 0.12	1.37 ± 0.62	1.27 ± 0.68
systolic blood pressure	131 ± 2	111 ± 6	124 ± 5	111 ± 13
diastolic blood pressure	67 ± 2	69 ± 3	65 ± 3	66 ± 4

Table 6.1 Baseline characteristics of all subjects.

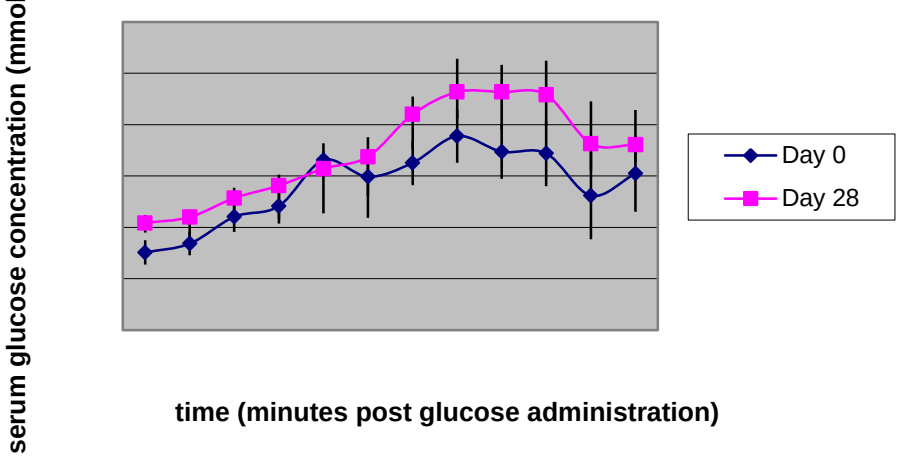
6.3.2 Changes in Serum Glucose during OGTT

Mean serum glucose was higher after treatment at baseline (time 0) in women receiving GH (p=0.01) and at 60 minutes post glucose administration in men

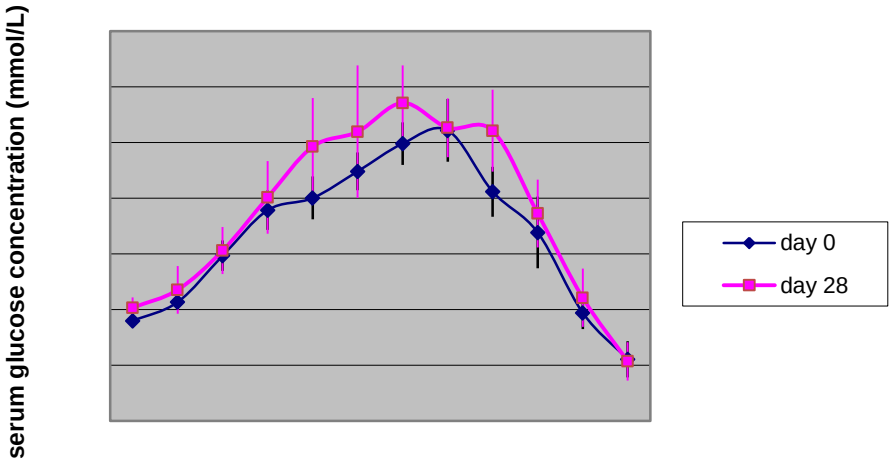
receiving GH (p=0.05) but not at any other time point during the OGTT. There was no change in the placebo group in either men or women. The serum glucose concentrations during OGTT are shown in figure 6.1.



Women receiving GH: mean serum glucose concentration during GTT pre (day 0) and post (day 28) drug administration



Men receiving placebo: mean glucose concentration during OGTT pre (day 0) and post (day 28) drug administration



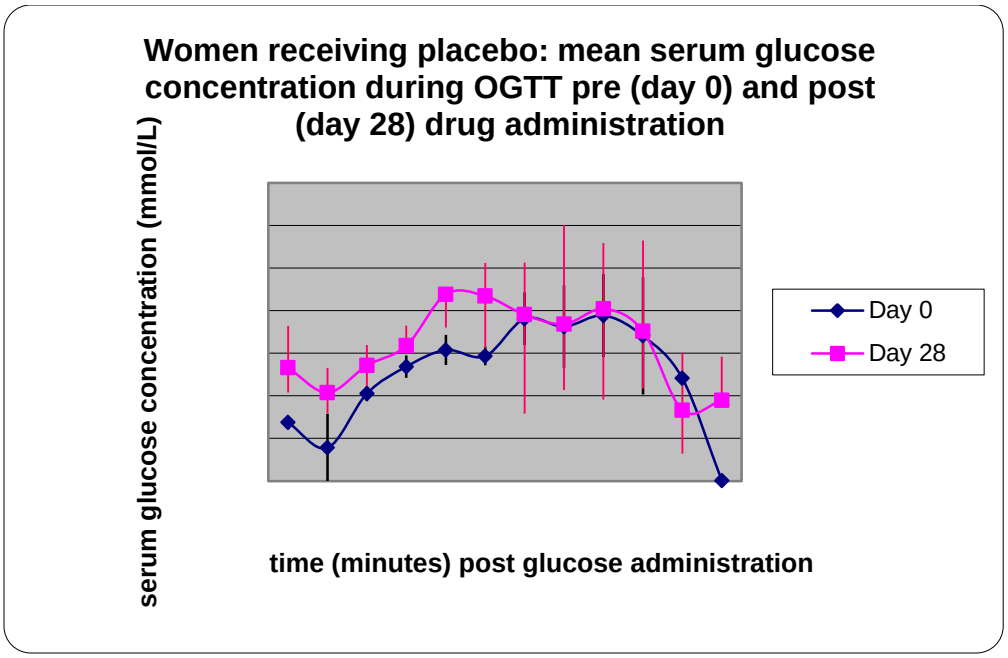


Figure 6.1 Serum glucose concentrations (mmol/L) fasting and at 5, 10, 15, 20, 30, 35, 40, 60, 90 and 120 minutes post glucose administration on pre and post treatment (day 0 and day 28 respectively).

The mean differences between day 0 and day 28 are shown in table 6.2.

Statistical significance data for glucose concentration change from day 0 to 28					
		time points (mins post glucose)	Mean difference between day 0 and 28 (mmol/L)	SEM	p value
GH	men	0	-0.19	0.29	0.54
		60	1.07	0.5	0.05
		120	0.17	0.57	0.73
	women	0	0.56	0.17	0.01
		60	0.92	0.53	0.13
		120	0.27	0.41	0.53

Table 6.2 The mean difference in serum glucose concentration (mmol/L) at 0, 30, 60 minutes post glucose administration pre and post treatment (day 0 and day 28 respectively) and the statistical significance data for the change (paired t test).

6.3.2.1 Area under curve (AUC) for glucose during OGTT

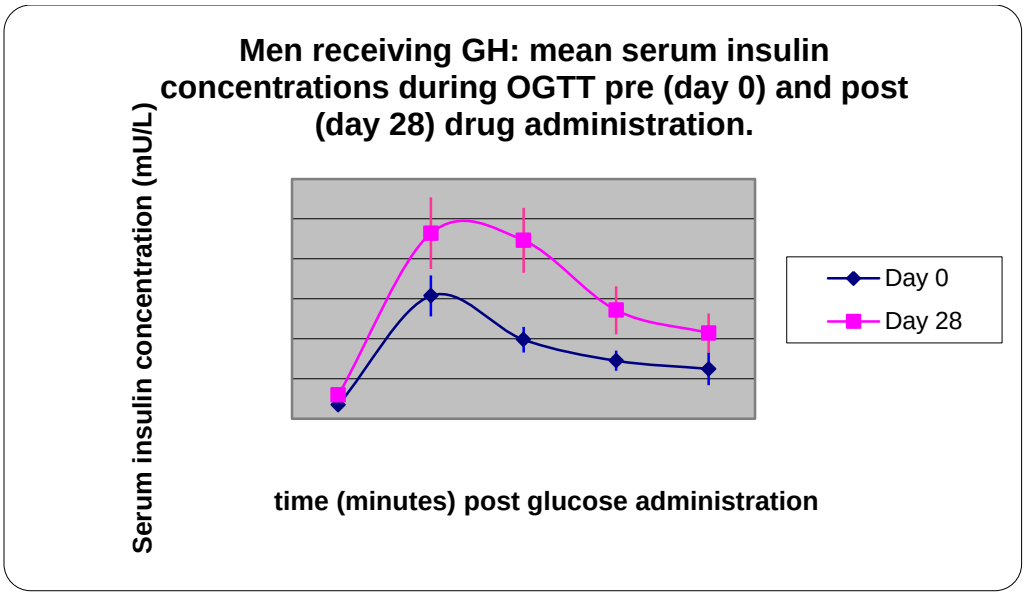
There was no statistically significant change in the AUC of any group in this study. The data for mean AUC glucose are shown in table 6.3.

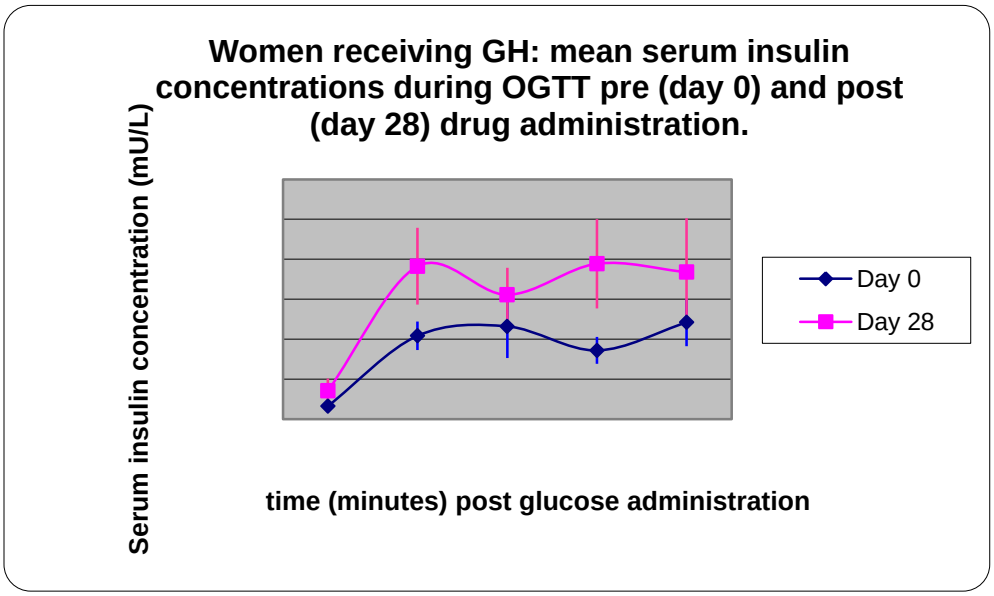
Mean AUC for glucose (mmol/L/hr)				
	GH		placebo	
	men	Women	men	women
	n=13	n=9	n=7	n=2
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
day 0	734 ± 0.25	714 ± 37	720 ± 37	666 ± 71
day 28	749 ± 38	757 ± 36	757 ± 36	7.4 203
	p=0.09	p=0.07	p=0.45	p=0.82

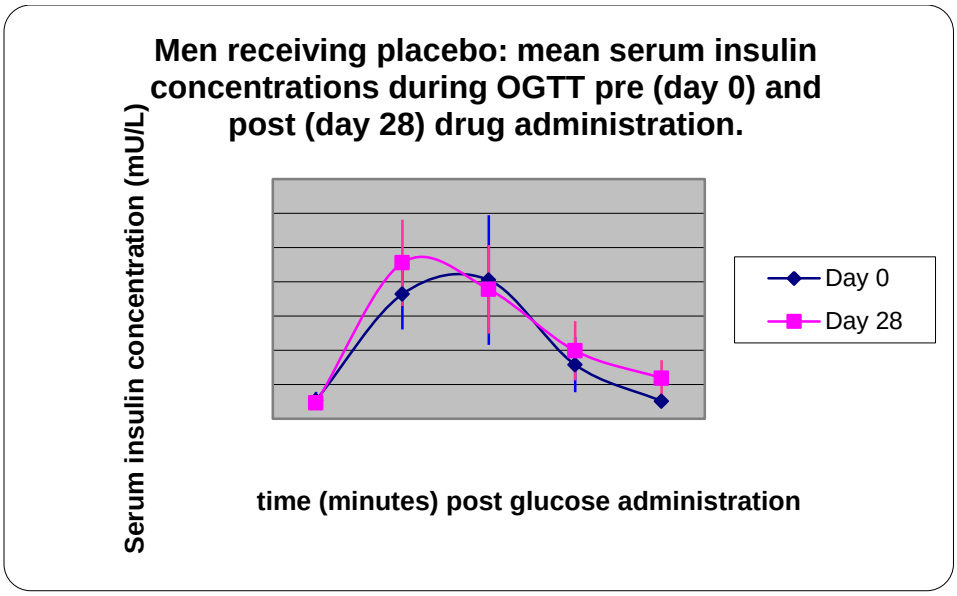
Table 6.3 Area under the curve (AUC) in serum glucose concentrations (mmol/L/hr) during the OGTT pre and post treatment (day 0 and 28 respectively) in all groups, and the statistical significance data for the change (paired t tests).

6.3.3 Changes in Serum Insulin during OGTT

There was a significant rise in serum insulin concentration after treatment at 60 minutes post glucose administration in men receiving GH but not at any other time point or in any other group. Serum insulin concentrations in all groups are shown in the figure 6.2 and mean difference data in table 6.4.


Men receiving GH: mean serum insulin concentrations during OGTT pre (day 0) and post (day 28) drug administration.
Serum insulin concentration (mU/L)
Day 0
Day 28
time (minutes) post glucose administration


Women receiving GH: mean serum insulin concentrations during OGTT pre (day 0) and post (day 28) drug administration.
Serum insulin concentration (mU/L)
Day 0
Day 28
time (minutes) post glucose administration



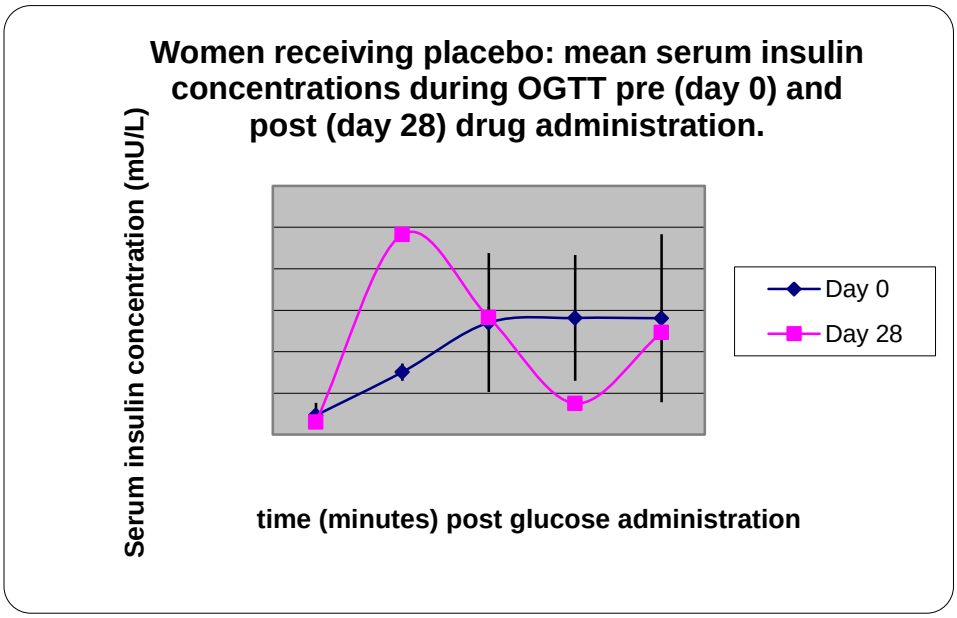


Figure 6.2 Serum insulin concentrations (mU/L) during OGTT pre and post treatment (day 0 and 28 respectively) in all groups at time 0, 30, 60, 90 and 120 minutes post glucose administration.

Statistical significance data for insulin concentration change from day 0 to 28					
		minutes post glucose	Mean difference between day 0 and 28 (mU/L)	SEM	p value
GH	men	0	2.89	2.6	0.42
		60	48.92	17.0	0.01
		120	21.49	8.37	0.08
	women	0	6.11	4.66	0.07
		60	24.39	24.8	0.73
		120	14.14	14.23	0.69

Table 6.4 The mean difference in serum insulin concentrations (mU/L) in the GH groups pre and post treatment (day 0 and day 28 respectively).

6.3.4 Area under curve (AUC) for insulin during OGTT

There was a significant rise is AUC insulin in men on GH after treatment (comparing day 0 and day 28, log transformed) (p=0.003). There was no change in women receiving GH (p=0.09) or men receiving placebo (p=0.27). The area under the curve for insulin during the OGTT is shown in the table 6.5.

Mean AUC for insulin (mU/L/hr)	GH		placebo	
	men	women	men	women
	n=13	n=9	n=6	n=1
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
day 0	4.48 ± 0.64	4.48 ± 0.7	5.56 ± 2.17	5.36
day 28	8.4 ± 1.35	7.87 ± 1.99	6.74 ± 2.28	6.01
	p=0.003	p=0.09	p=0.27	none available

Table 6.5 AUC for insulin (mU/L/hr) during the OGTTs pre and post treatment (day 0 and 28 respectively) in all groups.

6.3.5 Change in HOMA and QUICKI following treatment.

There was no change in HOMA2 or QUICKI in any group. There was only one woman in the placebo group so no comparison could be made. These results are shown in tables 6.6 and 6.7.

HOMA2	GH		placebo	
	men	women	men	women
	n=12	n=8	n=6	n=1
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM
day 0	0.92 ± 0.12	0.71 ± 0.12	1.37 ±0.62	1.27
day 28	1.58 ± 0.32	1.67 ± 0.65	1.64 ± 0.55	0.8
	p=0.30	p=0.22	p=0.65	none available

Table 6.6 Changes in HOMA2 for all groups pre and post GH administration (day 0 and 28 respectively) in all groups.

QUICKI	GH		placebo	
	men	women	men	women
	n=13	n=8	n=6	n=1
	mean ± SEM	Mean ± SEM	mean ± SEM	mean ± SEM
day 0	1.36 ± 0.47	2.59 ± 0.39	1.97 ±0.29	-424.52
day 28	1.77 ± 0.26	1.68 ± 0.26	1.8 ± 0.27	1.8
	p=0.63	p=0.07	p=0.84	none available

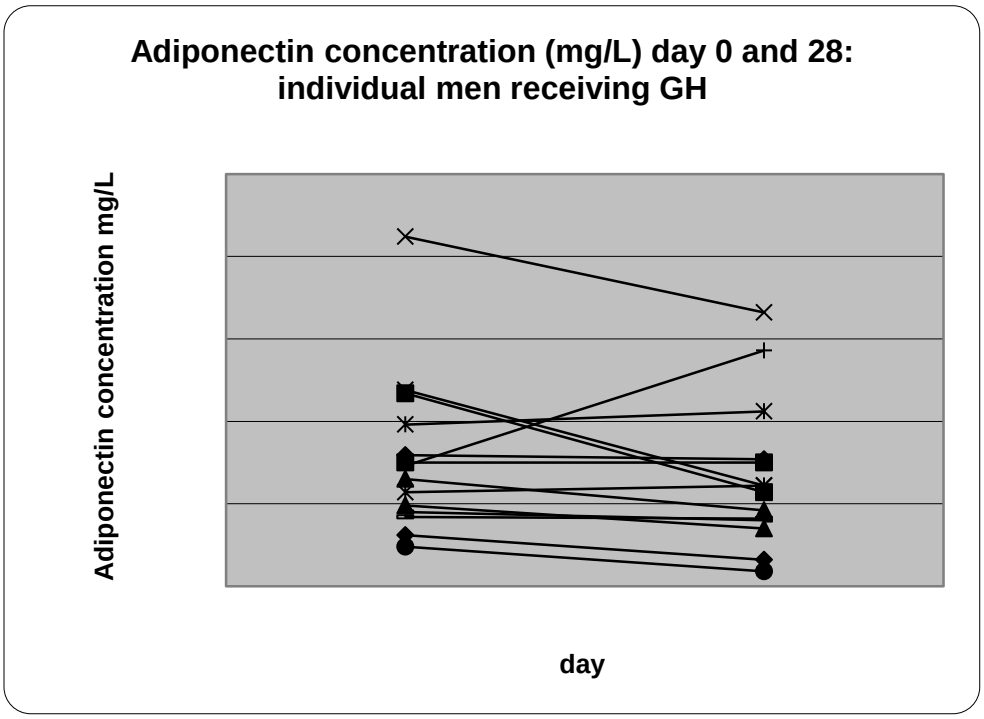
Table 6.7 QUICKI calculations pre and post GH administration (day 0 and 28 respectively) in all groups.

6.3.6 Changes in Insulin: glucose ratio (I: G ratio)

There was no change in fasting insulin to glucose ratio (I:G) in any group after treatment. However, a significant rise was seen in I: G ratio at 60, 90 and 120 minutes post glucose administration in men receiving GH (p= 0.02, 0.05, 0.04), but not in women receiving GH (p= 0.36, 0.8, 0.88), or men receiving placebo (p=0.66, 0.62, 0.63).

6.3.7 Adiponectin concentrations

Adiponectin concentrations were assessed in the fasting state before and after GH administration in the treatment groups but not measured in the placebo group. See figure 6.3.



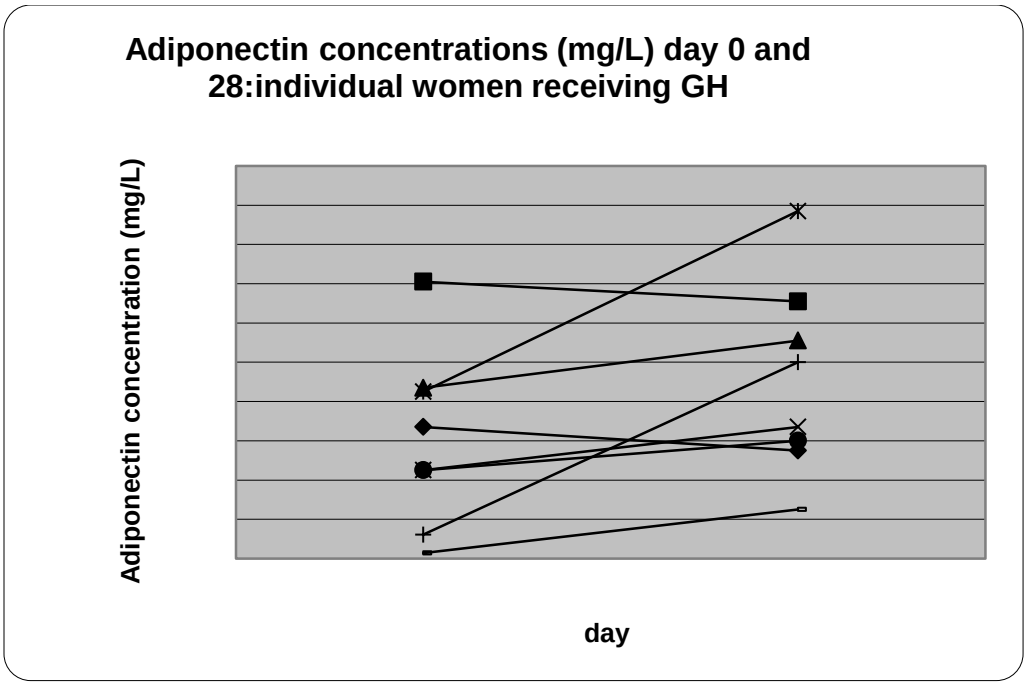


Figure 6.3 Individual adiponectin concentrations (mg/L) pre and post treatment (day 0 and 28 respectively) in men and women receiving GH.

Mean values for the treatment groups are shown in table 6.8

Fasting Adiponectin concentration (mg/L)	GH	
	men n=14	women n=8
	mean ± SEM	mean ± SEM
day 0	7.76 ± 1.3	7.75 ± 1.39
day 28	6.66 ± 1.2	9.08 ± 1.73

Table 6.8 Fasting adiponectin concentrations (mg/L) pre and post GH administration (day 0 and 28 respectively).

6.3.8 Regression analyses.

There was no significant correlation between adiponectin on day 28 and any other variable by linear regression analysis.

Statistical data correlating serum adiponectin (day 28) with other variables in subjects on GH							
		GH					
		men			women		
		n	p value	R square	n	p value	R square
BMI		7	0.479	0.051	7	0.768	0.013
total body fat							
	anthropometry	7	0.467	0.049	7	0.359	0.121
	bioelectrical impedance	7	0.256	0.115	7	0.492	0.082
	BODPOD®	7	0.871	0.003	7	0.845	0.007
HOMA		7	0.704	0.015	7	0.832	0.01
AUC glucose		7	0.225	0.13	7	0.199	0.305
AUC insulin		7	0.327		7	0.52	
systolic BP day 28		7	0.3	0.089	7	0.663	0.034
diastolic BP day 28		7	0.697	0.013	7	0.66	0.456

Table 6.9. Statistical correlation data between serum adiponectin concentration (log transformed) post treatment (day 28) and other variables (log transformed).

When combining the genders and performing paired ttests the results are shown in table 6.10. There is no statistically significant change seen.

Statistical significance testing for measures of body fat and insulin resistance combining male and female gender groups		p value	n
GH	fasting NEFA	1.00	16
	NEFA t-120 mins	0.20	13
	Glucose t= 120 mins	0.52	19
	Insulin t=120 mins	0.07	18
	fasting glucose	0.83	22
	fasting insulin	0.06	18
Placebo	fasting NEFA	0.18	7
	NEFA t-120 mins	0.69	6
	Glucose t= 120 mins	0.97	6
	Insulin t=120 mins	0.37	5
	fasting glucose	0.19	9
	fasting insulin	0.71	4

Table 6.10. Statistical significance data (paired ttests) for parameters of insulin resistance using male and female genders combined. All parameters are log transformed and compare day 0 (pre treatment) and day 28 data (post treatment).

There was a statistically significant correlation between serum IGF-I concentration on day 28 (log transformed) and AUC insulin on day 28 (p=0.019) in men receiving GH but not in women receiving GH (p=0.296). There was a correlation between AUC NEFA on day 28 in both men and women receiving GH

(p=0.036, 0.015 respectively). There was no correlation between serum IGF-I and any other variable of insulin resistance. Please see table 6.11, 6.12.

Statistical data correlating IGF-I (day 28) (log transformed) with other variables (linear regression analysis) in subjects on GH						
dependent variable = log transformed IGF-I day 28	men			women		
day 28	n	p	r^2	n	p	r r^2
HOMA	7	0.065	0.3	7	0.665	0.046
AUC glucose	7	0.645	0.02	7	0.254	0.001
AUC insulin	7	0.019	0.407	7	0.296	0.044
AUC NEFA	7	0.036	0.368	7	0.064	0.252

Table 6.11. Statistical correlation data between serum IGF-I concentration (log transformed) post treatment (day 28) and other variables (log transformed) in subjects on GH.

Statistical data correlating IGF-I (day 28) (log transformed) with other variables (linear regression analysis) in subjects on placebo						
dependent variable = log transformed IGF-I day 28	men			women		
day 28	n	p	r^2	n	p	r^2
HOMA	4	0.643	0.04	1	None available	None available
AUC glucose	4	0.94	0.25	1	none available	None available
AUC insulin	4	0.653	0.214	1	none available	None available
AUC NEFA	4	0.683	0.653	1	none available	None available

Table 6.12. Statistical correlation data between serum IGF-I concentration (log transformed) post treatment (day 28) and other variables (log transformed) in subjects on GH

There is a statistically significant correlation fasting fat oxidation on day 28 and AUC glucose in men receiving placebo (p=0.046) but not in any other group. There was no correlation between fat oxidation at 60 or 120 minutes post glucose administration and AUC glucose on day 28.There was no correlation between fat oxidation at any time point and AUC insulin on day 28.

6.4 Discussion

This study has shown that 28 days of supraphysiological rhGH administration leads to a reduction in some but not all of the measures of insulin sensitivity in men and women. This was most notable in post prandial glucose levels, and there was a rise in the area under the curve for insulin, a rise in glucose and insulin at 60 minutes post glucose administration, and in insulin to glucose ratio at 60, 90 and 120 minutes during the rapid sampling OGTT. This was not associated with any changes in HOMA or QUICKI. There was some evidence of reduced insulin sensitivity in women with a rise in baseline/fasting glucose concentrations on day 28 compared to day 0. There was no significant change in HOMA or QUICKI post GH administration. Although changes in whole body insulin sensitivity can be linked to changes in body composition, there was no reported change in whole body fat percentage and only a reduction in hip circumference on anthropometry. No correlation was found by regression analysis between serum adiponectin concentrations after GH administration and any changes in body fat indices or insulin sensitivity. However, there was a correlation between IGF-I on day 28 and AUC insulin on day 28 in men receiving GH, and between AUC NEFA on day 28 and AUC insulin on day 28 in men receiving GH.

GH infusion has been shown to be associated with a fall in glucose uptake in muscle and a fall in glucose oxidation secondary to a rise in FFA and competition at the level of the Randles cycle in some studies (Moller et al., 1990, Moller, 1990, Djurhuus, 2004). Glucose uptake accounts for approximately 15 to 20% of substrate for muscle metabolism in the basal state with lipid constituting up to 80% (Tancredi, 1976). This reduced glucose uptake is thought to be either a direct effect of GH or secondary to increased lipid oxidation rates (Dagenais, 1976). Serum glucose concentration is preserved in the face of reduced glucose uptake and reduced glucose utilization and this suggests another tissue utilizing the glucose. There is no evidence for increased lipogenesis in adipose tissue or liver lipogenesis as GH reduces and not increases respiratory exchange ratio (Frayne, 1983). After large GH pulses there have been reports of a reduction in post absorptive splanchnic glucose output suggesting increased glucose uptake and in vitro studies have reported increased renal gluconeogenesis (Rogers, 1990). Dogs have doubling of liver glycogen content post exposure and in human subjects radiolabelled glucose studies have confirmed increased gluconeogenesis, and hepatic lipogenesis increased after months of therapy with GH (Butler, 1991).

GH deficiency closely resemble the metabolic syndrome with increased central adiposity and impaired physical fitness, reduced insulin sensitivity. This is likely to be a result of prolonged GH deficiency and the inherent lack of the lipolytic and anabolic effects of GH as well as the underlying disease state itself. Discontinuation studies show improvement of insulin sensitivity after 1 year off therapy despite reaccumulation of body fat mass and imply an inherent insulin resistance with GH therapy (Johannsson, 1999). FFA play a vital role and inhibition of lipolysis with acipimox restores insulin sensitivity (Nielsen, 2001).

After commencement of GH replacement in GHDA there is a transient rise in plasma glucose and insulin resistance parameters and results correlate positively with the dosage administered and negatively with the duration of therapy (Woodmansee, 2009). After longer term exposure improvements in lean body mass and waist to hip ratio lead to improved insulin sensitivity with normalisation of serum glucose and HbA1c (Fideleff, 2008, Abs, 1999, Woodmansee, 2009). Acute and chronic GH replacement leads to impaired insulin sensitivity with increased endogenous production and reduction in peripheral glucose uptake secondary to increased rates of lipolysis and competitive inhibition of glucose metabolism at the level of the glucose fatty acid cycle (Bramnert, 2003, Nielsen, 2001, Piatti, 1999). Administration of peroxisome-proliferator-activated receptor agonists lead to improvement in insulin resistance in GHD adults (Krag, 2008).

GH replacement in GHDA has not been shown to influence glucose oxidation during exercise (Brandou and J.F., 2006) and Kanaley et al reported no change in glucose appearance or uptake from the circulation (Kanaley, 2004). However Gibney et al found raised plasma glucose levels in resting and exercise conditions (Gibney et al., 2003).

Active acromegaly is associated with hyperglycaemia despite hyperinsulinemia, impaired glucose tolerance, and hepatic and peripheral insulin resistance (Nabarro, 1987, Sonksen, 1967). Hansen et al documented a higher prevalence of abnormal glucose tolerance pre treatment (57.9 *vs.* 20.6%) as well as lower glucose to insulin ratios in clamp studies and elevated hepatic glucose production rates in active acromegalic patients (Hansen, 1986). Skeletal muscle uptake studies reported increased insulin resistance with reduced non oxidative glucose disposal rates (Foss, 1991, Kasayama, 2000, Moller, 1992).

Adiponectin concentrations have been shown to be correlate well with insulin resistant states independent of BMI in a non obese GH replete populations (Kern et al., 1989). One of the hypotheses of this study is that changes in insulin sensitivity are mediated by changes in body fat which is in turn mediated by adiponectin. Regression analysis did not support adiponectin as the mediator of these changes, nor indeed does it support changes in body fat being due to changes in adiponectin in that study. Most studies in GHDA to date have showed no changes in adiponectin despite changes in body composition. Svennson et al postulating that changes in adiponectin concentration with GH replacement in GHD adults occurs by GH independent means. They reported decreased glucose tolerance on OGTT after 1 week of GH replacement in GHD individuals in the absence of any changes in adiponectin(Svensson, 2005). No changes in adiponectin concentrations were seen despite increases in IR measured by HOMA after 5 days of rhGH administration in GHD individuals (Schmid, 2005). A study of 12 months therapy showed no change in IR or adiponectin concentrations despite changes in body composition in GHD individuals (Sinha et al., 1989). Fukuda et al demonstrated lower levels of adiponectin in individuals with GHD and healthy controls compared to individuals with GH excess (acromegaly) but in acromegaly IR was not closely associated with adiponectin levels compared to assessment of BMI (Fukuda, 2002). Giavoli et al found no difference in adiponectin levels in untreated GHD patients compared to controls despite higher percentage body fat, BMI and visceral obesity (Giavoli, 2004).

Subjects in this study are non Caucasian and that as such have differing metabolic profiles to Caucasian subjects. Fasting serum insulin can be up to two fold higher in African American compared to Caucasian American individuals of otherwise normal glucose tolerance. There were no significant differences in serum glucose or C-peptide during fasting or during OGTT, with a 45% lower post-prandial hepatic insulin clearance and 33% lower basal insulin clearance in African American subjects (Osei and Schuster, 1994). African Americans athletes

have been shown to have higher fasting insulin and lower C peptide levels than Hispanic Americans and Caucasians (Erotokritou-Mulligan, 2008).

As with preceding chapters the issues of compliance, small study numbers, crude measures of insulin sensitivity and short duration of exposure are likely to be relevant to the lack of changes in overall insulin resistance. It is possible that the duration of exposure was too short. Some studies in the literature showed changes at 1 year that were not seen at 8 weeks which were thought to be due to the changes necessary in body habitus which are longer term changes and require longer exposure. The oral glucose tolerance test and HOMA/QUICKI are not the gold standard for the detection of small changes in glucose tolerance and particularly for the identification of changes in hepatic compared to peripheral insulin resistance. Use of clamp studies would be considered the gold standard and were not performed in this study. The OGTT and HOMA index reflects hepatic insulin sensitivity and basal hepatic glucose production with a correlation coefficient of 0.88 ($p<0.0001$) (Matthews et al., 1985) and 0.59 ($p<0.0005$) (Stumvoll et al., 2000).

In summary there was evidence of small changes in insulin sensitivity in men receiving GH and less so in women receiving GH but no change in overall whole body insulin resistance and no evidence that these changes are mediated by changes in adiponectin concentration was found in this study. However there was some evidence that the changes were mediated by changes in serum IGF-I and NEFA concentrations.

Chapter 7

Conclusion

7.1 Discussion

In conclusion there was no evidence found in this study of an effect of ethnicity on the response of IGF-I and P-III-NP to 28 days of rhGH administration in non Caucasian athletes compared with white European athletes. There was a rise in IGF-I, P-III-NP and the GH2000 score in both the low and high dosing GH groups. The placebo group and baseline concentrations in the treatment groups were lower than the corresponding GH2000 cohort but peak concentrations of IGF-I and P-III-NP were similar to the GH2000 group. However, the peak GH2000 score was lower in men than in the GH2000 group. There was no evidence of an effect of ethnicity on maximal response to rhGH which could potentially invalidate a test for GH abuse among athletes.

GH has been known for some time to confer some aspects of physical performance advantages to people with GH deficiency. It would seem naive to either assume that either GH was not being used or that the athletes are somehow “misguided” and that GH would not confer advantages. Especially in view of the level of expertise within the athletic community in detecting small changes in performance, circumstantial evidence that they have been using GH for a number of decades for its performance enhancing effects, and the ever increasing financial rewards and public acclaim associated with sporting excellence. Similarly naive would be the assumption that societal morals about “cheating” would deter usage. The fear of detection and subsequent disgrace and loss of income are more significant deterrents than the thought of personal harm. In a survey by Sports Illustrated, 195 out of 198 athletes said that they would take a performance-enhancing drug if they were guaranteed to win and not be caught. 50% stated that they would still take the substance even if they would

die from a side effect of the drug after 5 years of successful competition (Bamberger and Yaeger, 1997).

This study has demonstrated a number of these advantageous processes in normal men receiving supraphysiological GH that could be considered performance enhancing. Exercise capacity is determined by the pulmonary respiratory exchange, increased substrate and oxygen delivery to exercising muscle and subsequently uptake by the muscle cells and conversion into kinetic energy. GH could potentially deliver a performance advantage by improved stroke volume through increased cardiac preload secondary to water retention. It has a direct or IGF-I mediated anabolic effect on the cardiac muscle. It reduces systemic vascular resistance by relaxation of the endothelium with increased blood in the muscle vascular bed and increases red cell mass by increased erythropoiesis and thus delivery of oxygen and substrate to the exercising muscles. (Sacca`, 1994)(Maison and Chanson, 2003). It also provides increased substrate availability by increased lipolysis and serum fatty acid concentration, hyperglycaemia, and hyperinsulinaemia increasing glycogen stores which is carbohydrate sparing and potentially anabolic. The effect of GH is seen most dramatically in adults with GH deficiency (GHDA) where it leads to increased muscle mass, enhanced use of lipids as a fuel source, improved thermal regulation, increased cardiac output, and improved wound healing and ligamentous strength (Salomon, 1989). The replacement of GH for 6 months in men and women can lead to an 8.8% increase in the muscle mass and 14.4% loss in fat mass (Salomon, 1989).

There was no change found in this study in VO_2 maximum or time to exhaustion during a treadmill running test after GH treatment. There is some evidence in the literature that GH may reduce exercise capacity. Berggren et al studied 30 healthy volunteers after 28 days of GH administration and found no significant

difference in exercise capacity or power output (Berggren, 2005). Exercising lactate levels were shown to be higher in 2 studies compared to controls (Lange et al., 2002, Hansen, 2005) which has been shown to be associate with reduced stamina and early fatigue. One study showed improved maximum inspiratory and expiratory pressure in GH treated participants without pre-existing GHD but there was no change in VO_2 maximum (Graham, 2007b, Graham, 2007a). A single blind study by Graham et al on former anabolic androgenic steroid users found a rise in maximum inspiratory and expiratory pressures but most other studies found no change in aerobic capacity in healthy GH replete individuals post GH administration. Growth hormone administration (2mg/day) to recreationally trained men increased sprint capacity by 3.9% but co-administration with testosterone (250mg/wk) increased sprint capacity to 8.3% Neither treatment group showed sustained effect at 6 weeks post cessation of treatment (Meinhardt, 2010). Pulmonary capacity is generally not a significant limiting factor at sea level in healthy individuals during training due to an existing pulmonary reserve capacity. Thus the contribution of GH to ventilatory capacity may be limited and studies certainly show it can increase lactate and fatigue and thus inhibit rather than facilitate exercise capacity (Brooks, 2006).

Use of GH has been reported in sporting disciplines where muscle mass and strength confers an advantage such as body building (Spellwin, 2006, Fainura-Wada, 2006). There was no evidence found in this study to support this hypothesis, with no increased muscle mass measured by midarm circumference, no fall in urinary nitrogen or rise in basal metabolic rate to indicate increased muscle bulk in men receiving GH. There was some evidence of anabolism in women with reduced urinary nitrogen but no change in mid arm circumference or basal metabolic rate in these subjects. Yarasheski et al reported no increase in muscle protein synthesis or reduction in protein breakdown in college football players or weight lifters (Yarasheski, 1993). Other studies have shown growth hormone therapy in young lean physically fit individuals leads to an increase in

lean body mass and approached statistical significance in terms of fat mass reduction. However studies in similar subjects reported no increase in muscle strength after 42 or 84 days rhGH therapy in 1-repetition maximum voluntary strength testing and no improvement in biceps, or quadriceps strength, 7 other muscle groups and no change in 4 circumference measures (Yarasheski, 1992, Deyssig, 1993). Thus it appears GH may increase muscle bulk but not strength. This would be consistent with changes seen in chronic acromegaly with weak hypertrophied muscles. Early acromegaly may well present a window of performance opportunity as anecdotally seen in one elite rower, whereby muscle is hypertrophied and has yet to lose strength (Sharp, 2009).

Following 28 days of GH administration in GH replete individuals in this study there was evidence of increased glucose and FFA availability to act as substrates for the increased metabolic demands of exercising muscle. There is evidence of hyperinsulinaemia which could facilitate the anabolic effects of insulin and/or glycogen storage capabilities of insulin, and reduced systemic vascular resistance and systolic blood pressure seen in this study to increase pooling of blood in the muscle bed. GH has been shown to lead to reduced carbohydrate utilisation and hyperinsulinaemia allowing relative glycogen sparing or “bulking up” of carbohydrate stores. It has also been shown to potentiate lipid utilisation which is further carbohydrate sparing and may be particularly advantageous for sporting performance.

GH also reduces body fat mass. There were some changes in this study in body fat as waist to hip circumference was reduced in men receiving GH but no change in whole body fat percentage. It is likely that this represents redistribution rather than a loss of total body fat. GH is known to lead to a redistribution of body fat from central to peripheral depots in GHD individuals (Salomon, 1989). Loss of body fat is performance enhancing in sporting

disciplines such as body building where it increases muscle definition and improves appearance, and sports such as boxing and athletics for reduced body weight. There was a reduction in fat mass of -0.9 kgs but it did not reach statistical significance in the 10 studies in the met analysis by Liu (Liu, 2008).

In this study there was some evidence of changes in glucose metabolism with a higher fasting glucose, insulin, area under the curve for insulin during OGTT but there were no changes in overall whole body insulin resistance as measured by HOMA or QUICKI. There is thus evidence of circulating hyperinsulinaemia without insulin resistance which may potentiate the effects of insulin and be performance enhancing. It is most likely that changes were too early and of small calibre to affect whole body insulin resistance and HOMA and QUICKI were not sensitive enough to detect such changes.

Despite higher concentrations of circulating glucose and fatty acids there was no evidence of a change in respiratory quotient and increased lipid oxidation. In other studies lean physically fit GH replete individuals treated with GH had higher lactate, plasma free fatty acids and glycerol concentrations during exercise but similarly no change in respiratory exchange ratio during exercise in GH treated individuals compared to placebo (Hansen, 2005, Lange et al., 2002, Healy, 2006).

There was increased body weight in men receiving GH in this study. It is likely to be secondary to water retention in the absence of changes in metabolic rate to indicate increased muscle mass or changes in urinary nitrogen or mid arm circumference. However, there was no change in percentage body water on bioelectrical impedance analysis, and only 2 volunteers reported pedal oedema. Increased water could potentially contribute to circulating blood volume and preload but the effects of this on performance are controversial (Gibney, 2007).

7.2 Limitations of this study

The study has a number of limitations. Study numbers were low. The study did not achieve the requisite 90 volunteer numbers. 62 subjects were recruited for the study but only 36 subjects completed the study. Volunteers were recruited but because of a problem with the supply and distribution on the part of the pharmaceutical company Serono, delays of up to 6 months were incurred and volunteers were sometimes unavailable to continue the study at that stage. 18 volunteers dropped out in this manner.

Most subjects were men and thus data and conclusions on the effect of GH on women were limited. Only 2 Oriental subjects were recruited so this population was under-represented in the study.

More robust measures of insulin resistance such as clamp studies would have allowed determination of small magnitude change as well as the relative contribution of hepatic and peripheral insulin resistance sites.

The study duration may well have been too short and dosing too low to allow for detection of changes in muscle mass by the methods chosen, e.g. circumference measurement. Changes may be of higher magnitude and be easier to detect after longer duration of exposure. However, it is important to be mindful of the adverse effects of GH administration in a healthy population. There is a higher incidence of oedema, arthralgia, carpal tunnel syndrome and fatigue among GH treated individuals (Abs, 1999, Carroll et al., 1998).

Measures of body fat used were too crude for the degree of changes that occurred with such a short study, and the BODPOD® would need to be in working

order, more reliable and the testing environment more regulated. Dual energy X-ray absorptiometry (DEXA) is currently considered the gold standard. Future studies would be advised to assess body composition by DEXA or other radiographic imaging to allow detection of early and small magnitude changes. Lactate studies, as well as amino acid measurements would have allowed more meaningful detection of small metabolic changes.

Similarly methods of assessment were also too crude to detect a number of early or small changes in substrate utilisation. From the view point of the metabolic studies urinary nitrogen should have been measured before and after OGTT on pre and post GH days and a more detailed diary of dietary protein intake performed.

I would like to acknowledge the many subjects who volunteered, and especially those who completed the study. Unfortunately a significant number were disappointed and could not take part in the study because of the difficulties with the supply of GH. Most who did partake were very obliging of their time and I am very grateful. However during the course of this study some proved to be a particularly somewhat unreliable. They often did not turn up on pre-arranged testing days, lost GH pens on trains etc. and changed their mobile phones very frequently as companies offered incentives which made it difficult to contact them. Many did not attend for day 28 on the required day and had to be removed from the analysis. Subjects who attended more than 1 day after the true day 28 were excluded. In hindsight I should have been more insistent on receiving empty vials, measured the drug quantity present and not permitted any excuses or delays in getting the day 28 testing completed. Spending some time finding out what would motivate the participants and what they wanted to get from the study may have improved compliance. Offering ongoing regular incentives may also have improved compliance, e.g. more regular VO_2 maximum testing, feedback on body fat changes and serum markers would all have contributed to keeping their interest alive and allowed regular contact with study

personnel thus encouraging compliance and rewarding the contribution of the subjects. Some other authors also studied a student population and had a better compliance rate. I can only comment that the students in our study who were under direct tutorage in our department had increased compliance rates with attendances etc. Those who were recruited from fresher's fayre, etc. were less so. Perhaps the longevity and type of relationship with the study personnel is a contributing factor to compliance rates in other student studies.

Compliance was assessed by return of unused vials and serum IGF-I and P-III-NP concentrations. In practice subjects often failed to return any vials and/or broken vials. Many returned partially filled vials and it was difficult to assess the amount of GH/placebo remaining in partially filled vials with any degree of accuracy. Thus compliance data are incomplete. It appears from the analysis of IGF-I and P-III-NP that some volunteers stopped the GH prior to day 28 and this may well influence the metabolic variables measured. Assays were performed in batches after completion of the whole study to maintain blinding and use of the same assay batch for all the results but meant that there was a delay in the compliance issue coming to light and it was then too late to adjust the protocol and more stringent implementation of compliance assessment. Means of increasing and monitoring compliance would include supervised drug administration and/or involve the candidate or other individual calling out to take interval samples as opposed to the subject attending the Wellcome Trust Clinical Research Facility. It would, however, be difficult to administer an OGTT or body fat assessment using BODPOD® outside a clinical research facility.

There are little published or reliable data on the real life doping regimens used by athletes, so dosing regimens used were low for safety reasons and if small doses could be detected safely and accurately than larger doses would also be detectable. Saugy et al reported dosing regimens of 15 to 189 g/kg (Saugy,

2006). Anecdotal reports also suggest polypharmacy in doping regimens (Fainura-Wada, 2006). That was not addressed in this study.

Samples were analysed in batches but each individual sample set was not necessarily analysed on the same assay run in order to maintain blinding. This is current antidoping protocol. Therefore there is potential inter-assay variability. The GH2000 IGF-I samples were assayed using the Nichols IGF-I RIA assay which was commercially unavailable when the GH2004 assays were performed and hence the DSL IRMA assay was used. Therefore adjustments had to be made to convert the DSL results to their appropriate Nichols result. Of all commercially available assays the DSL offers most concordance with the Nichols IGF-I assay but is still 28.6% higher which amounted to a mean difference in this study of 30.8µg/L in this study. This assay difference reduces precision of results. WADA has requested a further assessment of commercial assay validation.

Ethnicity is a difficult concept to quantify and in real life many athletes have complex mixed race parentage. A defence lawyer could potentially argue insufficient validation of test methods in an athlete of a rare mixed parentage.

The study was performed on amateur athletes but will need to be applied to professional elite athletes who are potentially of different physique. It is morally and ethically unsound to administer a potentially performance enhancing agent to competing athletes even for research purposes. As part of this wider project the author performed a cross sectional analysis of serum IGF-I and P-III-P in elite athletes of different ethnicities within 2 hours of competing at national or international events The influence of ethnicity on insulin like growth factor-I and procollagen III peptide in elite athletes and its effect on the ability to detect GH

abuse (Erotokritou-Mulligan, 2009). As previous studies involved predominantly white European elite athletes, it was necessary to validate the method in other ethnic groups.

In men, IGF-I was 21.7 ± 2.6% lower in Afro-Caribbeans than white Europeans ($p<0.0001$) but there were no differences between other ethnic groups. In women, IGF-I was 14.2 ± 5.1 % lower in Afro-Caribbeans ($p=0.005$) and 15.6 ± 7.0% higher in Orientals ($p=0.02$) compared with white Europeans. P-III-P was 15.2 ± 3.5%, 26.6 ± 6.6 % and 19.3 ± 5.8% lower in Afro-Caribbean ($p<0.0001$), Indo-Asian ($p<0.0001$) and Oriental men ($p=0.001$) respectively compared with white European men. In women, P-III-P was 15.7 ± 4.7% lower in Afro-Caribbeans compared to white Europeans ($p=0.0009$) but there were no differences between other ethnicities. Despite these differences, most observations were below the upper 99% prediction limits derived from white European athletes. All GH-2000 scores lay below the cut-off limit proposed for doping. In conclusion the GH-2000 detection method based on IGF-I and P-III-P would be valid in all ethnic groups.

Another potential legal impediment to the successful implementation of the marker method as a test for GH abuse among athletes were addressed as part of the overall project undertaken by the author. The data was not included as part of this thesis. Skeletal or soft tissue injury may alter IGF-I and P-III-NP concentrations. Elevations in either of these proteins after injury could lead to a false accusation of doping with GH. A longitudinal observational study following sporting injury was performed at Southampton General Hospital and the British Olympic Medical Centre (Erotokritou-Mulligan, 2008). 127 male (age 29.6 ± 0.9 yrs, range 17 – 68 yrs) and 30 female (age 32.0 ± 2.1 yrs, range 19 – 63) amateur sportsmen and women were recruited from the Accident & Emergency outpatient clinic or orthopaedic fracture clinic at the Southampton University Hospitals Trust, UK. 16 male (age 23.8 ± 1.4 yrs) and 10 female (24.7 ± 1.6 yrs)

professional athletes were also recruited from the British Olympic Medical Centre, Northwick Park Hospital, UK. All subjects were recruited within 10 days of a bony or soft tissue musculoskeletal injury. Blood samples were taken for IGF-I and P-III-NP at regular intervals for 84 days post injury.

There was no change in IGF-I concentration following an injury. By contrast, P-III-P concentrations rose by 41.1 ± 16.6% reaching a peak around 14 days following an injury. The rise in P-III-P varied according to injury type and severity. This rise had a trivial effect on the GH-2000 discriminant function score and no subject reached the threshold needed for a doping offence. It was thus concluded that although there was a rise in P-III-P following injury, this was insufficient to invalidate the GH-2000 detection method based on IGF-I and P-III-P concentrations.

Further research is necessary using the real life doping regimens in terms of doses and mix of agents used for performance enhancement purposes. It would best involve a longer duration of dosing and higher dosing regimens. More frequent sample interval testing, strict adherence to protocol with some ongoing measures of compliance to identify subjects who are not adhering to the protocol.

More precise measures of assessing protein metabolism would be more beneficial with studies of amino acid constituents. Direct testing of sporting performance itself such a tests of muscle tensile strength, fatigability, or running times etc. would also be more definitive to determine actual translation of metabolic changes into sporting enhancement. Such measurements should have been included in this study at the outset. Use of one assay maintained in house by WADA would allow them to retain control, and avoid commercial complications as occurred with Nichols. Larger numbers of volunteers with mixed

ethnicities and mixed nationalities as well as more elite athletes would ensure that legal challenges to any positive test were minimized.

Performance enhancement is becoming increasing sophisticated as the financial and professional gains associated with sport ever increase. Athletes have extensive financial reserves and new drugs or doping methods are not subject to the rigors of scientific research prior to utilisation and can thus be on the market rapidly. Widespread usage can occur before authorities are aware and/or antidoping measures have been developed and verified. Testing is only recently being used and already one athlete has had a positive result. There is a school of thought, albeit somewhat cynical, that the athlete who wins no longer has the best physiology but the best pharmacologist.

Appendix

Papers published as a result of work within this thesis.

Original paper

The GH-2004 project: the response of IGF-I and type III pro-collagen to the administration of exogenous growth hormone (GH) in non-Caucasian amateur athletes Richard Holt, Ioulietta Erotokritou-Mulligan, Cathy McHugh, E Bassett, Christiaan Bartlett, Adam Fityan, Jenny Bacon, David Cowan, and Peter Sonksen. Eur J Endocrinol (163) 45-54. (Holt, 2010)

Review articles

Challenges in Detecting the Abuse of Growth Hormone in Sport. Cathy M. McHugh, Roderick T. Park, Peter H. Sonksen, and Richard I.G. Holt. Clinical Chemistry 51:9, 1587–1593 (2005). (McHugh, 2005a)

Moving one step closer to catching the GH cheats: The GH-2004 experience. Holt RI, Bassett EE, Erotokritou-Mulligan I, McHugh C, Cowan D, Bartlett C, Sönksen PH; GH-2004 group. Growth Horm IGF Res. 2009 Aug;19(4):346-51. Epub 2009 May 24. (Holt, 2009)

Book Chapter

Growth hormone and the metabolic syndrome. McHugh, C.M., Holt, R.I.G. Editors Byrne, C., Wild, S. in “The Metabolic Syndrome”. Wiley Publications 2005. Chapter 14. pg 353-380. (McHugh, 2005b)

References

ABELLAN, R., VENTURA, R., PICHINI, S., PASCUAL, J. A., PACIFICI, R., DI CARLO, S., BACOSI, A., SEGURA, J. & ZUCCARO, P. (2005) Evaluation of immunoassays for the measurement of insulin-like growth factor-I and procollagen type III peptide, indirect biomarkers of recombinant human growth hormone misuse in sport. *Clin Chem Lab Med,* 43**,** 75-85.

ABS, R., BENGTSSON, B.A., HERNBERG-STAHL, E., MONSON, J.P., TAUBER, J.P., WILTON, P., WUSTER, C. (1999) GH replacement in 1034 growth hormone deficient hypopituitary adults: demographic and clinical characteristics, dosing and safety. *Clin Endocrinol,* 50**,** 703-713.

ALBERTI, K. G. M. M. & ZIMMET, P. Z. (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. *Diabetic Medicine,* 15**,** 539-553.

ALBINI, C. H., QUATTRIN, T., VANDLEN, R. L. & MACGILLIVRAY, M. H. (1988) Quantitation of urinary growth hormone in children with normal and abnormal growth. *Pediatr Res.,* 23**,** 89-92.

ALEXANDER, M. K. (1964) The postmortem estimation of total body fat, muscle and bone. *Clin Sci,* 26**,** 193-202.

ALTMAN, P. L. & DITTMER, D. (1968) Metabolism. IN BETHESDA, M. D. (Ed.) *Federation of American Societies for Experimental Biology.*

AMA, P. F., SIMONEAU, J. A., BOULAY, M. R., SERRESSE, O., THERIAULT, G. & BOUCHARD, C. (1986) Skeletal muscle characteristics in sedentary black and Caucasian males. *J Appl Physiol,* 61**,** 1758-1761.

AMATO, G., CARELLA, C., FAZIO, S., LAMONTAGNA, G., CITTADINI, A., SABATINI, & D., MARCIANOMONE, C., SACCA, L., BELLASTELLA, A. (1993) Body composition, bone metabolism, and heart structure and function in growth hormone(GH)-deficient adults before and after GHreplacement therapy at low doses *J Clin Endocrinol Metab* 77**,** 1671-1676.

AMERICAN ASSOCIATION OF PHYSICAL ANTHROPOLOGY (1996) AAPA Statement on Biological Aspects of Race. *Am J Phy Anthropol,* 101**,** 569-570.

AMERICAN DIABETES ASSOCIATION (1997) American Diabetes Association Consensus Development Conference on Insulin Resistance. *American*

Diabetes Association Consensus Development Conference on Insulin Resistance. Diabetes Care 1998.

ARITA, Y., KIHARA, S., OUCHI, N., TAKAHASHI, M., MAEDA, K., MIYAGAWA, J., HOTTA, K., SHIMOMURA, I., NAKAMURA, T., MIYAOKA, K., KURIYAMA, H., NISHIDA, M., YAMASHITA, S., OKUBO, K., MATSUBARA, K., MURAGUCHI, M., OHMOTO, Y., FUNAHASHI, T., MATSUZAWA, Y. (1999) Paradoxical decrease of an adipose-specific prtein, adiponectin, in obesity. *Biochem Biophy Res Commun,* 257**,** 79-83.

ASTRAND, I. (1960) Aerobic work capacity in men and women with special reference to age. *Acta Physiologica Scandinavica,* 49**,** 1-92.

BAGG, W., AOINA, J., CROSS, P.A., WHALLEY, G.A., GAMBLE, G.D., DOUGHTY, R.N., HOLDAWAY, I.M. (2006) Serum IGF-I levels are similar in Samoan, Maori and European populations despite differences in body composition. *Growth Horm IGF Res,* 16**,** 57-60.

BAK, J. F., . MØLLER, N., SCHMITZ, O. (1991) Effects of growth hormone on fuel utilization and muscle glycogen synthase activity in normal humans. *Am J Physiol,* 260**,** E736–E742.

BAMBERGER, M. & YAEGER, D. (1997) Over the Edge. *Sports Illustrated.*

BANFI, G., CASARI, E., MURONE, M. & BONINI, P. (1990) Discrepancies among commercial kits for assaying growth hormone. *Clin Chem,* 36**,** 402.

BANFI, G., MARINELLI, M., ROI, G. S., COLOMBINI, A., PONTILLO, M., GIACOMETTI, M. & WADE, S. (1994) Growth hormone and insulin-like growth factor I in athletes performing a marathon at 4000 m of altitude. *Growth Regul,* 4**,** 82-86.

BANG, P., BRANDT, J., DEGERBLAD, M., ENBERG, G., KAIJSER, L., THORE´N, M., & HALL, K. (1990) Exercise-induced changes in insulin-like growth factors and their low molecular weight binding protein in healthy subjects and patients with growth hormone deficiency. *Eur J Clin Invest,* 20**,** 285-292.

BARLE, H., ESSEN, P., NYBERG, B., OLIVECRONA, H., TALLY, M., MCNURLAN, M.A., WERNERMAN, J., GARLICK, P.J. (1999) Depression of liver protein synthesis during surgery is prevented by growth hormone. *Am J Physiol Endocrinol Metab,* 276**,** E620-627.

BARLE, H., GAMRIN, L., ESSEN, P., MCNURLAN, M.A., GARLICK, P.J., WERNERMAN, J. (2001) Growth hormone does not affect albumin synthesis in the critically ill. *Intensive Care Med,* 27**,** 836-843.

BARROSO, O., SCHAMASCH, P., RABIN, O. (2009) Detection of GH abuse in sport: past, present and future. *Growth Hormone & IGF Research,* 19**,** 369-374.

BASKIN, D. G., PORTE, D. JR., GUEST, K., DORSA, D.M. (1983) Regional concentrations of insulin in the rat brain. *Endocrinology***,** 898–903.

BASTARD, J. P., RABASA-LHORET, R., MAACHI, M., DUCLUZEAU, P. H., ANDREELI, F., VIDAL, H. & LAVILLE, M. (2003) What kind of simple fasting index should be used to estimate insulin sensitivity in humans? *Diabetes Metab.,* Jun; 29**,** 285-288.

BATTEZZATI, A., BENEDINI, S., FATTORINI, A., LOSA, M., MARTINI, P., BERTOLI, S., LANZI, R., TESTOLIN, G., BIOLO, G., LUZI, L. (2003) Insulin action on protein metabolism in acromegalic patients. *Am J Physiol Endocrinol Metab,* 284**,** E823—E829.

BAXTER, R. C. (1994) Insulin like growth factor binding proteins in the human circulation: a review. *Horm Res,* 42.

BEAUVILLE, M., HARANT, I., CRAMPES, F., RIVIERE, D., TAUBER, M.T., TAUBER, J.P., GARRIGUES, M. (1192) Effect of long-term rhGH administration in GH-deficient adults on fat cell epinephrine response. *Am J Physiol,* 263.

BEMBEN, M. G., MASSEY, B. H., BEMBEN, D. A., BOILEAU, R. A. & MISNER, J. E. (1995) Age related patterns in body composition for men aged 20-79 yr. *Med Sci Sports Exerc,* 27**,** 264-269.

BENGTSSON, B., ABS, R., BENNMARKER, H., MONSON, J. P., FELDT-RASMUSSEN, U., HERNBERG-STAHL, E., WESTBERG, B., WILTON, P. & WUSTER, C. (1999) The Effects of Treatment and the Individual Responsiveness to Growth Hormone (GH) Replacement Therapy in 665 GH-Deficient Adults. *J Clin Endocrinol Metab,* 84**,** 3929-3935.

BENGTSSON, B. A., BRUMMER, R.J., BOSAEUS, I. (1990) Growth hormone and body composition. *Hormone Research,* 33**,** 19-24.

BENGTSSON, B. A., BRUMMER, R.J., EDEN, S., BOSAEUS, I., LINDSTEDT, G. (1989) Body composition in acromegaly: the effect of treatment. *Clinical Endocrinology,* 31**,** 481-490.

BENGTSSON, B. A., EDEN, S., LONN, L., KVIST, H., STOKLAND, A., LINDSTEDT, G., BOSAEUS, I., TOLLI, J., SJOSTROM, L., ISAKSSON, O.G. (1993) Treatment of adults with growth hormone deficiency with recombinant human GH. *J Clin Endocrinol Metab,* 76**,** 309-317.

BERGGREN, A., EHRNBORG, C., ROSE´N, T., ELLEGÅRD, L., BENGTSSON, B.A., CAIDAHL, K. (2005) Short-term administration of supraphysiological recombinant human GH (growth hormone) does not increase maximum endurance exercise capacity in healthy, active young men and women with normal GH-insulin-like growth factor I axes. *J Clin Endocrinol Metab,* 90**,** 3268-3273.

BESHYAH, S. A., FREEMANTLE, C., SHAHI, M., ANYAOKU, V., MERSON, S., LYNCH, S., SKINNER, E., SHARP, P., FOALE, R., JOHNSTON, D.G. (1995) Replacement treatment with biosynthetic human growth hormone in growth hormone-deficient hypopituitary adults. *Clin Endocrinol(Oxf),* 42**,** 73-84.

BILLAT, V. L., SIRVENT, P., PY, G., KORALSZTEIN, J.P., MERCIER, J. (2003) The concept of maximal lactate steady state: a bridge between biochemistry, physiology and sport science. *Sports Med,* 33**,** 407-426.

BINNERTS, A., SWART, G. R., WILSON, J. H. P., HOOGERBRUGGE, N., POLS, H. A., BIRKENHAGER, J. C. & LAMBERTS, S. W. (1992) The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. *Clin Endocrinol (Oxf),* 37**,** 79-87.

BLACKMAN, M. R., SORKIN, J.D., MUNZER, T., BELLANTONI, M.F., BUSBY-WHITEHEAD. J,, STEVENS, T.E., JAYME, J., O'CONNOR, K.G., CHRISTMAS, C., TOBIN, J.D., STEWART, K.J., COTTRELL, E., ST CLAIR, C., PABST, K.M., HARMAN, S.M. (2002) Growth hormone and sex steroid administration in healthy aged women and men: a randomized controlled trial. *JAMA,* 288**,** 2282-2292.

BLETHEN, S. L., ALLEN, D. B., GRAVES, D., AUGUST, G., MOSHANG, T. & ROSENFELD, R. (1996) Safety of recombinant deoxyribonucleic acid-derived growth hormone: The National Cooperative Growth Study experience. *J Clin Endocrinol Metab,* 81**,** 1704-1710.

BLUM, W. F. & BREIER, B. H. (1994) Radioimmunoassays for IGFs and IGFBPs. *Growth Regul,* 4**,** 11-19.

BLUNDELL, T. L., BEDARKAR, S., RINERKNECHT, E. & HUMBEL, R. E. (1978) Insulin-like growth factor: a model for tertiary structure accounting for immunoreactivity and receptor binding. *Proc Natl Acad Sci U S A,* 75**,** 180-184.

BODYSTAT INC. (2000) *User's Guide for Bodystat 1500®. Body composition analysis*.

BÖGER, R. H., SKAMIRA, C., BODE-BÖGER, S.M., BRABANT, G., VON ZUR MUHLEN, A., FROLICH, J.C. (1996) Nitric oxide may mediate the

hemodynamic effects of recombinant growth hormone in patients with acquired growth hormone deficiency. A double-blind, placebo-controlled study. *J Clin Invest,* 98**,** 2706-2713.

BOILEAU, R. A., MAYHEW, J. L., RINER, W. F. & LUSSIER, L. (1982) Physiological characteristics of elite middle and long distance runners. *Can J Appl Sport Sci,* 7**,** 167-171.

BOUCHARD, C. (1985) Reproducibility of Body Composition and Adipose Tissue Measurement in Humans. IN ROCHE, A. (Ed.) *Body Compositon Assessment in Youth and Adults.* Columbus, Ohio, Ross laboraties.

BOULWARE, S. D., TAMBORLANE, W. V., MATTHEWS, L. S. & SHERWIN, R. S. (1992) Diverse effects of insulin-like growth factor I on glucose, lipid, and amino acid metabolism. *Am J Physiol Endocrinol Metab,* 262**,** E130-133.

BOWTELL, J. L., LEESE, G.P., SMITH, K., WATT, P.W., NEVILL, A., ROOYACKERS, O., WAGENMAKERS, A.J., RENNIE, M.J. (1998) Modulation of whole body protein metabolism, during and after exercise, by variation of dietary protein. *J Appl Physiol,* 85**,** 1744-1752.

BOWTELL, J. L., LEESE, G.P., SMITH, K., WATT, P.W., NEVILL, A., ROOYACKERS, O., WAGENMAKERS, A.J., RENNIE, M.J. (2000) Effect of oral glucose on leucine turnover in human subjects at rest and during exercise at two levels of dietary protein. *J Physiol,* 525**,** 271-281.

BRAMNERT, M., SEGERLANTZ, M., LAURILA, E., DAUGAARD, J.R., MANHEM, P., GROOP, L. (2003) Growth hormone replacement therapy induces insulin resistance by activating the glucose-fatty acid cycle. *J Clin Endocrinol Metab,* 88**,** 1455-1463.

BRANDOU, F., ALOULOU, I., RAZIMBAUD, A., FACDOU, C., MERCIER, J., BRUN, J.F. (2006) Lower ability to oxidize lipids in adult patients with growth hormone (GH) deficiency: reversal under GH treatment. *Clin Endocrinol (Oxf)*

BRILL, K. T., WELTMAN, A.L., GENTILI, A., PATRIE, J.T., FRYBURG, D.A., HANKS, J.B., URBAN, R.J., VELDHUIS, J.D. (2002) Single and combined effects of growth hormone and testosterone administration on measures of body composition, physical performance, mood, sexual function, bone turnover, and muscle gene expression in healthy older men. *J Clin Endocrinol Metab,* 87**,** 5649–5657.

BROOKS, G., FAHEY, T., BALDWIN, K. (2006) Exercise Physiology: Human Bioenergetics and Its Applications. 4th ed. New York, McGraw-Hill.

BRUNDIN, T. & CERNIGLIARO, C. (1975) The effect of physical training on sympathoadrenal response to exercise. *Scandinavian Journal of Clinical and Laboratory Investigation,* 35**,** 525-530.

BUIJS, M. M., DE LEEUW, P.W., HOUBEN, A.J., KROON, A.A., FRÖLICH, M., PIJL, H., MEINDERS, AE. (2005) Renal contribution to increased clearance of exogenous growth hormone in obese hypertensive patients. *J Clin Endocrinol Metab,* 90**,** 795-799.

BUNT, J. C., BOILEAU, R. A., BAHR, J. M. & NELSON, R. A. (1986) Sex and training differences in human growth hormone levels during prolonged exercise. *J Appl Physiol,* 61**,** 1796-1801.

BUNT, J. C., LOHMAN, T. G. & BOILEAU, R. A. (1989) Impact of total body water fluctuations on estimations of body fat from body density. *Med Sci Sports Exerc,* 21**,** 96-100.

BURGI, H., MULLER, W. A., HUMBEL, R. E., LABHART, A. & FROESCH, E. R. (1996) Non-suppressible insulin-like activity of human serum. I. Physiochemical properties, extraction and partial purification. *Biochem. Biophys. Acta,* 121**,** 349-359.

BUTLER, P., KRYSHAK, E., RIZZA, R. (1991) Mechanism of growth hormone induced postprandial carbohydrate intolerance in humans. *Am J Physiol* 260**,** E513–E520.

CAGNACCI, A., PAOLETTI, A. M., ARANGINO, S., MELIS, G. B. & VOLPE, A. (1999) Effect of ovarian suppression on glucose metabolism of young lean women with and without ovarian hyperandrogenism. *Human Reproduction,* 14**,** 893-897.

CAHILL, G. F. J., MARLISS, E. B. & AOKI, T. T. (1970) Fat and nitrogen metabolism in fasting man. *Horm Metab Res,* 2**,** 181-185.

CALLAWAY, C. W., CHUMLEA, W. C. & BOUCHARD, C. (1988) Circumferences. IN T.G., L., ROCHE, A. F. & MARTORELL, R. (Eds.) *Anthromometric Standardization Reference Manual.* Champaign, Illinois: Human Kinetics Books.

CAMERON, D. (2000) Olympic Jitters at Power Drug Theft. *Sydney Morning Herald.* Sydney.

CANADIAN ASSOCIATION OF SPORT SCIENCES (1987) Canadian Standardized Test of Fitness. IN GOVERNMENT OF CANADA, F. A. A. S. (Ed.).

CARROLL, P. V., CHRIST, E. R., BENGTSSON, B. Å., CARLSSON, L., CHRISTIANSEN, J. S., CLEMMONS, D., HINTZ, R., HO, K., LARON, K.,

SIZONENKO, P., SÖNKSEN, P. H., TANAKA, T. & THORNER, M. (1998) Growth Hormone Deficiency in Adulthood and the Effects of Growth Hormone Replacement: A Review. *J Clin Endocrin and Metab,* 83**,** 382-395.

CATON, J. R., MOLE, P. A., ADAMS, W. C. & HEUSTIS, D. S. (1988) Body composition analysis by bioelectrical impedance: effect of skin temperature. *Med Sci Sports Exerc,* 20**,** 489-491.

CAVALLI-SFORZA, L. L. & FELDMAN, M. W. The application of molecular genetic approaches to the study of human evolution.

CHALMERS, R. J., BLOOM, S. R., DUNCAN, G., JOHNSON, R.H., SULAIMAN, W.R. (1979) The effect of somatostatin on metabolic and hormonal changes during and after exercise. *Clin Endocrinol,* 10**,** 451-458.

CHANG, S., WU, X., YU, H. & SPITZ, M. R. (2002) Plasma Concentrations of Insulin-like Growth Factors among Healthy Adult Men and Postmenopausal Women: Associations with Body Composition, Lifestyle, and Reproductive Factors. *Cancer Epidemiol Biomarkers Prev,* 11**,** 758-766.

CHANOINE, J. P. (2005) Ghrelin in growth and development. *Horm Res,* 63**,** 129-138.

CHAPMAN, I. M., HARTMAN, M.L., STRAUME, M., JOHNSON, M.L., VELDHUIS, J.D., THORNER, M.O. (1994) Enhanced sensitivity growth hormone (GH) chemiluminescence assay reveals lower postglucose nadir GH concentrations in men than women. *J of Clin Endocrin Metab,* 78**,** 1312-1319.

CHIHARA, K., KODAMA, H., KAJI, H., KITA, T., KASHIO, Y., OKIMURA, Y., ABE, H., FUJITA, T. (1985) Augmentation by propranolol of growth hormone releasing hormone-(1–44)-NH2-induced growth hormone release in normal short and normal children. *J Clin Endocrinol Metab***,** 229-233.

CHRIST, E. R., CUMMINGS, M. H., WESTWOOD, N. B., SAWYER, B. M., PEARSON, T. C., SONKSEN, P. H. & RUSSELL_JONES, D. L. (1997) The importance of growth hormone in the regulation of erythropoiesis, red cell mass, and plasma volume in adults with growth hormone deficiency. *J Clin Endocrin and Metab,* 82**,** 2985-2990.

CHRISTENSEN, S. E., JORGENSEN, O.L., MOLLER, N., ORSKOV, H. (1984) Characterization of growth hormone release in response to external heating. Comparison to exercise induced release. *Acta Endocrinol (Copenh),* 107**,** 295-301.

CHUMLEA, W. C., BAUMGARTNER, R. M. & ROCHE, A. F. (1988) Specific resistivity used to estimate fat-free mass from segmental body measures of bioelectric impedance. *Am J Clin Nutr,* 48**,** 7-15.

CLEMMONS, D. R., THISSON, J. P., MAES, M., KETELSLEGERS, J. M. & UNDERWOOD, B. E. (1989) Insulin-like growth factor infusion into hypophysectomized or protein deprived rates induces specific IGF binding proteins in serum. *Endocrinology,* 125**,** 2967-2972.

COLAO, A., CUOCOLO, A., DI SOMMA, C., CERBONE, G., MORTE, A.M., PIVONELLO, R., NICOLAI, E., SALVATORE, M., LOMBARDI, G. (2000) Does the age of onset of growth hormone deficiency affect cardiac performance? A radionuclide angiography study. *Clin Endocrinol (Oxf),* 52**,** 447-455.

COLAO, A., CUOCOLO, A., MARZULLO, P., NICOLAI, E., CERONE, G., MORTE, D., PIVONELLO, R., NICOLAI, E., SALVATORE, M., LOMBARDI, G. (1999) Impact of patient's age and disease duration on cardiac performance in acromegaly: a radionuclide angiography study. *J Clin Endocrinol Metab* 84**,** 1518-1523.

COLAO, A., DI SOMMA, C., CUOCOLO, A., FILIPPELLA, M., ROTA, F., ACAMPA, W., SAVASTANO, S., SALVATORE, M., LOMBARDI, G. (2004) The severity of growth hormone deficiency correlates with the severity of cardiac impairment in 100 adult patients with hypopituitarism: an observational, case-control study. *J Clin Endocrinol Metab* 89**,** 5998-5960.

COLAO, A., FERONE, D., MARZULLO, P., LOMBARDI, G. (2004) Systemic complications of acromegaly: epidemiology, pathogenesis, and management. *Endocr Rev,* 25**,** 102-152.

COLAO, A., MARZULLO, P., DI SOMMA, C. & LOMBARDI, G. (2001) Growth hormone and the heart. *Clin Endocrinol (Oxf),* 54**,** 137-154.

COMBS, T.P. (2001), Endogenous glucose production is inhibited by the adipokine derived protein ACRP 30. *J Clin Invest*, 108, 1875-1881

CONOVER, C. A. (1992a) Potentiation of insulin-like growth factor IIGF) action by IGF-binding protein-3: studies of underlying mechanism. *Endocrinology,* 130.

CONOVER, C. A. (1996) The role of insulin-like growth factors and binding proteins in bone cell biology. IN BILEZIKIAN, J. P., RAISZ, L. G. & RODAN, G. A. (Eds.) *Principles of bone biology.* San Diego, Academic Press.

CONOVER, C. A., LEE, P.D., KANALEY, J.A., CLARKSON, J.T., JENSEN, M.D. (1992b) Insulin regulation of insulinlike growth factor binding protein-1 in obese and nonobese humans. *J Clin Endocrinol Metab* 74**,** 1355-1360.

CONVERTINO, V. A. (1991) Blood volume: its adaptation to endurance training. *Medicine and Science in Sports and Exercise,* 23**,** 1338-1348.

COPELAND, K. C. & NAIR, K. S. (1994) Recombinant human insulin-like growth factor-I increases forearm blood flow. *J Clin Endocrinol Metab,* 79**,** 230-232.

CORDIDO, F., PEINO, R., PENALVA, A., ALVAREZ, C.V., CASANUEVA, F.F., DIEGUEZ, C. (1996) Impaired growth hormone secretion in obese subjects is partially reversed by acipimox-mediated plasma free fatty acid depression. *J Clin Endocrinol and Metab,* 81**,** 914-918.

CORPAS, E., HARMAN, S. M. & BLACKMAN, M. R. (1993) Human growth hormone and human ageing. *Endocr Rev.,* 14**,** 20-39.

COSTILL, D. L., DANIELS, J., EVENA, W., FING, W., KRAHENBURHL, G. & SALTIN, B. (1976) Skeletal muscle enzymes and fiber compositon in male and female track athletes. *J Appl Physiol,* 40**,** 149-154.

COWELL, C. T. & DIETSCH, S. (1995) Adverse events during growth hormone therapy. *Pediatr Endocrinol Metab,* 8**,** 243-252.

CRAPO, R. O., MORRIS, A. H., CLAYTON, P. D. & NIXON, C. R. (1982) Lung volumes in healthy non smoking adults. *Bull Eur Physiopathol Respir,* 18**,** 419-425.

CRAWFORD, B., MARTIN, J., HOWE, C., HANDELSMAN, D. & BAXTER, R. (1992) Comparison of extraction methods for insulin-like growth factor-I in rat serum. *J Endocrinol,* 134**,** 169-176.

CRIST, D. M., PEAKE, G.T., EGAN, P.A., WATERS, D.L. (1988) Body composition response to exogenous GH during training in highly conditioned adults. *J Appl Physiol,* 65**,** 579-584.

CUMMINGS, D. E., MERRIAM, G.R. (2003) Growth hormone therapy in adults. *Annu Rev Med,* 54**,** 513-533.

CUNEO, R. C., JUDD, S., WALLACE, J. D., PERRY-KEENE, D., BURGER, H., LIM-TIO, S., STRAUSS, B., STOCKIGT, J., TOPLISS, D., ALFORD, F., HEW, L., BODE, H., CONWAY, A., HANDELSMAN, D., DUNN, S., BOYAGES, S., WAH CHEUNG, N. & HURLEY, D. (1998) The Australian Multicenter trial of growth hormone (GH) treatment in GH-deficient adults. *J Clin Endocrin Metab,* 83**,** 107-116.

CUNEO, R. C., SALOMON, F., WILES, C.M., HESP, R., SONKSEN, P.H. (1991) Growth hormone treatment in growth hormone-deficient adults. II. Effects on exercise performance. *J Appl Physiol,* 70**,** 695-700.

DAGENAIS, G. R., TANCREDI, R.G., ZIERLER, K.L. (1976) Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the human forearm. *J Clin Invest* 58**,** 421-431.

DALL, R., LONGOBARDI, S., EHRNBORG, C., KEAY, N., ROSEN, T., JORGENSEN, J. O. L., CUNEO, R. C., BOROUJERDI, M. A., CITTADINI, A., NAPOLI, R., CHRISTIANSEN, J. S., BENGTSSON, B. A., SACCA, L., BAXTER, R. C., BASSET, E. E. & SONKSEN, P. H. (2000) The Effect of Four Weeks of Supraphysiological Growth Hormone Administration on the Insulin-Like Growth Factor Axis in Women and Men. *J Clin Endocrinol Metab,* 85**,** 4193-4200.

DANIELS, F. & ALBERTY, R. A. (1967) *Physical Chemistry,* New York, John Wiley and Sons.

DAVIDSON, M. B. (1987) Effect of growth hormone on carbohydrate and lipid metabolism. *Endocr Rev,* 8**,** 115-131.

DAVIES, J. S., KOTOKORPI, P., ECCLES, S.R., BARNES, S.K., TOKARCZUK, P.F., ALLEN, S.K., WHITWORTH, H.S., GUSCHINA, I.A., EVANS, B.A., MODE, A., ZIGMAN, J.M., WELLS, T. (2009) Ghrelin induces abdominal obesity via GHS-R-dependent lipid retention. *Mol Endocrinol,* 23**,** 914–924.

DE BOER, H., BLOK, G., VAN DER VEEN, E.A. (1995) Clinical aspects of growth hormone deficiency in adults. *Endocr Rev* 16**,** 63-86.

DEGERBLAD, M., ALMKVIST, O., GRUNDITZ, R., HALL, K., KAIJSER, L., & KNUTSSON, E., RINGERTZ, H., THORE´NM (2007) Physical and psychological capabilities during substitution therapy with recombinant growth hormone in adults with growth hormone deficiency. *Acta Endocrinol (Copenh)* 123**,** 185-193.

DEGERBLAD, M., ALMQVIST, O., GRUNDITZ, R., HALL, K., KAIJSER, L., KNUTSSON, E., RINGERTZ, H. & THOREN, M. (1990) Physical and psychological capabilities during substitution therapy with recombinant growth hormone in adults with growth hormone deficiency. *Acta Endocrinol (Copenhagen),* 123**,** 185-193.

DELELLIS, K., INGLES, S., KOLONEL, L., MCKEAN-COWDIN, R., HENDERSON, B., STANCZYK, F., PROBST-HENSCH. N.M. (2003) IGF1 genotype, mean plasma level and breast cancer risk in the Hawaii/Los Angeles multiethnic cohort. *Br J Cancer* 88**,** 277–282.

DEMPSEY, R. L., MAZZONE, M. F. & MEURER, L. N. (2002) Does oral creatine supplementation improve strengths? A meta-analysis. *J Family Pract,* 51**,** 945-951.

DEMPSTER, P. & AITKENS, S. (1995) A new air displacemnt method for the determination of human body composition. *Med Sci Sports Exerc,* 27**,** 1962-1967.

DEURENBERG, P., WESTSTRATE, J. A., PAYMANS, I. & VAN DER KOOY, K. (1988) Factors affecting bioelectrical impedance measurements in humans. *Eur J Clin Nutr,* 42**,** 1017-1022.

DEWIT, O., FULLER, N. J., FEWTRELL, M. S., ELIA, M. & WELLS, J. C. K. (2000) Whole body air displacement plethysmography compared with hydrodensitometry for body composition analysis. *Arch Dis Child,* 82**,** 159-164.

DEYSSIG, R., FRISCH, H., BLUM, W.F., WALDHO¨R, T. (1993) Effect of growth hormone treatment on hormonal parameters, body composition and strength in athletes. *Acta Endocrinol (Copenh),* 128**,** 313-318.

DJURHUUS, C. B., GRAVHOLT, C.H., NIELSEN, S., PEDERSEN, S.B., MOLLER, N., SCHMITZ, O. (2004) Additive effects of cortisol and growth hormone on regional and systemic lipolysis in humans. *Am J Physiol Endocrinol Metab* 286**,** E488-E494.

DOHERTY, R. O., STEIN, D. & FOLEY, J. (1997) Insulin resistance. *Diabetologia,* 40**,** B10-B15.

DRAKE, W. M., COYTE, D., CAMACHO-HUBNER, C., JIVANJI, N. M., KALTSAS, G., WOOD, D. F., TRAINER, P. J., GROSSMAN, A. B., BESSER, G. M. & MONSON, J. P. (1998) Optimizing Growth Hormone Replacement Therapy by Dose Titration in Hypopituitary Adults. *J Clin Endocrinol Metab,* 83**,** 3913-3919.

DRESNER, A., LAURENT, D., MARCUCCI, M., GRIFFIN, M. E., DUFOUR, S., CLINE, G. W., SLEZAK, L. A., ANDERSEN, D. K., HUNDAL, R. S., ROTHMAN, D. L., PETERSEN, K. F. & SHULMAN, G. I. (1999) Effects of free fatty acids on glucose transport and IRS-1–associated phosphatidylinositol 3-kinase activity. *J. Clin. Invest.,* 103**,** 253-259.

DUBOIS, D. & DUBOIS, E. F. (1916) A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med,* 17**,** 863-871.

DUCHAINE, D. (1983) *Underground steroid handbook,* Venice, California, HLR Technical Books.

EDWARDS, D. A. W. (1950) *Clinical Science*.

EHRNBORG, C., ELLEGÅRD, L., BOSAEUS, I., BENGTSSON, B.A., ROSE´N, T. (2005) Supraphysiological growth hormone: less fat, more extracellular fluid but uncertain effects on muscles in healthy, active young adults. *Clin Endocrinol (Oxf),* 62**,** 449-457.

ELIAKIM, A., BRASEL, J.A., MOHAN, S., BARSTOW, T.J., BERMAN, N., COOPER, D.M. (1996) Physical fitness, endurance training, and the growth hormone-insulin-like growth factor I system in adolescent females. *J Clin Endocrinol Metab,* 81**,** 3986-3992.

ELIAKIM, A., SCHEETT, T. P., NEWCOMB, R., MOHAN, S. & COOPER, D. M. (2001) Fitness, Training, and the Growth Hormone{->}Insulin-Like Growth Factor I Axis in Prepubertal Girls. *J Clin Endocrinol Metab,* 86**,** 2797-2802.

ELIAS, A. N., WILSON, A.F., NAQVI, S., PANDIAN, M.R. (1997) Effects of blood pH and blood lactate on growth hormone, prolactin, and gonadotropin release after acute exercise in male volunteers. *Proc Soc Exp Biol Med,* 214**,** 156-160.

ELSEN, R., SIU, M. L., PINEDA, O. & SOLOMONS, N. W. (1987) Sources of variability in bioelectrical impedance determinations in adults. IN ELLIS, K. J., YASAMURA, S. & MORGAN, W. D. (Eds.) *In vivo body composition studies.* London, The Institute of Physical Sciences.

EROTOKRITOU-MULLIGAN, I., BASSETT, E.E., BARTLETT, C., COWAN, D., MCHUGH, C., SEAH, R., CURTIS, B., WELLS, V., HARRISON, K., SÖNKSEN, P.H., HOLT, R.I. (2008) The effect of sports injury on insulin-like growth factor-I and type 3 procollagen: implications for detection of growth hormone abuse in athletes. *J Clin Endocrinol Metab,* 93**,** 2760-2763.

EROTOKRITOU-MULLIGAN, I., BASSETT, E.E., COWAN, D,A., BARTLETT, C., MCHUGH, C., SÖNKSEN, P.H., HOLT, R.I. (2009) Influence of ethnicity on IGF-I and procollagen III peptide (P-III-P) in elite athletes and its effect on the ability to detect GH abuse. *Clin Endocrinol (Oxf),* 70**,** 161-168.

FAINARU-WADA, M. & WILLIAMS, L. (2004) Sprinter admitted use of BALCO 'magic potion' November testimony to federal grand jury contradicts record-holder's public denials. *San Francisco Chronicle.* San Francisco.

FAINURA-WADA, M., WILLIAMS, L. (2006) Game of Shadows. New York, Gotham.

FALKNER, B., KUSHNER, H., TULENKO, T., SUMNER, A. E. & MARSH, J. B. (1995) Insulin sensitivity, lipids and blood pressure in young. *Arterioscler Throm Vasc Biol,* 15.

FALLS, H. B. & HUMPHREY, L. D. (1976) Energy cost of running an walking in young women. *Med Sci Sports,* 8**,** 9-13.

FARIA, A. C., BEKENSTEIN, L.W., BOOTH, R.A. JR, VACCARO, V.A., ASPLIN, C.M., VELDHUIS, J.D., THORNER, M.O., EVANS, W.S. (1992) Pulsatile growth hormone release in normal women during the menstrual cycle. *Clin Endocrin,* 36**,** 591-596.

FAZIO, S., CITTADINI, A., BIONDI, B., PALMIERI, E. A., RICCIO, G., BONE, F., OLIVIERO, U. & SACCA, L. (2000) Cardiovascular Effects of Short-Term Growth Hormone Hypersecretion. *J Clin Endocrinol Metab,* 85**,** 179-182.

FAZIO, S., CITTADINI, A., CUOCOLO, A., MEROLA, B., SABATINI, D., COLAO, A., BIONDI, B., LOMBARDI, G., SACCA, L. (1994) Impaired cardiac performance is a distinct feature of uncomplicated acromegaly. *J Clin Endocrinol Metab* 79**,** 441-446.

FEDERICA, M., LAURO, D., D'ADAMO, M., GIOVANNONE, B., PORZIO, O., MELLOZZI, M., TAMBURRANO, G., SBRACCIA, P., SESTI, G. (1998) Expression of insulin/IGF-I hybrid receptors is increased in skeletal muscle of patients with chronic primary hyperinsulaemia. *Diabetes,* 47**,** 87-92.

FELIG, P., WAHREN, J., SHERWIN, R. & PALAIOLOGOS, G. (1977) Amino acid and protein metabolism in diabetes mellitus. *Arch Intern Med,* 137**,** 507-513.

FELSING, N. E., BRASEL, J.A., COOPER, D.M. (1992) Effect of low and high intensity exercise on circulating growth hormone in men. *J Clin Endocrinol Metab,* 75**,** 157-162.

FERNANDO, D. P., ARGENTINO, D. P., MUNOZ, M. C., MIQUET, J. G., SOTELO, A. I. & TURYN, D. (2005) Influence of the cross talk between growth hormone and insulin signalling on th modulation of insulin sensitivity. *Growth Hormone & IGF Research,* 15**,** 324-336.

FERRANNINI, E., BUZZIGOLI, G., BONADONNA, R., GIORICO, M. A., OLEGGINI, M., GRAZIADEI, L., PEDRINELLI, R., BRANDI, L. & BEVILACQUA, S. (1987) Insulin resistance in essential hypertension. *New England Journal of Medicine,* 317**,** 350-357.

FESTEN, D. A. M., TOORENENBERGEN, A.V., DUIVENVOORDEN, H.J., HOKKEN-KOELEGA, A.C.S. (2007) Adiponectin Levels in Prepubertal Children with Prader-Willi Syndrome before and during Growth Hormone Therapy *J Clin Endocrinol Metab,* 92**,** 1549-1554.

FIDANZA, F. (1991) Anthropometric Methodology. *Nutritional Status Assessment.* London, Chapman and Hall.

FIDELEFF, H. L., BOQUETE, H.R., STALLDECKER, G., GIACCIO, A.V., SOBRADO, P.G. (2008) Comparative results of a 4-year study on cardiovascular parameters, lipid metabolism, body composition and bone mass between untreated and treated adult growth hormone deficient patients. *Growth Horm IGF Res,* 18**,** 318-324.

FIELDS, D. A., GORAN, M. I. & MCCRORY, M. A. (2002) Body-composition assessment via air-displacement plethysmography in adults and children: a review. *Am J Clin Nutr,* 75**,** 453-467.

FINEBERG, S. E., . MERIMEE, T.J. (1974) Acute metabolic effects of human growth hormone. *Diabetes,* 23**,** 499-504.

FLANCBAUM, L., CHOBAN, P. S., SAMBUCCO, S., VERDUCCI, J. & BURGE, J. C. (1999) Comparison of indirect calorimetry, the Fick method, and prediction equations in estimating the energy requirements of critically ill patients. *Am J Clin Nutr,* 69**,** 461-466.

FLYVBJERG, A., JORGENSEN, K. D., MARSHALL, S. M. & ORSKOV, H. (1991) Inhibitory effect of octreotide on growth hormone-induced IGF-I generation and organ growth in hypophysectomized rats. *Am J Physiol,* 260**,** E568-E574.

FORSTNER, K. (1999) Errors in blood pressure measuring with modern automated machines. Effects of errors in non-invasive oscillometric blood pressure measuring. *Fortschr Med,* 117**,** 22-25.

FOSS, M. C., SAAD, M.J., PACCOLA, G.M., PAULA, F.J., PICCINATO, C.E., MOREIRA, A.C. (1991) Peripheral glucose metabolism in acromegaly. *J Clin Endocrinol Metab,* 72**,** 1048-1053.

FOWELIN, J., ATTVALL, S., VON SCHENCK, H., SMITH, U. & LAGER, I. (1991) Characterization of the insulin-antagonistic effect of growth hormone in man. *Diabetologia,* 34**,** 500-506.

FRANSSILA_KALLUNKI, A., RISSANEN, A., EKSTRAND, A., OLLUS, A. & GROOP, L. (1992) Effects of weight loss on substrate oxidation, energy expenditure, and insulin sensitivity in obese individuals. *Am J Clin Nutr,* Feb 55**,** 356-361.

FRAYNE, K. N. (1983) Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 55**,** 638-634.

FRAYNE, K. N. (1996) *Metabolic Regulation, A Human Perspective,* London, Blackwell Science.

FREDA, P. U., SHEN, W., HEYMSFIELD, S.B., REYES-VIDAL, C.M., GEER, E.B., BRUCE, J.N., GALLAGHER, D. (2008) Lower visceral and

subcutaneous but higher intermuscular adipose tissue depots in patients with growth hormone and insulin-like growth factor I excess due to acromegaly. *J Clin Endocrinol Metab,* 93**,** 2334-2343.

FROESCH, E. R., BURGI, H., RAMSEIER, E. B., BALLY, P. & LABHART, A. (1963) Antibody-Suppressible and non suppressible insulin-like activities in human serum and their physiologic significance. An insulin assay with adipoe tissue of increased precision and specificity. *J Clin Invest,* 42**,** 1816-1834.

FRUEBIS, J., TSAO, T.S. , JAVORSCHI, S., EBBETS-REED, D., ERICKSON, M.R.S., YEN, F.T., BIHAIN, B.E., LODISH, H.F. (2001) Proteolytic cleavage product of 30 kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc natl Acad Sci USA,* 98**,** 2005-2010.

FRYBURG, D. A., BARRETT, E.J. (1993) Growth hormone acutely stimulates skeletal muscle but not whole-body protein synthesis in humans. *Metabolism,* 42**,** 1223-1227.

FRYBURG, D. A., GELFAND, R. A. & BARRETT, E. J. (1991) Growth hormone acutely stimulates forearm muscle protein synthesis in normal humans. *Amer J Physiol,* 260**,** E499-E504.

FRYSTYK, J. (2004) Free insulin-like growth factors – measurements and relationships to growth hormone secretion and glucose homeostasis. *Growth Horm IGF Res* 14**,** 337–375.

FRYSTYK, J., SKJAERBAEK, C., VESTBO, E., FISKER, S., ORSKOV, H. (1999) Circulating levels of free insulinlike growth factors in obese subjects: the impact of type 2 diabetes. *Diabetes Metab Res,* 15**,** 314-322.

FUKAGAWA, N. K., MINAKER, K. L., ROWE, J. W., GOODMAN, M. N., MATTHEWS, D. E., BIER, D. M. & YOUNG, V. R. (1985) Insulin-mediated reduction of whole body protein breakdown. Dose-response effects on leucine metabolism in postabsorptive men. *J Clin Invest,* 76**,** 2306-2311.

FUKUDA, I., HIZUKA, N., ISHIKAWA, Y., ITOH, E., YASUMOTO, K., MURAKAMI, Y., SATA, A., TSUKADA, J., KURIMOTO, M., OKUBO, Y., TAKANO, K. (2002) Serum adiponectin levels in adult growth hormone deficiency and acromegaly. *Growth Horm IGF Res,* 14**,** 449-454.

FULLER, N. J. & ELIA, M. (1989) Potential use of bioelectrical impedance of "whole body" and of body segments for the assessment of body composition: A comparison with densitometry and anthropometry. *Eur J Clin Nutr,* 432**,** 779-791.

GAPSTUR, S. M., KOPP, P., CHIU, B. C., GANN, P. H., COLANGELO, L. A., LIU, K. (2004) Longitudinal Associations of Age, Anthropometric and Lifestyle Factors with Serum Total Insulin-Like Growth Factor-I and IGF Binding Protein-3 Levels in Black and White Men: the CARDIA Male Hormone Study. *Cancer Epidemiol Biomarkers Prev,* 13**,** 2208-2216.

GARIBOTTO, G., BARRECA, A., RUSSO, R., SOFIA, A., ARAGHI, P., CESARONE, A., MALASPINA, M., FIORINI, F., MINUTO, F. & TIZIANELLO, A. (1997) Effects of Recombinant Human Growth Hormone on Muscle Protein Turnover in Malnourished Hemodialysis Patients. *J. Clin. Invest,* 99**,** 97-105.

GIANNOULIS, M. G., SONKSEN, P.H., UMPLEBY, M., BREEN, L., PENTECOST, C., WHYTE, M., MCMILLAN, C.V., BRADLEY, C., MARTIN, F.C. (2006) The effects of growth hormone and/or testosterone in healthy elderly men: a randomized controlled trial. *J Clin Endocrinol Metab,* 91**,** 477-484.

GIAVOLI, C., CAPIELLO, V., CORBETTA, S., RONCHI, C.L., MORPURGO, C., FERRANTE, E., BECK-PECCOZ, P., SPADA, A. (2004) Different effects of short and long-term recombinant hGH administration on ghrelin and adiponectin levels in GH-deficient adults. *Clin Endocrin,* 61**,** 81-87.

GIBNEY, J., HEALY, M. L., STOLINSKI, M., BOWES, S. B., PENTECOST, C., BREEN, L., MCMILLAN, C., RUSSELL-JONES, D. L., SONKSEN, P. H. & UMPLEBY, A. M. (2003) Effect of Growth Hormone (GH) on Glycerol and Free Fatty Acid Metabolism during Exhaustive Exercise in GH-Deficient Adults. *J Clin Endocrinol Metab,* 88**,** 1792-1797.

GIBNEY, J., HEALY, M.L., SÖNKSEN, P.H. (2007) The Growth Hormone/Insulin-Like Growth Factor-I Axis in Exercise and Sport. *Endocr. Rev,* 28**,** 603-624.

GIUSTINA, A., VELDHUIS, J.D. (1998) Pathophysiology of the neororegulation of growth hormone secretion in experimental animals and the human. *Endocr Rev,* 19**,** 717-797.

GLEDHILL, N., COX, D. & JAMNIK, R. (1994) Endurance athletes; stroke volume does not plateau: major advantage is diastolic function. *Medicine and Science in Sports and Exercise,* 26**,** 1116-1121.

GLEICHAUF, C. N. & ROSE, D. A. (1989) The menstrual cycle's effect on the reliability of bioimpedance measurements for assessing body composition. *Am J Clin Nutr,* 50**,** 903-907.

GOMEZ, J. M., GOMEZ, N., FITER, J. & SOLER, J. (2000) Effects of long-term treatment with GH in the bone mineral density of adults with

hypopituitarism and GH deficiency and after discontinuation of GH replacement. *Horm Metab Res,* 32**,** 66-70.

GOODMAN-GRUEN, D. & BARRETT-CONNOR, E. (1997) Epidemiology of insulin-like growth factor-I in elderly men and women. The Rancho Bernardo Study [published erratum appears in Am J Epidemiol 1997 Aug 15;146(4):357]. *Am. J. Epidemiol.,* 145**,** 970-976.

GOODMAN, H. M., GRICHTING, G. (1983) Growth hormone and lipolysis: a reevaluation. *Endocrinology,* 113**,** 1697-1702.

GRAHAM, M. R., BAKER, J.S., EVANS, P., KICMAN, A., COWAN, D., HULLIN, D., DAVIES, B. (2007a) Evidence for a decrease in cardiovascular risk factors following recombinant growth hormone administration in abstinent anabolic-androgenic steroid users. *Growth Horm IGF Res,* 17**,** 201-209.

GRAHAM, M. R., BAKER, J.S., EVANS, P., KICMAN, A., COWAN, D., HULLIN, D., DAVIES, B. (2007b) Short-term recombinant human growth hormone administration improves respiratory function in abstinent anabolic-androgenic steroid users. *Growth Horm IGF Res,* 17**,** 328-335.

GRAHAM, M. R., DAVIES, B., KICMAN, A., COWAN, D., HULLIN, D., BAKER, J.S. (2007c) Recombinant human growth hormone in abstinent androgenic-anabolic steroid use: psychological, endocrine and trophic factor effects. *Curr Neurovasc Res,* 4**,** 9-18.

GRAVES, J. E., POLLOCK, M. L., COLVIN, A. B., VAN LOAN, M. & LOHMAN, T. G. (1989) Comparison of different bioelectrical impedance analyzers in the prediction of body composition. *Amer J Hum Biol***,** 603-611.

GRAY, D. S., BRAY, G. A., GEMAYEL, N. & KAPLAN, K. (1989) Effect of obesity on bioelectrical impedance. *Am J Clin Nutr,* 50**,** 225-260.

GUHA, N., EROTOKRITOU-MULLIGAN, I., BURFORD, C., STROBRIDGE, G., BRIGG, J., DRAKE, T., BASSETT, E.E., COWAN, D., BARTLETT, C., SÖNKSEN, P.H., HOLT, R.I. (2010) Serum insulin-like growth factor-I and pro-collagen type III N-terminal peptide in adolescent elite athletes: implications for the detection of growth hormone abuse in sport. *J Clin Endocrinol Metab,* 95**,** 2969-2976.

HAGBERG, J. M., DOUGLAS, R., SEALS, D.R., YERG, J.E., GAVIN, J., GINGERICH, R., PREMACHANDRA, & B., H., J.O. (1988) Metabolic responses to exercise in young and older athletes and sedentary men. *J Appl Physiol* 65**,** 900-908.

HALLORAN, B. P. & SPENCER, E. M. (1988) Dietary phosphorus and 1,25-dihydroxyvitamin D metabolism: influence of insulin-like growth factor I. *Endocrinology,* 123**,** 1225-1229.

HANSEN, I., TSALIKIAN, E., BEAUFRERE, B., GERICH, J., HAYMOND, M., RIZZA, R. (1986) Insulin resistance in acromegaly: defects in both hepatic and extrahepatic insulin action. *Am J Physiol Endocrinol Metab,* 250**,** E269–E273.

HANSEN, M., MORTHORST, R., LARSSON, B., DALL, R., FLYVBJERG, A., RASMUSSEN, M.H, ORSKOV, H., KJAER, M., LANGE, K.H.W. (2005) No effect of growth hormone administration on substrate oxidation during exercise in young, lean men. *J Physiol,* 567**,** 1035-45.

HARANT, I., BEAUVILLE, M., CRAMPES, F., RIVIERE, D., TAUBER, M.T., TAUBER, J.P., GARRIGUES, M. (1994) Response of fat cells to growth hormone (GH): effect of long term treatment with recombinant human GH in GH-deficient adults. *J Clin Endocrinol Metab* 78**,** 1392-1395.

HARMAN, M. L. (2001) Review of HypoCSS data. *Proceedings of the AM Endo Soc Conference.*

HARTGENS, F. & KUIPERS, H. (2004) Effects of androgenic-anabolic steroids in athletes. *Sports Med,* 34**,** 513-554.

HARTMAN, M. L., FARIA, A.C., VANCE, M.L., JOHNSON, M.L., THORNER, M.O.,VELDHUIS, J.D. (1991) Temporal structure of in vivo growth hormone secretory events in humans. *Am J Physiol,* 260**,** E101–E110.

HEALY, M. L., GIBNEY, J., RUSSELL-JONES, D. L., PENTECOST, C., CROOS, P., SONKSEN, P. H. & UMPLEBY, A. M. (2003) High Dose Growth Hormone Exerts an Anabolic Effect at Rest and during Exercise in Endurance-Trained Athletes. *J Clin Endocrinol Metab,* 88**,** 5221-5226.

HEALY, M. L., GIBNEY, J., PENTECOST, C., CROOS, P., RUSSELL-JONES, D.L., SO¨NKSEN, P.H., UMPLEBY, A.M. (2006) Effects of high-dose growth hormone on glucose and glycerol metabolism at rest and during exercise in endurance-trained athletes. *J Clin Endocrinol Metab,* 91**,** 320-327.

HEALY, M. L., GIBNEY, J., RUSSELL-JONES, D.L., PENTECOST, C., CROOS, P.H., SONKSEN, P.H., UMPLEBY, A.M. (2003) High Dose Growth Hormone Exerts an Anabolic Effect at Rest and during Exercise in Endurance-Trained Athletes *J Clin Endocrinol Metab* 88**,** 5221-5226.

HEALY, M. L. D., R., GIBNEY, J., BASSETT, E., EHRNBORG, C., PENTECOST, C., ROSEN, T., CITTADINI, A., BAXTER, R.C., SO¨NKSEN, P.H. (2005) Toward the development of a test for growth hormone (GH) abuse: a study of extreme physiological ranges of GH-dependent markers in 813 elite athletes in the postcompetition setting. *J Clin Endocrinol Metab* 90**,** 641-649.

HEILING, V., CAMPBELL, P., GOTTESMAN, I., TSALIKIAN, E., BEAUFRERE, B., GERICH, J. & HAYMOND, M. (1993) Differential effects of hyperglycemia and hyperinsulinemia on leucine rate of appearance in normal humans. *J Clin Endocrinol Metab,* 76**,** 203-206.

HERMANSEN, L. (1974) *Individual differences,* New York, Macmillan.

HEYWARD, V. H. & STOLARCZYK, L. M. (1996) *Applied Body Composition Assessment,* Champaign, Illinois, Human Kinetics.

HIGGINS, P. B., FIELDS, D. A., HUNTER, G. R. & GOWER, B. A. (2001) Effect of Scalp and Facial Hair on Air Displacement Plethysmography Estimates of Percentage of Body Fat. *Obes Res,* 9**,** 326-330.

HINTZ, R. L., CLEMMONS, D. R., UNDERWOOD, L. E. & VAN WYK, J. J. (1972) Competitive binding of somatomedin to the insulin receptor of adipocytes, chondrocytes, and liver membranes. *Proc. Natl. Acad. Sci. U. S. A.,* 69**,** 2351–2353.

HO, K. K., O'SULLIVAN, A. J. & HOFFMAN, D. M. (1996) Metabolic actions of growth hormone in man. *Endocr J.,* 43**,** S57-S63.

HO, K. K., O'SULLIVAN, A.J., HOFFMAN, D.M. (1996) Metabolic actions of growth hormone in man. *Endocr J,* 43 (Suppl).

HOFFMAN, D. M., CRAMPTON, L., SERNIA, C., NGUYEN, T. V. & HO, K. K. (1996) Short-term growth hormone (GH) treatment of GH-deficient adults increases body sodium and extracellular water, but not blood pressure. *J Clin Endocrinol Metab,* 81**,** 1123-1128.

HOLLY, J. M. & WASS, J. A. (1989) Insulin-like growth factors; autocrine, paracrine or endocrine? New perspectives of the somatomedin hypothesis in the light of recent developments. *J Endocrinol,* 122.

HOLLOSZY, J. O. & COYLE, E. F. (1984) Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol,* 56**,** 831-838.

HOLT, R. I., BASSETT, E.E., EROTOKRITOU-MULLIGAN, I., MCHUGH, C., COWAN, D., BARTLETT, C., SÖNKSEN, P.H. (2009) Moving one step closer to catching the GH cheats: The GH-2004 experience. *Growth Horm IGF Res,* 19**,** 346-351.

HOLT, R. I., EROTOKRITOU-MULLIGAN, I., MCHUGH, C., BASSETT, E., BARTLETT, C., COWAN, D., SONKSEN, P (2004a) GH2000 A methodology for the detection of doping with growth hormone and related substance. GH administration in ethnically diverse amateur athletes. Southampton.

HOLT, R. I., EROTOKRITOU-MULLIGAN, I., MCHUGH, C., BASSETT, E., BARTLETT, C., COWAN, D., SONKSEN, P (2004b) GH administration in ethnically diverse amateur athletes. Southampton.

HOLT, R. I., EROTOKRITOU-MULLIGAN, I., MCHUGH, C., BASSETT, E., BARTLETT, FITYAN, A., BACON, J., DAVID COWAN, D., SONKSEN, P. (2010) The GH-2004 project: the response of IGF-I and type III pro-collagen to the administration of exogenous growth hormone (GH) in non-Caucasian amateur athletes. *Eur J Endocrinol,* 163**,** 45-54.

HOLT, R. I. G. (2004c) The Metabolic Effects of Growth Hormone. *CME Bulletin in Endocrinology and Diabetes,* 5**,** 11-17.

HOLT, R. I. G., WEBB, E., PENTECOST, C. & SONKSEN, P. H. (2001) Ageing and Physical Fitness Are More Important Than Obesity in Determining Exercise-Induced Generation of GH. *J Clin Endocrinol Metab,* 86**,** 5715-5720.

HOMAYOUN, P., DAHER, R., VAN LENTE, F., FAIMAN, C. & GUPTA, M. K. (1996) Immunoradiometric assay measurements of insulin-like growth factor-I (IGF-I): comparison with radioimmunoassays using native or des (1-3) IGF-I as radioligands. *J Clin Lab Anal,* 10**,** 446-450.

HORBER, F. F., HAYMOND, M.W. (1990) Human growth hormone prevents the catabolic side-effects of prednisone in humans. *J Clin Invest* 86**,** 265-272.

HORBER, F. F., KOHLER, S. A., LIPPUNER, K. & JAEGER, P. (1996) Effect of regular physical training on age-associated alteration of body composition in men. *Eur J Clin Invest.,* 26**,** 279-285.

HOUSSAY, B. A. & BIASOTTI, A. (1930) La diabetes pancreatica de los perros hipofiseoprivos. *Rev Soc Argent Biol,* 6**,** 251-296.

HU, H. Y., YAMAMOTO, H., SOHMIYA, M., ABE, T., MURAKAMI, Y., KATO, Y. (1994) Body composition assessed by bioelectrical impedance analysis (BIA) and the correlation with plasma insulin-like growth factor I (IGF-I) in normal Japanese subjects and patients with acromegaly and GH deficiency. *Endocrin J*.

HUNTER, W. M., FONSEKA, C.C., PASSMORE, R. (1965) Growth hormone: important role in muscular exercise in adults. *Science* 150**,** 1051-1053.

IKKOS, D., LUFT, R. & GEMZELL, C.-A. (1959) The effect of human growth hormone in man. *Acta Endocrinol (Copenhagen),* 32**,** 341-361.

IRVING, B. A., PATRIE, J.T., ANDERSON, S.M., WATSON-WINFIELD, D.D., FRICK, K.I., EVANS, W.S., VELDIUS, J.D., WELTMAN, A. (2004) The effects of time following acute growth hormone administration on

metabolic and power output measures during acute exercise. *J Clin Endocrinol Metab,* 89**,** 4298-4305.

ISGAARD, J., TIVESTEN, A., FRIBERG, P. & BENGTSSON, B. A. (1999) The role of the GH/IGF-I axis for cardiac function and structure. *Horm Metab Res,* 31**,** 50-54.

JACKSON, A. S., POLLOCK, M. L., GRAVES, J. E. & MAHAR, M. T. (1988) Reliability and validity of bioelectrical impedance in determining body composition. *J Appl Physiol***,** 529-534.

JACOB R, HU, X., NIEDERSTOCK D, HASAN S, MCNULTY PH, SHERWIN RS, ET AL. (1996) IGF-I stimulation of muscle protein synthesis in the awake rat: permissive role of insulin and amino acids. *American Journal of Physiology,* 270**,** E60-66.

JANSSEN, Y. J., DOORNBOS, J., ROELFSEMA, F. (1999) Changes in muscle volume, strength, and bioenergetics during recombinant human growth hormone (GH) therapy in adults with GH deficiency. *J Clin Endocrinol Metab* 84**,** 279-284.

JEUKENDRUP, A. E. (2002) Regulation of fat metabolism in skeletal muscle. *Ann NY Acad Sci,* 967**,** 217-235.

JOHANNSSON, G., ALBERTSSON-WIKLAND, K., BENGTSSON, B.A. (1999) Discontinuation of growth hormone (GH) treatment: metabolic effects in GH-deficient and GH-sufficient adolescent patients compared with control subjects. Swedish Study Group for Growth Hormone Treatment in Children. *J Clin Endocrinol Metab,* 84**,** 4516-4524.

JOHANNSSON, G., GRIMBY, G., SUNNERHAGEN, K.S., BENGTSSON, B.A. (1997) Two years of growth hormone (GH) treatment increase isometric and isokinetic muscle strength in GH-deficient adults. *J Clin Endocrinol Metab* 82**,** 2877-2884.

JOHANSSON, A. G., ENGSTROM, B. E., LJUNGHALL, S., KARLSSON, F. A. & BURMAN, P. (1999) Gender Differences in the Effects of Long Term Growth Hormone (GH) Treatment on Bone in Adults with GH Deficiency. *J Clin Endocrinol Metab,* 84**,** 2002-2007.

JONES, J. I., CLEMMONS, D.R. (1995) Insulin like growth factors and their binding proteins:biolobical actions. *Endocrin Rev,* 16.

JONES, N. L. (1988) *Clinical exercise testing,* Philadelphia, W. B. Saunders.

JONES, N. L., MAKRIDES, L., HITCHCOCK, C., CHYPCHAR, T., MCCARTNEY, N. (1985) Normal standards for an incremental progressive cycle ergometer test. *Am Rev Respir Dis,* 131**,** 700-708.

JORGENSEN, J., MOLLER, J., ALBERTI, K., SCHMITZ, O., CHRISTIANSEN, J., ORSKOV, H. & MOLLER, N. (1993) Marked effects of sustained low growth hormone (GH) levels on day-to- day fuel metabolism: studies in GH-deficient patients and healthy untreated subjects. *J Clin Endocrinol Metab,* 77**,** 1589-1596.

JORGENSEN, J. O., PEDERSEN, S. A., THUESEN, L., JORGENSEN, J., INGEMANN_HANSEN, T., SKAKKEBAEK, N. E. & CHRISTIANSEN, J. S. (1989) Beneficial effects of growth hormone treatment in GH-deficient adults. *Lancet,* 1**,** 1221-1225.

JORGENSEN, J. O. L., PEDERSEN, S.B., BORGLUM, J., MOLLER, N., SCHMITZ, O., CHRISTIANSEN, J.S., RICHELSEN, B. (1994a) Fuel metabolism, energy expenditure, and thyroid function in growth hormone-treated obese women: a double-blind placebo-controlled study. *Metab,* 43**,** 872-877.

JORGENSEN, J. O. L., THUESEN, L., MULLER, J., OVESEN, P., SKAKKEBAEK, N.E., CHRISTIANSEN, J.S. (1994b) Three years of growth hormone treatment in growth hormone-deficient adults: near normalization of body composition and physical performance. *Eur J Endocrinol,* 130**,** 224-228.

JUNGAS, R. L., HALPERIN, M.L, BROSNAN, J.T. (1992) Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. *Physiol Rev*, 72, 419-448.

KAMEGAI, J., TAMURA, H., SHIMIZU, T., ISHII, S., TATSUGUCHI, A., SUGIHARA, H., OIKAWA, S., KINEMAN, R. (2004) The role of pituitary ghrelin in growth hormone secretion:GH-releasing hormone dependent regulation of pituitary ghrelin gene expression and peptide content. *Endocrinol,* 145**,** 3731-3738.

KANALEY, J. A., DALL, R., MOLLER, N., NIELSEN, S.C., CHRISTIANSEN, J.S., JENSEN, M.D., JORGENSEN, J.O. (2004) Acute exposure to GH during exercise stimulates the turnover of free fatty acids in GH-deficient men. *J Appl Physiol,* 96**,** 747-753.

KANALEY, J. A., WEATHERUP-DENTES, M.M., JAYNES, E.B., HARTMAN, M.L. (1999) Obesity attenuates the growth hormone response to exercise. *J Clin Endocrinol Metab,* 84**,** 3156-3161.

KANALEY, J. A., WELTMAN, J.Y., VELDHUIS, J.D., ROGOL, A.D., HARTMAN, M.L., WELTMAN, A. (1997) Human growth hormone response to repeated bouts of aerobic exercise. *J Appl Physiol,* 83**,** 1756-1761.

KASAYAMA, S., OTSUKI, M., TAKAGI, M., SAITO, H., SUMITANI, S., KOUHARA, H., KOGA, M., SAITOH, Y., OHNISHI, T., ARITA, N. (2000)

Impaired B-cell function in the presence of reduced insulin sensitivity determines glucose tolerance status in acromegalic patients.

KATZ, A., NAMBI, S. S., MATHER, K., BARON, A. D., FOLLMANN, D. A., SULLIVAN, G. & QUON, M. J. (2000) Quantative insuiln sensitivity check index; a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab,* 85**,** 2402-2410.

KELLY, P. J., EISMAN, J.A., STUART, M.C., POCOCK, N.A., SAMBROOK, P.N., & GWINN, T. H. (1990) Somatomedin-C, physical fitness, and bone density. *J Clin Endocrinol Metab,* 70**,** 718-723.

KERN, P. A., SVOBODA, M. E., ECKEL, R. H. & VAN VYK, J. J. (1989) Insulin-like growth factor action and production in adipocytes and endothelial cells from human adipose tissue. *Diabetes,* 38**,** 710-717.

KEYS, A., FIDANZA, F., KARVONEN, M. J., KIMURA, N. & TAYLOR, H. L. (1972) Indices of relative weight and obesity. *J Chron Dis,* 25**,** 329-343.

KICMAN, A. T., MIELL, J. P., TEALE, J. D., POWRIE, J., WOOD, P. J., LAIDLER, P., MILLIGAN, P. J. & COWAN, D. A. (1997) Serum IGF-I and IGF binding proteins 2 and 3 as potential markers of doping with human GH. 47**,** 43-50.

KIILAVUORI, K., SOVIJARVI, A., NAVERI, H., IKONEN, T., LEINONEN, H. (1996) Effect of physical training on exercise capacity and gas exchange in patients with chronic heart failure. *Chest,* 110**,** 985-991.

KING, J. C. (1981) *The Biology of Race,* Berkeley, CA, Univ of California Pr.

KNAPIK, J., MEREDITH, C., JONES, B., FIELDING, R., YOUNG, V., EVANS, W. (1991) Leucine metabolism during fasting and exercise. *J Appl Physiol,* 70**,** 43-47.

KNIESS, A., ZIEGLER, E., KRATZSCH, J., THIEME, D. & MULLER, R. K. (2003) Potential parameters for the detection of hGH doping. *Anal Bioanal Chem,* 376**,** 696-700.

KNUTTGEN, H. G. & EMERSON, K. J. (1974) Physiological response to pregnancy at rest and duringe exercise. *J Appl Physiol,* 36**,** 549.

KOISTINEN, H., KOISTINEN, R., SELENIUS, L., YLIKORKALA, Q. & SEPPALA, M. (1996) Effect of marathon run on serum IGF-I and IGF-binding protein 1 and 3 levels. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology,* 80**,** 760-764.

KOK, P., ROELFSEMA, F., FRÖLICH, M., VAN PELT, J., MEINDERS, A.E., PIJL, H. (2008) Short-term treatment with bromocriptine improves impaired

circadian growth hormone secretion in obese premenopausal women. *J Clin Endocrinol Metab* 93**,** 3455–3461.

KOLLER, E. A., GREEN, L., GERTNER, J. M., BOST, M. & MALOZOWSKI, S. N. (1998) Retinal changes mimicking diabetic retinopathy in two nondiabetic, growth hormone-treated patients. *J Clin Endocrinol Metab,* 83**,** 2380-2383.

KOMI, P. V., RUSKO, H., VOS, J. & VIHKO (1977) Anaerobic performance capacity in athletes. *Acta Physiol Scand,* 100**,** 107-114.

KOZIRIS, L. P., HICKSON, R.C., CHATTERTON, JR. R.T., GROSETH, R.T., CHRISTIE, J.M., GOLDFLIES, D.G., UNTERMAN, T.G. (1999) Serum levels of total and free IGF-I and IGFBP-3 are increased and maintained in long-term training. *J Appl Physiol,* 86**,** 1436-1442.

KRAG, M. B., NIELSEN, S., GUO, Z., PEDERSEN, S.B., SCHMITZ, O., CHRISTIANSEN, J.S., JORGENSEN, J.O. (2008) Peroxisome proliferatoractivated receptor agonism modifies the effects of growth hormone on lipolysis and insulin sensitivity. *Clin Endocrinol (Oxf),* 69**,** 452-461.

KRAJCIK, R. A., BOROFSKY, N. D., MASSARDO, S. & ORENTREICH, N. (2002) Insulin-like Growth Factor I (IGF-I), IGF-binding Proteins, and Breast Cancer. *Cancer Epidemiol Biomarkers Prev,* 11**,** 1566-1573.

KREITSCHMANN-ANDERMAHR, I., SUAREZ, P., JENNINGS, R., EVERS, N., BRABANT, G. (2010) GH/IGF-I Regulation in Obesity – Mechanisms and Practical Consequences in Children and Adults. *Horm Res Paediatr,* 73**,** 153-160.

KUSHNER, R. F. (1992) Bioelectrical impedance analysis; A review of principles and applications. *J Am Coll Nutr,* 11**,** 199-209.

LAAGER, R., NINNIS, R. & KELLER, U. (1993) Comparison of the effects of recombinant human IGF on glucose and leucine kinetics in humans. *J Clin Invest,* 92**,** 1903-1909.

LAMBERTS, S. W., VAN DEN BELD, A. W. & VAN DER LELY, A. J. (1997) The endocrinology of ageing. *Science,* 278**,** 419-424.

LANDIN-WILHELMSEN, K., WILHELMSEN, L., LAPPAS, G., ROSEN, T., LINDSTEDT, G., LUNDBERG, P. A. & BENGTSSON, B. A. (1994) Serum insulin-like growth factor I in a random population sample of men and women: relation to age, sex, smoking habits, coffee consumption and physical activity, blood pressure and concentrations of plasma lipids, fibrinogen, parathyroid hormone and osteocalcin. *Clin Endocrinol (Oxf),* 41**,** 351-357.

LANGE, K. H., LARSSON, B., FLYVBJERG, A., DALL, R., BENNEKOU, M., RASMUSSEN, M. H., ORSKOV, H. & KJAER, M. (2002) Acute growth hormone administration causes exaggerated increases in plasma lactate and glycerol during moderate to high intensity bicycling in trained young men. *J Clin Endocrinol Metab,* 87**,** 4966-4975.

LANNING, N. J. & CARTER-SU, C. (2006) Recent advances in growth hormone signaling. *Rev Endocr Metab Disord* 7**,** 225-235.

LANZI, R., LUZI, L., CAUMO, A., ANDREOTTI, A.C., MANZONI, M.F., MALIGHETTI, M.E., SERENI, L.P., PONTIROLI, A.E. (1999) Elevated insulin levels contribute to the reduced growth hormone (GH) response to GH-releasing hormone in obese subjects. *Metab,* 48**,** 1152-1156.

LARIVIERE, F., WAGNER, D. A., KUPRANYCZ, D. HOFFER, L. J. (1990) Prolonged fasting as conditioned by prior protein depletion: effect on urinary nitrogen excretion and whole-body protein turnover. *Metab,* 39.

LASSARRE, C., GIRARD, F., DURAND, J., RAYNAUD, J. (1974) Kinetics of human growth hormone during submaximal exercise. *J Appl Physiol,* 37**,** 826-830.

LAURSEN, T., JØRGENSEN, J.O.L., CHRISTIANSEN, J.S. (1994) Metabolic effects of growth hormone administered subcutaneously once or twice daily to growth hormone deficient adults. *Clin Endocrinol (Oxf),* 41**,** 337-343.

LE ROITH D, BONDY, C., YAKAR S, LIU JL, BUTLER A (2001) The somatomedin hypothesis. *Endocr Rev* 22**,** 53-74.

LEBLANC, J., DIAMOND, P., COTE, J. & LABRIE, A. (1984) Hormonal factors in reduced post prandial heat production of exercise trained subjects. *J Appl Physiol,* 56**,** 772-776.

LEGRAND, V., BECKERS, A., PHAM, V.T., DEMOULIN, J.C., STEVENAERT, A. (1994) Dramatic improvement of severe dilated cardiomyopathy in an acromegalic patient after treatment with octreotide and trans-sphenoidal surgery. *Eur Heart J,* 15**,** 1286-1289.

LESAGE, R., SIMONEAU, J. A., JOBIN, J., LEBLANC, J. & BOUCHARD, C. (1985) Familial resemblance in maximal heart rate, blood lactate and aerobic power. *Human Heredity,* 35**,** 182-189.

LIEBERMAN, S. A., MITCHELL, A.M., MARCUS, R., HINTZ, R.L., HOFFMAN, A.R. (1994) The insulin-like growth factor I generation test: resistance to growth hormone with ageing and estrogen replacement therapy. *Horm Metab Res,* 26**,** 229-233.

LIFE MEASUREMENT INC. Body composition Tracking System Operator's Manual. 76.0001 Rev A.

LIGGETT, S. B., ST JOHN, R. E. & LEFRAK, S. S. (1987) Determination of resting energy expenditure utilizing the thermodilution pulmonary artery catheter. *Chest,* 91**,** 562-566.

LIU, H., BRAVATA, D.M., OLKIN, I., FRIEDLANDER, A., LIU, V., ROBERTS, B., BENDAVID, E., SAYNINA, O., SALPETER, S.R., GARBER, A.M., HOFFMAN, A.R. (2008) Systematic Review: The Effects of Growth Hormone on Athletic Performance. *Ann Intern Med,* 148**,** 747-758.

LIU, Z. & BARRETT, E. J. (2002) Human protein metabolism: its measurement and regulation. *Am J Physiol Endocrinol Metab,* 283**,** E1105-1112.

LIVESEY, G. & ELIA, M. (1988) Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry ; evaluation of errors with special reference to the detailed composition of foods. *Am J Clin Nutr,* 47**,** 608-628.

LJUNGHALL, S., JOHANSSON, A. G., BURMAN, P., KAMPE, O., LINDH, E. & KARLSSON, F. A. (1992) Low plasma levels of insulin-like growth factor 1 (IGF-1) in male patients with idiopathic osteoporosis. *J Intern Med,* 232**,** 59-64.

LONG, C. L., SCHAFFEL, N., GEIGER, J. W., SCHILLER, W. R. & BLAKEMORE, W. S. (1979) Metabolic response to injury and illness: estimation of energy and protein needs from indirect calorimetry and nitrogen balance. *J Parenter Enteral Nutr 1979.,* 3**,** 452-456.

LONGOBARDI, S., CUOCOLO, A., MEROLA, B., DI RELLA, F., COLAO, A., NICOLAI, E., CARDEI, S., SALVATORE, M., LOMBARDI, G. (1998) Left ventricular function in young adults with childhood and adulthood onset growth hormone deficiency. *Clin Endocrinol (Oxf),* 48**,** 137-143.

LONGOBARDI, S., KEAY, N., EHRNBORG, C., CITTADINI, A., ROSEN, T., DALL, R., BOROUJERDI, M. A., BASSETT, E. E., HEALY, M. L., PENTECOST, C., WALLACE, J. D., POWRIE, J., JORGENSEN, J. O. & SACCA, L. (2000) Growth Hormone (GH) Effects on Bone and Collagen Turnover in Healthy Adults and Its Potential as a Marker of GH Abuse in Sports: A Double Blind, Placebo-Controlled Study. *J Clin Endocrinol Metab,* 85**,** 1505-1512.

LUCIDI, P., LAUTERI, M., LAURETI, S., CELLENO, R., SANTONI, S., VOLPI, E., ANGELETTI, G., SANTEUSANIO, F. & DE FEO, P. (1998) A Dose-Response Study of Growth Hormone (GH) Replacement on Whole Body Protein and Lipid Kinetics in GH-Deficient Adults. *J Clin Endocrinol Metab,* 83**,** 353-357.

LUGER, A., WATSCHINGER, B., DEUSTER, P., SVOBODA, T., CLODI, M., CHROUSOS, & G.P. (1992) Plasma growth hormone and prolactin responses to graded levels of acute exercise and to a lactate infusion. *Neuroendocrinology,* 56**,** 112-117.

LUKASKI, H. C., JOHNSON, P. E., BOLONCHUK, W. W. & LYKKEN, G. I. (1985) Assessment of fat-free mass using bioelectrical impedance measurements of the human body. *Am J Clin Nutr,* 41**,** 810-817.

MACCARIO, M., GROTTOLI, S., PROCOPIO, M., OLEANDRI, S.E., ROSSETTO, R., GAUNA, C., ARVAT, E., GHIGO, E. (2000) The GH/IGF-I axis in obesity: influence of neuro-endocrine and metabolic factors. *Int J Obes Relat Metab Disord* 24(Suppl 2)**,** S96–S99.

MACKELVIE, K. J., MENEILLY, G.S., ELAHI, D., WONG, A.C., BARR, S.I., CHANOINE, J.P. (2007) Regulation of appetite in lean and obese adolescents after exercise: role of acylated and desacyl ghrelin. *J Clin Endocrinol Metab,* 92**,** 648-654.

MAISON, P. & CHANSON, P. (2003) Cardiac effects of growth hormone in adults with growth hormone deficiency: a meta-analysis. *Circulation,* 108**,** 2648-2652.

MALOZOWSKI, S., TANNER, L. A., WYSOWSKI, D. & FLEMING, G. A. (1993) Growth hormone, insulin-like growth factor I, and benign intracranial hypertension. *The New England Journal of Medicine,* 329**,** 665-666.

MARCUS, C., BOLME, P., MICHA-JOHANSSON, G., MARGERY, V., BRONNEGARD, M. (1994) Growth hormone increases the lipolytic sensitivity for catecholamines in adipocytes from healthy adults. *Life Sci,* 54**,** 1335-1341.

MARCUS, R., BUTTERFIELD, G., HOLLOWAY, L., GILLILAND, L., BAYLINK, D.J., HINTZ, R.L., SHERMAN, B.M. (1990) Effects of short-term administration of recombinant human growth hormone to elderly people. *J Clin Endocrinol Metab,* 70**,** 519-527.

MARCUS, R. & REAVEN, G. M. (1997) Growth Hormone-Ready for Prime Time? *J Clin Endocrin and Metab,* 82**,** 725-726.

MATTHEWS, D. R., HOSKER, J. P., RUDENSKI, A. S., NAYLOR, B. A., TREACHER, D.F., TURNER, R. C. (1985) Homeostasis model assessment: Insulin resistance and ß-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia 1985,* 28**,** 412-419.

MATTHEWS, K. G., DEVLIN, G. P., CONAGLEN, J. V., STUART, S. P., MERVYN_AITKEN, W. & BASS, J. J. (1999) Changes in IGFs in cardiac tissue following myocardial infarction. *J Endocrin,* 163**,** 433-445.

MCCRORY, M. A., GOMEZ, T. D., BERNAUER, E. M. & MOLE, P. A. (1995) Evaluation of a new air displacement plethysmograph for measureing human body compositon. *Med Sci Sports Exerc,* 27**,** 1686-1691.

MCGREEVY, K., HOEL, B., LIPSITZ, S., BISSADA, N., HOEL, D. Racial and anthropometric differences in plasma levels of insulin-like growth factor I and insulin-like growth factor binding protein-3. *Urology,* 66**,** 587-592.

MCHUGH, C. M., HOLT, R.I.G. (2005a) Growth hormone and the metabolic syndrome. IN BYRNE, C., WILD, S. (Ed.) *The Metabolic Syndrome.* Wiley Publications.

MCHUGH, C. M., PARK, R.T., SÖNKSEN, P.H., HOLT, R.I. (2005b) Challenges in detecting the abuse of growth hormone in sport. *Clin Chem,* 51**,** 1587-1593.

MEGYESI, K., KAHN, C. R., ROTH, J., NEVILLE, D. M., JR, NISSLEY, S. P., HUMBEL, R. E. & FROESCH, E. R. (1975) The NSILA-s receptor in liver plasma membranes. Characterization and comparison with the insulin receptor. *J. Biol. Chem.,* 250**,** 8990-8996.

MEHTA, D. K. (2000) Hypothalamic and anterior pituitary hormones and anti-oestrogens. IN MEHTA, D. K. (Ed.) *British National Formulary.* London, British Medical Association and the Royal Pharmaceutical Society of Great Britain.

MEINHARDT, U., NELSON, A.E., HANSEN, J.L. BIRZNIECE, V., CLIFFORD, D., LEUNG, K.C., GRAHAM, K., HO, K.K. (2010) The effects of growth hormone on body composition and physical performance in recreational athletes: a randomized trial. *Ann Intern Med,* 152**,** 568-577.

MEKALA, K. C., TRITOS, N.A. (2009) Effects of Recombinant Human Growth Hormone Therapy in Obesity in Adults: a meta analysis. *J Clin Endocrinol Metab,* 94**,** 130-137.

MILLS, J. L., FRADKIN, J., SCHONBERGER, L., GUNN, W., THOMSON, R.A., PIPER, J., WYSOWSKI, D., BROWN, P. (1990) Status report on the US human growth hormone recipient follow-up study. *Horm Res,* 33**,** 116-19.

MITCHELL, C. O., ROSE, J., FAMILONI, B., WINTERS, S. & LING, F. (1993) The use if multifrequency bioelectrical impedance analysis to estimate fluid volume changes as a function of the menstrual cycle. IN ELLIS, K. J. & EASTMAN, J. D. (Eds.) *Human body composition: In vitro methods, models and assessment.* New York, Plenum.

MITCHELL, J. H., SPROULE, B.J., CHAPMAN, C.B. (1958) The physiological meaning of the maximal oxygen intake test. *J Clin Invest,* 37**,** 538-547.

MITTELMAN, S. D., FU, Y.Y., REBRIN, K., STEIL, G., BERGMAN, R.N. (1997) Indirect Effect of Insulin to Suppress Endogenous Glucose Production Is Dominant, Even with Hyperglucagonemia. *J. Clin. Invest,* 100**,** 3121-3130.

MJAALAND, M., UNNEBERG, K., BJORO, T., REVHAUG, A. (1993) Growth hormone treatment after abdominal surgery decreased carbohydrate oxidation and increased fat oxidation in patients with total parenteral nutrition. *Metab,* 42**,** 185-190.

MOHAN, S. & BAYLINK, D. J. (1995) Development of a simple valid method for the complete removal of insulin-like growth factor (IGF)-binding proteins from IGFs in human serum and other biological fluids:comparison with acid-ethanol extraction treatment and C18 Sep-Pak separation. *J Clin Endocrinol Metab,* 80**,** 637-647.

MOLE, P. A. & HOFFMANN, J. J. (1999) VO(2) kinetics of mild exercise are altered by RER. *J Appl Physiol,* 87**,** 2097-2106.

MØLLER, J., FRANDSEN, E., FISKER, S., JØRGENSEN, J. O. & CHRISTIANSEN, J. S. (1996) Decreased plasma and extracellular Volume in growth hormone deficient adults and the acute and prolonged effects of GH administration: a controlled experimental study. *Clinical Endocrinology,* 44**,** 533-539.

MOLLER, N., BUTLER, P.C., ANTSIFEROV, M.A., ALBERTI, K.G. (1989) Efects of growth hormone on insulin sensitivity and forearm metabolism in normal man. *Diabetologia,* 32**,** 105-110.

MOLLER, N., JORGENSEN, J. O., SCHMITZ, O., MOLLER, J., CHRISTIANSEN, J., ALBERTI, K. G. & ORSKOV, H. (1990) Effects of a growth hormone pulse on total and forearm substrate fluxes in humans. *Amer J Physiol,* 258**,** E86-91.

MOLLER, N., JORGENSEN, J.O., ALBERTI, K.G., FLYVBJERG, A., SCHMITZ, O. (1990) Short-term effects of growth hormone on fuel oxidation and regional substrate metabolism in normal man. *J Clin Endocrinol Metab,* 70**,** 1179-1186.

MØLLER, N., MØLLER, J,. JØRGENSEN, J.O.L., OVESEN, P., SCHMITZ, O., ALBERTI, K.G., CHRISTIANSEN, J.S. (1993) Impact of 2 weeks growth hormone treatment on basal and insulin stimulated substrate metabolism in humans. *Clin Endocrinol (Oxf),* 39**,** 577-581.

MOLLER, N., SCHMITZ, O., JORGENSEN, J.O., ASTRUP, J., BAK, J.F., CHRISTENSEN, S.E., ALBERTI, K.G., WEEKE, J. (1992) Basal- and

insulin-stimulated substrate metabolism in patients with active acromegaly before and after adenomectomy. *J Clin Endocrinol Metab,* 74**,** 1012-1019.

MONSON, J. P., ABS, R., BENGTSSON, B.A., BENNMARKER, H., FELDT-RASMUSSEN, U., HERNBERG-STAHL, E., THOREN, M., WESTBERG, B., WILTON, P., WUSTER, C. (2000) Growth hormone deficiency and replacement in elderly hypopituitary adults. KIMS Study Group and the KIMS International Board. Pharmacia and Upjohn International Metabolic Database. . *Clin Endocrin,* 53**,** 281-289.

MONSON JP, JONSSON, P. (2003) Aspects of growth hormone (GH) replacement in elderly patients with GH deficiency: data from KIMS. *Horm Res.,* 60**,** 112-120.

MURPHY, L. J. (2003) The Role of the Insulin-Like Growth Factors and Their Binding Proteins in Glucose Homeostasis. *Experimental Diab. Res.,* 4**,** 213-224.

MUSEY, V. C., GOLDSTEIN, S., FARMER, P. K., MOORE, P. B. & PHILLIPS, L. S. (1993) Differential regulation of IGF-1 and IGF-binding protein-1 by dietary composition in humans. *Am J Med Sci,* 305**,** 131-138.

NABARRO, J. D. (1987) Acromegaly. *Clin Endocrinol (Oxf)***,** 481-512.

NAGULESPAREN, M., TRICKEY, R, DAVIES, M.J., JENKINS, J.S. (1976) Muscle changes in acromegaly. *Br Med J,* 2**,** 914-915.

NASS, R., HUBER, R.M., KLAUSS, V., MULLER, O.A., SCHOPOHL, J., STRASBURGER, C.J. (1995) Effect of growth hormone (hGH) replacement therapy on physical work capacity and cardiac and pulmonary function in patients with hGH deficiency acquired in adulthood. *J Clin Endocrinol Metab* 80**,** 552-557.

NELSON, A. E., HOWE, C.J., NGUYEN, T.V., LEUNG, K.C., TROUT, G.J., SEIBEL, M.J. BAXTER, R.C., HANDELSMAN, D.J., KAZLAUSKAS, R., HO. K.K. (2006) Influence of demographic factors and sport type on growth hormoneresponsive markers in elite athletes. *J Clin Endocrinol Metab,* 91**,** 4424-4432.

NIELSEN, S., MOLLER, N., CHRISTIANSEN, J.S., JORGENSEN, J.O. (2001) Pharmacological antilipolysis restores insulin sensitivity during growth hormone exposure. *Diabetes,* 50**,** 2301-2308.

NISHIYAMA, S., TOMOEDA, S., OHTA, T., HIGUCHI, A. & MATSUDA, I. (1988) Differences in basal and postexercise osteocalcin levels in athletic and nonathletic humans. *Calcif Tissue Int.,* 43**,** 150-154.

NORRELUND, H., FRYSTYK, J., JORGENSEN, J. O. L., MOLLER, N., CHRISTIANSEN, J. S., ORKSOV, H. & FLYVBJERG, A. (2009) The Effect of Growth Hormone on the Insulin-Like Growth Factor System during Fasting. *J Clin Endocrinol Metab,* 88**,** 3292-3298.

NORRELUND, H., NAIR, K.S., NIELSEN, S., FRYSTYK, J., IVARSEN, P., JORGENSEN, J.O., CHRISTIANSEN, J.S., MOLLER, N. (2003) The decisive role of free fatty acids for protein conservation during fasting in humans with and without growth hormone. *J Clin Endocrinol Metab,* 88**,** 4371-4378.

NUSSEY, S. S. & WHITEHEAD, S. A. (2001) The Pituitary Gland. IN NUSSEY, S. S. & WHITEHEAD, S. A. (Eds.) *Endocrinology an Integrated Approach.* 1st ed. Oxford, BIOS Scientific Publishers Limited.

NYGREN, J., THORELL, A., BRISMAR, K., ESSEN, P., WERNERMAN, J., MCNURLAN, M. A., GARLICK, P. J. & LJUNGQVIST, O. (2002) Glucose flux is normalized by compensatory hyperinsulinaemia in growth hormone-induced insulin resistance in healthy subjects, while skeletal muscle protein synthesis remains unchanged. *Clin Sci (Lond),* 102**,** 457-464.

NYSTRÖM, H. C., KLINTLAND, N., CAIDAHL, K., BERGSTRÖM, G., WICKMAN, A. (2005) Short-term administration of growth hormone (GH) lowers blood pressure by activating eNOS/nitric oxide (NO)-pathway in male hypophysectomized (Hx) rats. *BMC Physiol,* 5**,** 17.

O'BRIEN, E., WAEBER, B., PARATI, G., STAESSEN, J. & MYERS, M. G. (2001) Blood pressure measuring devices: recommendations of the European Society of Hypertension. *BMJ,* 322**,** 531-536.

O'SULLIVAN, A. J., KELLY, J.J., HOFFMAN, D.M., FREUND, J., HO, K.K. (1994) Body composition and energy expenditure in acromegaly. *J Clin Endocrinol Metab,* 78**,** 381-386.

ODLAND, L. M., HEIGENHAUSER, G. J. F., WONG, D., HOLLIDGE-HORVAT, M. G. & SPRIET, L. L. (1998) Effects of increased fat availability on fat-carbohydrate interaction during prolonged exercise in men. *Am J of Physiology,* 274**,** R894-R902.

OHTSUKA H, KOMIYA, I., AIZAWA, T, YAMADA T. (1995) Hypertension in acromegaly: hereditary hypertensive factor produces hypertension by enhancing IGF-I production. *Endoc Journal,* 42**,** 781-787.

OOI, G. T., BOISCLAIR, Y.R. (1999) Molcular biology of the insulin like growth factor binding proteins. IN ROSENFELD, R., . ROBERTS, J.C. (Ed.) *Cometmporary Endocrinology: the IGF System.* Totowa, NJ, Humana Press Inc.

OSEI, K. & SCHUSTER, D. P. (1994) Ethnic differences in secretion, sensitivity, and hepatic extraction of insulin in black and white Americans. *Diabet Med,* 11**,** 755-762.

OUCHI, N., KIHARA, S., ARITA, Y., OKAMOTO, Y., MAEDA, K., KURIYAMA, H., HOTTA, K., NISHIDA, M., TAKAHASHI, M., MURAGUCHI, M., OHMOTO, Y., NAKAMURA, T., YAMASHITA, S., FUNAHASHI, T., MATSUZAWA, Y. (2000) Adionectin, an adipocyte-derived plasma protein inhibits endothelial NF kappaB signaling through a cAMP-dependent pathway. *Circulation,* 102**,** 1296-1301.

PADAYATTY, S. J., PERRINS, E.J., BELCHETZ, P.E. (1996) Octreotide treatment increases exercise capacity in patients with acromegaly. *Eur J Endocrinol,* 134**,** 554-559.

PAPADAKIS, M. A., GRADY, D., BLACK, D., TIERNEY, M. J., GOODING, G. A., SCHAMBELAN, M. & GRUNFELD, C. (1996) Growth hormone replacement in healthy older men improves body composition but not functional ability. *Ann Intern Med,* 124**,** 708-716.

PARKINSON, C., RYDER, W. D. J. & TRAINER, P. J. (2001) The Relationship between Serum GH and Serum IGF-I in Acromegaly Is Gender-Specific. *J Clin Endocrinol Metab,* 86**,** 5240-5244.

PATE, R. R., PRATT, M., BLAIR, S. N., HASKELL, W. L., MACERA, C. A., BOUCHARD, C., BUCHNER, D., ETTINGER, W., HEATH, G. H., KING, A. C., KRISKA, A., LEON, A. S., MARCUS, B. H., MORRIS, J., PAFFENBARGER, R. S., PATRICK, K., POLLOCK, M. L., RIPPE, J. M., SALLIS, J. & WILMORE, J. H. (1995) Physical activity and public health: a recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA,* 273**,** 402-407.

PATEL, M. B., ARDEN, N.K., PHILLIPS, D.I., SWAMINATHAN, R., SYDDAL, H.E., BYRNE, C.D., WOOD, P.J., COOPER, C., HOLT, R.I. (2005) Investigating the role of growth hormone-insulin-like growth factor (GH-IGF) axis as a determinant of male bone mineral density (BMD). *Bone* 37**,** 833-841.

PIATTI, P. M., MONTI, L.D., CAUMO, A., CONTI, M., MAGNI, F., GALLI-KIENLE, M., FOCHESATO, E., PIZZINI, A., BALDI, L., VALSECCHI, G., PONTIROLI, A.E. (1999) Mediation of the hepatic effects of growth hormone by its lipolytic activity. *J Clin Endocrinol Metab,* 84**,** 1658-1663.

PINCUS, S. M., GEVERS, E.F., ROBINSON, I.C., VAN DEN BERG, G., ROELFSEMA, F., HARTMAN, M.L., VELDHUIS, J.D. (1996) Females secrete growth hormone with more process irregularity than males in both humans and rats. *Amer J Physiol,* 270**,** 107-115.

POEHLMAN, E. T., COPELAND, K.C. (1990) Influence of physical activity on insulin-like growth factor-I in healthy younger and older men. *J Clin Endocrinol Metab,* 71**,** 1468-1473.

POWRIE, J. K., BASSETT, E.E., ROSEN, T., JORGENSEN, J.O., NAPOLI, R., SACCA, L., CHRISTIANSEN, J.S., BENGTSSON, B.A., SÖNKSEN, P.H. (2007) GH-2000 Project Study Group. Detection of growth hormone abuse in sport. *Growth Horm IGF Res,* 17**,** 220-226.

PRATLEY, R., NICKLAS, B., RUBIN, M., MILLER, J., SMITH, A., SMITH, M., HURLEY, B. & GOLDBERG, A. (1994) Strength training increases resting metabolic rate and norepinephrine levels in healthy 50-65 year old men. *J Appl Physiol,* 73**,** 133-137.

PRITZLAFF, C. J., WIDEMAN, L., WELTMAN, J.Y., ABBOTT, R.D., GUTGESELL, M.E., & HARTMAN, M. L., VELDHUIS, J.D., WELTMAN, A. (1999) Impact of acute exercise intensity on pulsatile growth hormone release in men. *J Appl Physiol* 87**,** 498-504.

RADIKOVA, Z. (2003) Assessment of insulin sensitivity/resistance in epidemiological studies. *Endocr Regul.,* 37**,** 189-194.

RANDAL PJ (1963) The glucose fatty acid cycle: its role in insulin sensitivity an the metabolic disturbance of diabetes mellitus. *Lancet,* 1**,** 785-789.

RAPA, A., MONZANI, A., MOIA, S., VIVENZA, D., BELLONE, S., PETRI, A., TEOFOLI, F., CASSIO, A,. CESARETTI, G., CORRIAS, A., DE SANCTIS, V., DI MAIO, S., VOLTA, C., WASNIEWSKA, M., TATÒ, L., BONA, G. (2009) Subclinical hypothyroidism in children and adolescents: a wide range of clinical, biochemical, and genetic factors involved. *J Clin Endocrinol Metab,* 94**,** 2414=2420.

RASMUSSEN, M. H., HVIDBERG, A., JUUL, A., MAIN, K.M., GOTFREDSEN, A., SKAKKEBAEK, N.E., HILSTED, J., SKAKKEBAE, N.E. (1995) Massive weight loss restores 24-hour growth hormone release profiles and serum insulin-like growth factor-I levels in obese subjects. *J Clin Endocrinol Metab,* 80**,** 1407-1415.

RAU, H., FISCHER, H., SCHMIDT, K., LEMBCKE, B., ALTHOFF, P.H. (1991) Effect of bromocriptine withdrawal in acromegaly on body composition as assessed by bioelectrical impedance analysis. *Acta Endocrinologica,* 125**,** 273-279.

RAVEN, P. B. & MITCHELL, J. H. (1980) Effect of ageingon the cardiovascular response to dynamic and static exercise. IN WEISFELDT, M. L. (Ed.) *The ageing heart: its function and response to stress.* New York, Raven Press.

RAVEN, P. B. & POTTS, J. T. (1998) Cardiovascular responses to exercise and training. IN HARRIS, M., WILLIAMS, C., STAVISH, W. & MICHELI, L. (Eds.) *Oxford Textbook of sport medicine.* 2nd ed. Oxford.

RAVISSIN, E., SMITH, S.R. (2002) Increased fat intake, impaired fat oxidation and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. *Ann NY Acad Sci,* 968**,** 363-378.

RAYNAUD, J., DROUET, L., MARTINEAUD, J.P., BORDACHAR, J., COUDERT, J., DURAND, J. (1981) Time course of plasma growth hormone during exercise in humans at altitude. *J Appl Physiol,* 50**,** 229-233.

REBRIN, K., STEIL, G.M., GETTY, L., BERGMAN, R.N. (1995) Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes,* 44**,** 1038-1045.

RECHLER, M. M. (1993) Insulin –like growth factor binding proteins. *Vitamins and Hormones* 47.

RENNIE, M. J. (2003) Claims for the anabolic effects of growth hormone: a case of the Emperor's new clothes? *Br J Sports Med,* 37**,** 100-105.

RENNIE, M. J., EDWARDS, R.H., KRYWAWYCH, S., DAVIES, C.T., HALLIDAY, D., WATERLOW, J.C., MILLWARD, D.J. (1981) Effect of exercise on protein turnover in man. *Clin Sci (Lond),* 61**,** 627-639.

RENSBERGER, B. (1990) Racial odyssey. IN WHITTEN, P. & HUNTER, D. E. (Eds.) *Anthropology: Contemporary Perspectives.* Boston, Little, Brown.

RICART, W., FERNANDEZ_REAL, J.M. (2001) No decrease in free IGF-I with increasing insulin in obesity-related insulin resistance. *Obes Res,* Oct 9**,** 631-636.

RIEDL, M., LUDVIK, B., PACINI, G., CLODI, M., KOTZMANN, H., WAGNER, O., KAUTZKY-WILLER, A., PRAGER, R. & LUGER, A. (2000) The increased insulin sensitivity in growth hormone-deficient adults is reduced by growth hormone replacement therapy. *Eur J Clin Invest.,* 30**,** 771-778.

RIZZA, R. A., MANDARINO, L.J., GERICH, J.E. (1982) Effects of growth hormone on insulin action in man. Mechanisms of insulin resistance, impaired suppression of glucose production, and impaired stimulation of glucose utilization. *Diabetes,* 31**,** 663-669.

RODEN, M., PRICE, T., PERSEGHIN, G., PETERSEN, K.F., ROTHMAN, D.L., CLINE, G.W., SHULMAN, G.I. (1996) Mechanism of Free Fatty Acid-induced Insulin Resistance in Humans. *J. Clin. Invest,* 97**,** 2859-2865.

RODRIGUEZ-ARNAO, J., JABBAR, A., FULCHER, K., BESSER, G.M., ROSS, R.J. (1999) Effects of growth hormone replacement on physical performance and body composition in GH deficient adults. *Clin Endocrinol (Oxf)* 51**,** 53-60.

ROELEN, C. A., DE_VRIES, W. R., KOPPESCHAAR, H. P., VERVOORN, C., THIJSSEN, J. H. & BLANKENSTEIN, M. A. (1997) Plasma insulin-like growth factor-I and high affinity growth hormone-binding protein levels increase after two weeks of strenuous physical training. *Int J Sports Med,* 18**,** 238-241.

ROEMMICH, J.N, ROGEL, A. (1997) Exercise and growth hormone: does one affect the other? *J Pediatr.,* 131**,** S75-80.

ROGERS, S. A., MILLER, S.B., HAMMERMAN, M.R. (1990) Growth hormone stimulates IGF I gene expression in isolated rat renal collecting duct. *Am J Physiol,* 259**,** F474–F479.

ROSE, S., KAMIN, L. J. & LEWONTIN, R. C. (1984) *Not in our genes: biology, ideology and human nature,* New York, Penguin.

ROSEN, T., BOSAEUS, I., TOLLI, J., LINDSTEDT, G., BENGTSSON, B.A. (1993) Increased body fat mass and decreased extracellular fluid volume in adults with growth hormone deficiency. *Clin Endocrinol (Oxf),* 38**,** 63-71.

RUDMAN, D., FELLER, A. G., NAGRAJ, H. S., GERGANS, G. A., LALITHA, P. Y., GOLDBERG, A. F., SCHLENKER, R. A., COHN, L., RUDMAN, I. W. & MATTSON, D. E. (1990) Effects of human growth hormone in men over 60 years old. *NEJM,* 323**,** 1-6.

SACCA, L., CITTADINI, A., FAZIO, S. (1994) Growth hormone and the heart. *Endocr Rev* 15**,** 555-573.

SALOMON, F., CUNEO, R.C., HESP, R., SONKSEN, P.H. (1989) The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *N Engl J Med,* 321**,** 1797-1803.

SAUGY, M., ROBINSON, N., SAUDAN, C., BAUME, N., AVOIS, L., MANGIN, P. (2006) Human growth hormone doping in sport. *Br J Sports Med,* 40**,** 135-139.

SCACCHI, M., PINCELLI, A.I., CAVAGNINI, F. (1999) Growth hormone in obesity. *Int J Obes Relat Metab Disord* 23**,** 260-271.

SCHAEFER, F., BAUMANN,,G., HAFFNER, D., FAUNT, L.M., JOHNSON, M.L., MERCADO, M., RITZ, E., MEHLS, O., VELDHUIS, J.D. (1996) Multifactorial control of the elimination kinetics of unbound (free) growth

hormone (GH) in the human: regulation by age, adiposity, renal function, and steady state concentrations of GH in plasma. *J Clin Endocrinol Metab,* 81**,** 22-31.

SCHERING (2002) *RIA-gnost® PIIIP Kit Handbook*.

SCHMID, C., BIANDA, T., ZWIMPFER, C., ZAPF, J., WIESLI, P. (2005) Changes in insulin sensitivity induced by short-term growth hormone (GH) and insulin-like growth factor I (IGF-I) treatment in GH-deficient adults are not associated with changes in adiponectin levels. *Growth Hormone and IGF Research,* 15**,** 300-303.

SCHNEIDER, H. J., SALLER, B., KLOTSCHE, J., MARZ, W., ERWA, W., WITTCHEN, H.U., STALLA, G.K. (2006) Opposite associations of age-dependent insulin-like growth factor-I standard deviation scores with nutritional state in normal weight and obese subjects. *Eur J Endocrinol,* 154**,** 699-706.

SCHUTTE, J. E., TOWNSEND, E. J., HUGG, J., SHOUP, R. F., MALINA, R. M. & BLOMQVIST, C. G. (1984) Density of lean body mass is greater in blacks than in whites. *J Appl Physiol,* 56**,** 1647-1649.

SCHWINN, G., SCHWARCK, H., MCINTOSH, C., MILSTREY , H. R., WILLMS, B. & KO¨BERLING, J. (1976) Effect of the dopamine receptor blocking agent pimozide on the growth hormone response to arginine and exercise and on the spontaneous growth hormone fluctuations. *J Clin Endocrin Metab,* 43**,** 1176-1183.

SEE, K. O. & WILSON, W. J. (1988) Race and ethnicity. IN SMELSER, N. J. (Ed.) *Handbook of Sociology.* Beverly Hills, Sage Publications.

SENIOR, P. A. & BHOPAL, R. (1994) Ethnicity as a variable in epidemiological research. *BMJ,* 309**,** 327-330.

SERONO, M. SPC Saizen 8mg click.easy. IN SERONO, M. (Ed.) Serono Ltd, Bedfont Cross, Stanwell Road, Feltham , Middlesex , TW14 8NX, England.

SHARP, R. J. (2009) Land of giants. *Growth Horm IGF Res,* 19**,** 291-293.

SHARP, T. A., BELL, M. L., GRUNWALD, G. K., SCHMITZ, K. H., SIDNEY, S., LEWIS, C. E., TOLAN, K. & HILL, J. O. (2002) Differences in Resting Metabolic Rate between White and African-American Young Adults. *Obes Res,* 10**,** 726-732.

SHEFFIELD-MOORE, M., YECKEL, C.W., VOLPI, E., WOLF, S.E., MORIO, B., CHINKES, D.L., PADDON-JONES, D., WOLFE, R.R. (2004) Postexercise

protein metabolism in older and younger men following moderate-intensity aerobic exercise. *Am J Physiol Endocrinol Metab,* 287**,** E513-E522.

SHORT, K. R., VITTONE, J.L., BIGELOW, M.L., PROCTOR, D.N., NAIR, K.S. (2004) Age and aerobic exercise training effects on whole body and muscle protein metabolism. *Am J Physiol Endocrinol Metab,* 286**,** E92–E101.

SIDDLE, K., SOOS, M. A., FIELD, C. E. & NAVE, B. T. (1994) Hybrid and atypical insulin/insulin-like growth factor I receptors. *Horm. Res.,* 41**,** 56-64.

SINHA, M. K., BUCHANAN, C., LEGGETT, N., MARTIN, L., KHAZANIE, P. G., DIMARCHI, R., PORIES, W. J. & CARO, J. F. (1989) Mechanism of IGF-1-stimulated glucose transport in adipocytes. Demonstration of specific IGF-1 receptors not involved in stimulation of glucose transport. *Diabetes,* 38**,** 1217-1225.

SIRI, W. E. (1961) Body composition from fluid spaces and density: analysis of methods. IN BROZEK, J. & HENSCHEL, A. (Eds.) *Techniques for measuring body composition.* Washington D.C., National Academy of Sciences.

SJOGREN, K., WALLENIUS, K., LIU, J. L., BOHLOOLY, Y. M., PACINI, G., SVENSSON, L., TORNELL, J., ISAKSSON, O. G., AHREN, B., JANSSON, J. O. & OHLSSON, C. (2001) Liver-derived IGF-I is of importance for normal carbohydrate and lipid metabolism. *Diabetes,* 50**,** 1539-1545.

SNEGOVSKAYA, V., VIRU, A. (1993) Elevation of cortisol and growth hormone levels in the course of further improvement of performance capacity in trained rowers. *Int J Sports Med,* 14**,** 202-206.

SNEL, Y. E., DOERGA, M.E., BRUMMER, R.M., ZELISSEN, P.M., KOPPESCHAAR, H.P. (1995) Magnetic resonance imageing-assessed adipose tissue and serum lipid and insulin concentrations in growth hormone-deficient adults. *Arterioscler Thromb Vasc Biol,* 15**,** 1543-1548.

SNELL, P. G., MARTIN, W. H., BUCKEY, J. C. & BLOMQVIST, C. G. (1987) Maximal vascular leg conductance in trained and untrained men. *Journal of Applied Physiology,* 62**,** 606-610.

SNELL, P. G. & MITCHELL, J. H. (1984) The role of maximal oxygen uptake in exercise performance. *Clinics in Chest Medicine***,** 51-62.

SOLINI, A., BONORA, E., BONADONNA, R., CASTELLINO, P., DEFRONZO, R.A. (1997) Protein metabolism in human obesity: relationship with glucose and lipid metabolism and with visceral adipose tissue. *J Clin Endocrinol Metab,* 82**,** 2552-2558.

SONKSEN, P. H., GREENWOOD, F.C., ELLIS, J.P., LOWY, C., RUTHERFORD, A., NABARRO, J.D. (1967) Changes of carbohydrate tolerance in acromegaly with progress of the disease and in response to treatment. *J Clin Endocrinol Metab,* 27**,** 1418-1430.

SOONTHORNPUN, S., SETASUBAN, W., ATCHARA, T., CHAYANUNNUKUL, W., RATTARASARN, C. & GEATER, A. (2003) Insulin sensitivity index derived from oral glucose tolerance test. *J Clin Endocrinol Metab,* 8**,** 1019-1023.

SPELLWIN, G. (2006) Chemical wizardry.

STEINMANN, B., SUPERTI-FURGA, A., JOLLER-JEMELKA, H., CETTA, G. & BYERS, P. H. (1989) Ehlers-Danlos syndrome type IV: A subset of patients distinguished by low serum levels of the amino terminal propeptide of type III procollagen. *Am J Med Genet,* 34**,** 68-7171.

STEPPAN, C. M., BAILEY, S.T., BHAT, S., BROWN, E.J., BANERJEE, R.R., WRIGHT, C.M., PATEL, H.R., AHIMA, R.S., LAZAR, M.A. (2001) The hormone resistin links obesity to diabetes. *Nature,* 409**,** 307-312.

STEWART, C. E. H., ROTWEIN, P. (1996) Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. *Physiol Rev,* 76**,** 1005-1026.

STOKES, K., NEVILL, M., CHERRY, P., LAKOMY, H. K. & HALL, G. (2004) Effect of 6Â weeks of sprint training on growth hormone responses to sprinting. *Eur J Appl Physiol,* 92**,** 26-32.

STUMVOLL, M. & GERICH, J. (2001) Clinical features of insulin resistance and beta cell dysfunction and the relationship to type 2 diabetes. *Clin Lab Med,* 21**,** 31-51.

STUMVOLL, M., MITRAKOU, A., PIMENTA, W., JENESSEN, T., YKI-JARVINEN, H., VAN HAEFTEN, T., RENN, W. & GERICH, J. (2000) Use of the oral glucoce tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care,* 23**,** 295-301.

SUCUNZA, N., BARAHONA, M.J., RESMINI, E., FERNANDEZ-REAL, J.M., FARRERONS, J., LLUCH, P., PUIG, T., WAGNER, A.M., RICART, W., WEBB, S.M. (2008) Gender dimorphism in body composition abnormalities in acromegaly: males are more affected than females. *Eur J Endocrinol,* 159**,** 773-779.

SUIKARRI, A.-M., SANE, T., SEPPÄLÄ, M., YKI-JÄRVINEN, H., KARONEN, S.-L. & KOIVISTO, V. A. (1989) Prolonged exercise increases serum insulin-like growth factor-binding protein concentrations. *J Clin Endocrinol Metab,* 68**,** 141-144.

SUSSENBACH, J. S., STEENBERGH, P. H., JANSEN, E., HOLTHUIZEN, P., MEINSMA, D., VAN DIJK, M. A. et al. (1991) Structural and regulatory aspects of the human genes encoding IGF-I and -II. *Adv Exp Med Biol,* 293**,** 1-14.

SUTTON, J. & LAZARUS, L. (1976) Growth hormone in exercise: comparison of physiological and pharmacological stimuli. *J Appl Physiol,* 41**,** 523-527.

SVENSSON, J., FOWELIN, J., LANDIN, K., BENGTSSON, B.A. & JOHANSSON, J, O. (2002) Effects of Seven Years of GH-Replacement Therapy on Insulin Sensitivity in GH-Deficient Adults. *J Clin Endocrinol Metab,* 87**,** 2121-2127.

SVENSSON, J., HERLITZ, H., LUNDBERG, P.A., JOHANNSSON, G. (2005) Adiponectin, leptin, and erythrocyte sodium/lithium countertransport activity, but not resistin, are related to glucose metabolism in growth hormone-deficient adults. *J Clin Endocrin Metab* 90**,** 2290-2296.

TAAFFE, D. R., JIN, I.H., VU, T.H., HOFFMAN, A.R., MARCUS, R. (1996) Lack of effect of recombinant human growth hormone (GH) on muscle morphology and GH-insulin-like growth factor expression in resistance-trained elderly men. *J Clin Endocrinol Metab,* 81**,** 421-425.

TAAFFE, D. R., PRUITT, L., REIM, J., HINTZ, R.L., BUTTERFIELD, G., HOFFMAN, A.R., MARCUS, R. (1994) Effect of recombinant human growth hormone on the muscle strength response to resistance exercise in elderly men. *J Clin Endocrinol Metab,* 79**,** 1361-1366.

TANCREDI, R. G., DAGENAIS, G.R., ZIERLER, K.L. (1976) Free fatty acid metabolism in the forearm at rest: muscle uptake and adipose tissue release of free fatty acids. *Johns Hopkins Med J,* 138**,** 167-179.

TAPPY, L., ACHESON, K. (1998) Role of substrate competition in the pathogenesis of insulin resistance in man. . *Eur J Endocrinol,* 138**,** 10-15.

TAYLOR, H. L., BUSKIRK, E., HENSCHEL, A. (1955) Maximal oxygen intake as an objective measure of cardio-respiratory performance. *J Appl Physiol,* 8**,** 73-80.

TERAMUKAI, S., ROHAN, T., EGUCHI, H., ODA, T., SHINCHI, K. & KONO, S. (2002) Anthropometric and Behavioral Correlates of Insulin-like Growth Factor I and Insulin-like Growth Factor Binding Protein 3 in Middle-aged Japanese Men. *Am. J. Epidemiol.,* 156**,** 344-348.

TESSARI, P., NISSEN, S.L., MILES, J.M., HAYMOND, M.W. (1986) Inverse relationship of leucine flux and oxidation to free fatty acid availability in vivo. *J Clin Invest* 77**,** 575-581.

THOMAS, C. L. (1993) *Taber's Cyclopedic Medical Dictionary,* Philadelphia.

THOMAS, S. G., WOODHOUSE, L.J., PAGURA, S.M., EZZAT, S. (2002) Ventilation threshold as a measure of impaired physical performance in adults with growth hormone excess. *Clin Endocrinol (Oxf)* 56**,** 351-358.

THOMPSON, D., WILLIAMS, C., MCGREGOR, S. J., NICHOLAS, C. W., MCARDLE, F., JACKSON, M. J. & POWELL, J. R. (2001) Prolonged vitamin C supplementation and recovery from demanding exercise. *Int J Sport Nutr Exerc Metab,* 11**,** 466-481.

THOMPSON, D. L., WELTMAN, J. Y., ROGOL, A. D., METZGER, D. L., VELDHUIS, J. D. & WELTMAN, A. (1993) Cholinergic and opioid involvement in release of growth hormone during exercise and recovery. *J Appl Physiol,* 75**,** 870-878.

THORNER, M. O., VANCE, M. L., LAWS, E. R. J., HORVATH, E. & KOVACS, K. (1998) The Anterior Pituitary. IN WILSON, J. D., FOSTER, D. W., KRONENBERG, H. M. & LARSEN, P. R. (Eds.) *Williams Textbook of Endocrinology.* 9th ed. Philadephia, W.B. Saunders Company.

THUM, T., FLEISSNER, F., KLINK, I., TSIKAS, D., JAKOB, M., BAUERSACHS, J., STICHTENOTH, D.O. (2007) Growth Hormone Treatment Improves Markers of Systemic Nitric Oxide Bioavailability via Insulin-Like Growth Factor-I. *J Clin Endocrin Metab,* 92**,** 4172-4179.

THUM, T., TSIKAS, D., FRÖLICH, J.C., BORLAK, J. (2003) Growth hormone induces eNOS expression and nitric oxide release in a cultured human endothelial cell line. *FEBS Letters,* 555**,** 567-571.

TOMINAGA, A., ARITA, .K, KURISU, K., UOZUMI, T., MIGITA, K., EGUCHI, K., IIDA, K., KAWAMOTO, H., MIZOUE, T. (1998) Effects of successful adenomectomy on body composition in acromegaly. *Endocr J* 45**,** 335-342.

TOOGOOD, A. A. (2003) Growth hormone (GH) status and body composition in normal ageing and in elderly adults with GH deficiency. *Horm Res* 60**,** 105-111.

TRIVEDI, P., HINDMARSH, P., RISTELI, J., RISTELI, L., MOWAT, A. P. & BROOK, C. G. D. (1989) Growth velocity, growth hormone therapy, and serum concentrations of the amino-terminal propeptide of type III procollagen. *J Pediatr,* 114**,** 225-230.

TURKALJ, I., KELLER, U., NINNIS, R., VOSMEER, S. & STAUFFACHER, W. (1992) Effect of increasing doses of recombinant human insulin-like growth factor-I on glucose, lipid, and leucine metabolism in man. *J Clin Endocrinol Metab,* 75**,** 1186-1191.

U.S. DEPT OF COMMERCE (1992) U.S. Census of Population. Detailed Ancestry Groups for States. Washington D.C., U.S. Dept of Commerce.

VALERA, A., RODERIGUEZ-GIL, J. E., YUN, J. S., MCGRANE, M. M., HANSON, R. W. & BOSCH, F. (1993) Glucose metabolism in transgenic mice containing chimeric P-enolpyruvate carboxykinase/bovine growth hormone gene. *FASEB J,* 7**,** 791-800.

VAN CAUTER, E., PLAT, L., COPINSCHI, G. (1998) Interrelations between sleep and the somatotropic axis. *Sleep,* 21**,** 553-66.

VAN DEN BERG, G., VELDHUIS, J.D., FROLICH, M., ROELFSEMA, F. (1996) An amplitude-specific divergence in the pulsatile mode of growth hormone (GH) secretion underlies the gender difference in mean GH concentrations in men and premenopausal women. *J Clin Endocrinol Metab,* 81**,** 2460-2467.

VELDHUIS, J. D., IRANMANESH, A., HO, K.K., WATERS, M.J., JOHNSON, M.L., LIZARRALDE, G. (1991) Dual defects in pulsatile growth hormone secretion and clearance subserve the hyposomatotropism of obesity in man. *J Clin Endocrinol Metab,* 72**,** 51-59.

VELDHUIS, J. D., LIEM, A.Y., SOUTH, S., WELTMAN, A., WELTMAN, J., CLEMMONS, D.A., ABBOTT, R., MULLIGAN, T., JOHNSON, M.L., PINCUS, S. (1995) Differential impact of age, sex steroid hormones, and obesity on basal versus pulsatile growth hormone secretion in men as assessed in an ultrasensitive chemiluminescence assay. *J Clin Endocrinol Metab,* 80**,** 3209-3222.

VELDHUIS, J. D., ROEMMICH, J. N., RICHMOND, E. J. & BOWERS, C. Y. (2006) Somatotropic and gonadotropic axes linkages in infancy, childhood, and the puberty-adult transition. *Endocr Rev* 27**,** 110-140.

VIRU, A., KARELSON, K., SMIRNOVA, T. (1992) Stability and variability in hormonal responses to prolonged exercise. *Int J Sports Med,* 13**,** 230-235.

VON LINNE, C. (1956) Systema Naturae; A photographic facsimile of the first volume of the 10th edition.

WALLACE, J. D., CUNEO, R. C., BAXTER, R., ORSKOV, H., KEAY, N., PENTECOST, C., DALL, R., ROSEN, T., JORGENSEN, J. O., CITTADINI, A., LONGOBARDI, S., SACCA, L., CHRISTIANSEN, J. S., BENGTSSON, B,A. & SONKSEN, P. H. (1999) Responses of the Growth Hormone (GH) and Insulin-Like Growth Factor Axis to Exercise, GH Administration, and GH Withdrawal in Trained Adult Males: A Potential Test for GH Abuse in Sport. *J Clin Endocrinol Metab,* 84**,** 3591-3601.

WALLACE, J. D., CUNEO, R. C., LUNDBERG, P. A., ROSEN, T., JORGENSEN, J. O. L., LONGOBARDI, S., KEAY, N., SACCA, L., CHRISTIANSEN, J. S., BENGTSSON, B.A. & SONKSEN, P. H. (2000) Responses of Markers of Bone and Collagen Turnover to Exercise, Growth Hormone (GH) Administration, and GH Withdrawal in Trained Adult Males. *J Clin Endocrinol Metab,* 85**,** 124-133.

WALLACE, J. D., CUNEO, R.C., LUNDBERG, P.A., ROSEN, T., JORGENSEN, J.O., LONGOBARDI, S., KEAY, N., SACCA, L., CHRISTIANSEN, J.S., BENGTSSON, B.A., SONKSEN, P.H. (2000) Responses of markers of bone and collagen turnover to exercise, growth hormone (GH) administration, and GH withdrawal in trained adult males. *J Clin Endocrinol Metab,* 85**,** 124-133.

WALLACE, T. M., LEVY, J. C. & MATTHEWS, D. R. (2004) Use and Abuse of HOMA Modelling. *Diabetes Care,* 27**,** 1487-1495.

WASSERMAN, K., HANSEN, J. E. & SUE, D. Y. (1991) Facilitation of oxygen consumption by lactic acidosis during exercise. *News in Physiologyical Sciences,* 6**,** 29-34.

WASSERMAN, K., HANSEN, J. E., SUE, D. Y. & WHIPP, B. J. (1987) *Principles of exercise testing and interpretation,* Philadelphia, Lea and Febiger.

WEISSBERGER, A. J., HO, K.Y. & LAZARUS, L. (1991) Contrasting effects of oral and transdermal routes of estrogen replacement therapy on 24-hour growth hormone (GH) secretion, insulin-like growth factor I, and GH-binding protein in postmenopausal women. *J Clin Endocrin Metab,* 72**,** 374-381.

WELTMAN, A., WELTMAN, J. Y., SCHURRER, R., EVANS, W. S., VELDHUIS, J. D. & ROGOL, A. D. (1992) Endurance training amplifies the pulsatile release of growth hormone: effects of training intensity. *J Appl Physiol,* 72**,** 2188-2196.

WELTMAN, A., WELTMAN, J.Y., HARTMAN, M.L., ABBOTT, R.D., ROGOL, A.D., EVANS, W. S., VELDHUIS, J.D. (1994) Relationship between age, percentage body fat, fitness, and 24-hour growth hormone release in healthy young adults: effects of gender. *J Clin Endocrinol Metab,* 78**,** 543-548.

WHELDON. A., S., R.L., SÖNKSEN, P.H., HOLT, R.I. (2006) Exercising in the cold inhibits growth hormone secretion by reducing the rise in core body temperature. *Growth Horm IGF Res,* 16**,** 125-131.

WHIPP, B. J. & WARD, S. A. (1990) Physiological determinants of pulmonary gas exchange kinetics during exercise. *Med Sci Sports Exerc,* 22**,** 62-71.

WHITEHEAD, H. M., BOREMAN, C., MCILRATH, E.M., SHERIDAN, B., KENNEDY, L., ATKINSON, A.B., HADDEN, D.R. (1992) Growth hormone treatment of adults with growth hormone deficiency: results of a 13-month placebo controlled cross-over study. *Clin Endocrinol (Oxf)* 36**,** 54-52.

WICKMAN, A., JONSDOTTIR, I.H., BERGSTROM, G., HEDIN, L. (2002) GH and IGF-I regulate the expression of endothelial nitric oxide synthase (eNOS) in cardiovascular tissues of hypophysectomized female rats. *Eur J Endocrinol,* 147**,** 523-533.

WIDDOWSON, W. M., HEALY, M.L., SONKSEN, P., GIBNEY, J. (2009) The physiology of growth hormone and sport. *Growth Horm. IGF Res,* 19**,** 308-319.

WIDEMAN, L., CONSITT, L., PATRIE, J., SWEARINGIN, B., BLOOMER, R., DAVIS, P., WELTMAN, A. (2006) The impact of sex and exercise duration on growth hormone secretion. *J Appl Physiol,* 101**,** 1641-1647.

WIDEMAN, L., WELTMAN, J.Y., SHAH, N., STORY, S., VELDHUIS, J.D., WELTMAN, & A. (1999) Effects of gender on exercise-induced growth hormone release. *J Appl Physiol,* 87**,** 1154-1162.

WITZIG, R. (1996) The Medicalization of Race: Scientific Legitimization of a Flawed Social Construct. *Ann Intern Med,* 125**,** 675-679.

WOITGE, H. W., FRIEDMANN, B., SUTTNER, S., FARAHMAND, I., MULLER, M., SCHMIDT-GAYK, H., BAERTSCH, P., ZIEGLER, R. & SEIBEL, M. J. (1998) Changes in bone turnover induced by aerobic and anaerobic exercise in young males. *J Bone Miner Res,* 13**,** 1797-1804.

WOLFE, R. R., KLEIN, S., CARRARO, F., WEBER, J.M. (1990) Role of triglyceride fatty acid cycle in controlling fat metabolism in humans during and after exercise. *Am J Physiol,* 258**,** E382-E389.

WOLTHERS, T., GROFTE, T., JORGENSEN, J. O. L. & VILSTRUP, H. (1997) Growth hormone prevents prednisolone-induced increase in functional hepatic nitrogen clearance in normal man. *Journal of Hepatology,* 27**,** 789-795.

WOODHOUSE, L. J., ASA, S.L., THOMAS, S.G., EZZAT, S. (1999) Measures of submaximal aerobic performance evaluate and predict functional response to growth hormone (GH) treatment in GH-deficient adults. *J Clin Endocrinol Metab,* 84**,** 4570-4577.

WOODHOUSE, L. J., MUKHERJEE, A., SHALET, S.M., EZZAT, S. (2006) The influence of growth hormone status on physical impairments, functional limitations, and health-related quality of life in adults. *Endocr Rev* 27**,** 287-317.

WOODMANSEE, W. W., HARTMAN, M.L., LAMBERTS, S.W., ZAGAR, A.J., CLEMMONS, D.R. (2009) Occurrence of impaired fasting glucose in GH-deficient adults receiving GH replacement compared to untreated subjects. *Clin Endocrinol,* 72**,** 59-69.

WOODSON, R. D. (1984) Hemoglobin concentration and exercise capacity. *Am Rev Respir Dis,* 129 (suppl)**,** 72.

WORLD ANTI-DOPING AGENCY (2005) The Prohibited List 2005. International Standard for Testing. Montreal, World Anti Doping Agency.

WORLD ANTIDOPING AGENCY (2010) Guidelines for application of hGH isoform differential immunoassays.

WU, R. H., ST. LOUIS, Y., DIMARTINO-NARDI, J., WESOLY, S., SOBEL, E. H., SHERMAN, B. & SAENGER, P. (1990) Preservation of physiological growth hormone (GH) secretion in idiopathic short stature after recombinant GH therapy. *J Clin Endocrinol Metab,* 70**,** 1612-1615.

WU, X., DEVANY, E., BALARINI, G., JUNNILA, R., BIDLINGMAIER, M., STRASBURGER, C. (2010) Specific monoclonal antibodies and ultrasensitive immunoassays for 20K and 22K human growth hormone. *Growth Horm IGF Res,* June 20**,** 239-244.

WYNDHAM, C. H. & HEGNS, A. J. A. (1969) Determinants of oxygen consumption and maximum oxygen intake of Caucasians and Bantu males. *Int Z Angew Physiol,* 27**,** 51.

YAMAUCHI, M. & MECHANIC, G. L. (1988) Cross-linking of collagen. IN ME, N. (Ed.) *Collagen.* Boca Raton, Florida, CRC press.

YARASHESKI, K. E., CAMPBELL, J.A., SMITH, K., RENNIE, M.J., HOLLOSZY, J.O., BIER, D.M. (2008) Effect of growth hormone and resistance exercise on muscle growth in young men. *Am J Physiol,* 262**,** E261-267.

YARASHESKI, K. E., CAMPBELL, J.A., SMITH, K., RENNIE, M.J., HOLLOSZY, J.O., BIER, D.M. (1992) Effect of growth hormone and resistance exercise on muscle growth in young men. *Am J Physiol,* 262**,** E261-267.

YARASHESKI, K. E., ZACHWEIJA, J.J., ANGELOPOULOS, T.J., BIER, D.M. (1993) Short-term growth hormone treatment does not increase muscle protein synthesis in experienced weight lifters. *J Appl Physiol,* 74**,** 3073-3076.

YU-YAHIRO, J. A., MICHAEL, R. H., DUBIN, N. H., FOX, K. M., SACHS, M., HAWKES, W. G., HEBEL, J. R., ZIMMERMAN, S. I., SHAPIRO, J. & MAGAZINER, J. (2001) Serum and Urine Markers of Bone Metabolism During the Year After Hip Fracture. *J Am Geriatr Soc,* 49**,** 877-883.

ZENOBI, P. D., GRAF, S., URSPRUNG, H., FROESCH, E.R. (1992) Effects of insulin-like growth factor-I on glucose tolerance, insulin levels, and insulin secretion. *J Clin Invest,* 89**,** 1908-1913.

ZENOBI, P. D., JAEGGI-GROISMAN, S. E., RIESEN, W. F., RODER, M. E. & FROESCH, E. R. (1992) Insulin-like growth factor-I improves glucose and lipid metabolism in type 2 diabetes mellitus. *J Clin Invest,* 90**,** 2234-2241.

ZUNTZ, N. (1901) Ueber die Bedeutung der verschiedenen Nährstoffe als Erzeuger der Muskelkraft. *Pflügers Arch Physiol,* 83**,** 557.