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Growth Hormone in Athletes

by

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Citius, Altius, Fortius

To Jessica
Amanda and Wilma

Abstract

Doping with growth hormone (GH) is a well-known problem both among elite athletes and among people training at gyms. It is mainly the anabolic and, to some extent, lipolytic effect of GH that is valued by its users. However, no reliable method to detect GH doping has been available, and the role of GH as an effective doping agent has been discussed.

The aim of this thesis was to investigate markers of the GH/IGF-I axis and specific bone markers in athletes in connection with a maximum exercise test and longitudinally for one year, to validate the use of these markers in a forthcoming doping test for GH. Furthermore, the effects of one month's administration of supraphysiological GH doses on body composition, exercise performance and IGFBP-4 and IGFBP-5 concentrations in well-trained healthy subjects were studied.

The response to a maximum exercise test displayed a fairly uniform pattern, with peak concentrations of markers of the GH/IGF-I axis and bone markers immediately after exercise, followed by a subsequent decrease to baseline levels. The time to peak value for GH was significantly shorter for females compared with males.

Some of the markers show strong evidence of high *inter*- or *intra*-individual variations in resting samples during one year, based on analyses focusing on the right tail of the distribution in relation to a normal distribution. Post-competition values differed from resting values for several of the GH/IGF-I axis and bone markers. Ranges for post competition values and for each marker in each gender at specific time points in connection with a maximum exercise test, are presented.

The administration of supraphysiological doses of growth hormone for one month causes a dramatic increase in IGF-I levels, a reduction in body fat and an increase in the extracellular water volume. However, no significant increase in intracellular water volume was found, indicating limited anabolic effects by the supraphysiological GH doses.

Administration of supraphysiological doses of GH during one month did not improve power output or oxygen uptake in a bicycle exercise test.

Serum levels of IGFBP-4 and IGFBP-5 are increased by supraphysiological GH doses. Some of the effect of GH on IGFBP-4 and IGFBP-5 appears to be IGF-I dependent. However, the results do not support an obvious role for IGFBP-4 and IGFBP-5 as potential markers in a test for detecting GH doping.

In conclusion, this thesis describes different aspects of markers of the GH/IGF-I axis and specific bone markers in connection to rest and exercise, to be used in a forthcoming test for GH doping, in which IGFBP-4 and IGFBP-5 do not seem to have a role. Finally, no obvious anabolic effects on body composition or performance-enhancing effects were seen with supraphysiological GH doses; thus, questioning the role of GH as a potent doping agent.

Key words: Growth hormone, IGF-I, bone markers, doping, athletes, maximum exercise test, variability, supraphysiological, body composition, physical performance, IGFBP-4, IGFBP-5.

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Ehrnborg C, Lange KH, Dall R, Christiansen JS, Lundberg PA, Baxter RC, Boroujerdi MA, Bengtsson BA, Healey ML, Pentecost C, Longobardi S, Napoli R, Rosen T; GH-2000 Study Group.
The growth hormone/insulin-like growth factor-I axis hormones and bone markers in elite athletes in response to a maximum exercise test.
J Clin Endocrinol Metab. 2003 Jan;88(1):394-401.

II. Ehrnborg C, Lange KHW, Longobardi S, Healy ML, Dall R, Johansson H, Oden A, Cittadini A, Pentecost C, Christiansen JS, Bengtsson BA, Sonksen P, Rosen T; GH-2000 Study Group.
The GH/IGF-I axis hormones and bone markers in elite athletes. A longitudinal study examining stability over 12 months in- and out-of-competition.
Submitted.

III. Ehrnborg C, Ellegard L, Bosaeus I, Bengtsson BA, Rosen T.
Supraphysiological growth hormone: less fat, more extracellular fluid but uncertain effects on muscles in healthy, active young adults.
Clin Endocrinol (Oxf). 2005 Apr;62(4):449-57.

IV. Berggren A, Ehrnborg C, Rosen T, Ellegard L, Bengtsson BA, Caidahl K.
Short-term administration of supraphysiological recombinant human growth hormone (GH) does not increase maximum endurance exercise capacity in healthy, active young men and women with normal GH-insulin-like growth factor I axis.
J Clin Endocrinol Metab. 2005 Jun;90(6):3268-73.

V. Ehrnborg C, Ohlsson C, Mohan S, Bengtsson BA, Rosen T.
Increased serum concentration of IGFBP-4 and IGFBP-5 in healthy adults during one month's treatment with supraphysiological doses of growth hormone.
Growth Horm IGF Res. 2007 Jun;17(3):234-41.

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Abbreviations

AAS	Anabolic-androgenic steroids
ALS	Acid-labile subunit
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
BIS	Bioelectrical impedance spectroscopy
BMI	Body mass index
BPM	Beats per minute
Code	World Anti-Doping Code
CV	Coefficient(s) of variation
DNA	Deoxyribonucleic acid
DXA	Dual-energy X-ray absorptiometry
ECW	Extracellular water
EU	European Union
FFA	Free fatty acid
FFM	Fat-free mass
GH	Growth hormone
GHD	GH deficiency
GHRH	GH releasing hormone
HR	Heart rate
ICTP	Carboxy-terminal cross-linked telopeptide of type I collagen
ICW	Intracellular water
IGF	Insulin-like growth factor
IGFBP	IGF-binding protein
IOC	International Olympic Committee
LBM	Lean body mass
NO	Nitric oxide
NS	Not significant
OC	Oral contraceptive(s)
PICP	Carboxy-terminal propeptide of type I procollagen
P-III-P	Procollagen type III
PV	Plasma volume
RAAS	Renin-angiotensin-aldosterone system
rhGH	Recombinant human GH
RQ	Respiratory quotient
SD	Standard deviation
SE	Standard error
TBW	Total body water
VO_2	Oxygen uptake
$VO_{2\ max}$	VO_2 at peak exercise
WADA	World Anti-Doping Agency

Introduction

Athletes should be freed from the use of clay and mud and other irksome medicines.
Gymnastikos, 200 A.D (Flavius Philostratus) (1)

Doping in sports

The use of performance-enhancing substances and other artificial methods to enhance physical performance in sport (doping) is not a new phenomenon. It has been a feature of human competition since the beginning of recorded history. In fact, it has even been argued that the first instance of doping occurred in the Garden of Eden when Adam and Eve ate the forbidden fruit to acquire godlike powers. Knowledge of different performance-enhancing substances has then evolved with time and the use of different drugs and substances has been described in several different cultures and times during history, such as the ancient Egyptians, ancient Greek athletes and Roman gladiators. However, quite contrary to the opinion today, the use of performance-enhancing drugs has not always been regarded as cheating (2, 3).

It is believed that the word doping is derived from the Dutch word '*dop',* a term that refers to a stimulant drink used in tribal ceremonies in South Africa in order to enhance prowess in battle. The word '*dop'* first appeared in an English dictionary in 1889 and was then described as a narcotic potion used for racehorses (3, 4).

In the 19th century, cyclists and different endurance athletes often used alcohol, caffeine, cocaine and strychnine to fortify their performances and the first restrictions regarding drug use in sports were presented. In 1928, the International Amateur Athletic Federation (IAAF) was the first international sport federation to ban the use of stimulating substances (doping). Several other federations followed suit, but as no tests were performed, the restrictions were ineffective. The advent of anabolic-androgenic steroids (AAS) in the 1930s made the problem of performance-enhancing drugs worse and the pressure to introduce drug tests was further increased by the findings of traces of amphetamine, in the autopsy of a Danish cyclist, who died while competing at the Olympic Games in Rome in 1960 (3).

The start of the systematic use of AAS in sport has been ascribed to reports of their use by the successful Soviet weight-lifting teams in the early 1950s and, at the World Championships in Weight-lifting in Vienna in 1954, a U.S. team physician was reportedly told by his Soviet counterpart that the Soviets were taking testosterone (2).

The International Olympic Committee (IOC) instituted a Medical Commission in 1963 and the first list of prohibited substances was introduced in 1967. Drug tests were then first introduced in 1968 at the Winter Olympic Games in Grenoble and at the Summer Olympic Games in Mexico City. A reliable test method for AAS was introduced in 1974 and the IOC included anabolic steroids in the list of prohibited substances in 1976 (3).

The World Anti-Doping Agency (WADA) was established in Lausanne in 1999 to promote and co-ordinate the fight against doping in sport internationally. WADA was set up on the initiative of the IOC with the support and participation of several organisations and governments and, since 2004, the list of prohibited substances has been administered by WADA. The list of prohibited substances includes both prohibited substances and prohibited methods and is updated annually. The prohibited list is an international standard identifying

the substances and methods prohibited in-competition, out-of-competition and in particular sports. The substances are classified by categories (e.g. steroids, stimulants, gene doping) and the list is divided into four major parts: substances and methods prohibited at all times (in- and out-of-competition), substances and methods prohibited in-competition, substances prohibited in particular sports and specified substances.

One important achievement in the fight against doping in sports was the launch of a set of anti-doping rules, the World Anti-Doping Code (Code) that came into force on January 1 2004. One major advantage of the introduction of the Code is the harmonisation of a system that previously had rules that varied and in some cases did not exist. The Code also formalises rules and respective responsibilities regarding "non-analytical" violations, such as refusing or failing a doping test without compelling justification.

There are strict rules about the use of performance-enhancing substances and methods in sport and doping is defined as the occurrence of one or more of the anti-doping rule violations set forth in specific articles of the Code (3).

Several different hormones are used as doping agents. The most common apart from AAS, are growth hormone (GH), insulin and erythropoietin (EPO). However, AAS have been and still are the most common doping substances used in and outside sports (2, 5). There is reason to believe that the use has decreased among elite athletes since the test methods have been improved. Furthermore, the top-rank results in track and field disciplines such as shotput and discus, where doping has been highly suspected, appear to be levelling off, indirectly speaking in favour of a decrease in AAS use among elite athletes. In spite of this, use among non-elite athletes in for example gyms, for example, may not have decreased.

Doping with GH is a well-known problem in the world of sports and it has been known for decades (6). GH was described as a potent performance-enhancing anabolic agent in *The Underground Steroid Handbook* first published in California in the early 1980s (7). Its misuse has since increased, especially since the advent of recombinant GH in the late 1980s, and users can currently be found both among elite athletes and among people training at gyms (8, 9). It is mainly the anabolic and, to some extent, lipolytic effect of GH that is valued by users.

GH - historical background

The existence of a growth-promoting substance in the anterior hypophysis was described in animals in the 1920s (10). Human GH was first isolated by Li *et al.* in 1956 and, in the early 1970s, the structure of GH was subsequently shown to consist of a single polypeptide chain of 191 amino acids with two disulphide bridges and a molecular weight of 22 kDa (11-13). It was then stated that 22kDa GH is the major isoform of GH but a 20kDa GH variant comprises 5-10% of the pituitary GH and that a number of other isoforms produced by the pituitary also exist (14, 15). The gene for GH has been cloned and characterised and synthetic GH is currently produced in bacteria using recombinant DNA technology (16).

Children with growth hormone deficiency (GHD) have been treated with GH since the 1950s, when it was demonstrated that treatment with human GH, purified from cadaver pituitaries increased linear growth (17). The first paper describing GH treatment in adults was presented in 1962 and described the treatment of a 35-year-old GHD woman with human GH. The patient noticed 'increased vigour, ambition and sense of well-being' after two months of treatment (18). Treatment with GH was however initially restricted by the limited supply of

GH, before the advent of widely available recombinant human GH in the mid-1980s. The introduction of recombinant GH made it possible to further study the effects of GH in adults and the consequences of the clinical entity of GHD, including its treatment, have been well described (19-22).

Physiology of GH secretion

GH is secreted in a pulsatile pattern from somatotrope cells in the anterior hypophysis, regulated in a complex pattern by two hypothalamic peptides; a stimulating hormone, GH releasing hormone (GHRH), and an inhibiting hormone, somatostatin (23-26).

GH secretion is influenced by several normal and pathophysiological conditions, such as gender, age, sleep, physical exercise, nutritional state and other metabolic factors.
Gender. A difference between men and women in the GH release at rest, with greater release in young women than in age-matched men, has been described (27-29). Gonadal steroids interact with GH and the administration of oestrogen increases serum levels of GH (30-34). Testostereone and GnRH treatment in hypogonadal men has been shown to increase GH secretion (35). Oestrogens enhance GH secretion, mainly indirectly by inducing GH resistance resulting in higher serum levels of GH in females of reproductive age, a somewhat different secretion pattern and GH production rate (26, 34).
Age. It has been estimated that there is a 14% decrease in GH secretion per decade of adult life, following a peak during puberty (36)
Sleep. The GH levels are highest during slow wave sleep and lowest during rapid eye movement sleep (37).
Physical exercise. Physical exercise has a stimulatory effect on GH secretion (38). The GH levels rise in response to acute exercise, with a threshold level of approximately 30% of VO_2 max (39), and a twofold rise in GH concentrations after a year of high-intensity aerobic training has been shown in subjects who exercised consistently above the lactate threshold (40).
Nutritional state and other metabolic factors. Fasting results in enhanced GH production (41), while it is suppressed by glucose (42) and fatty acids (43). Certain amino acids such as leucine and arginine enhance GH secretion (44, 45).
Hormones. Hyperthyroidism is associated with increased GH secretion (46), while hypothyroidism is associated with low GH levels (47). The net effect of corticoids is inhibition of the GH secretion (48).
Neurotransmittors. Both α_2-adrenergic agonists and cholinergic agents stimulate GH secretion (49), the latter probably via suppression of somatostatin release (50).

GH and muscles

GH is an important and powerful metabolic hormone. An anabolic effect by GH in normal adults was demonstrated in 1958 by Ikkos *et al*. who observed a nitrogen-retention effect after GH administration (51). Patients with untreated acromegaly have shown a markedly increased body cell mass estimated from assessments of total body potassium (52). The body cell mass in acromegalic patients decreases in response to surgical treatment (53). Furthermore, it has been shown that acromegaly causes myopathy with hypertrophic, but functionally weaker muscles (54, 55). This could indicate that there are negative effects on muscle function following exposure to high levels of GH for a long period of time.

GH promotes the positive protein balance in skeletal muscle by increasing protein synthesis and possibly by inhibiting protein breakdown (56).

Adult GHD patients have a reduced muscle mass, isometric muscle strength and functional exercise capacity compared with healthy controls (57, 58). Furthermore, isokinetic muscle strength and local muscle endurance are reduced or in the lower range (57, 59-61). The reduced muscle mass and isometric strength could be an effect of reduced muscle cross-sectional area in GHD patients (62), but it could also be caused by a reduction in the peak torque per muscle area (63), suggesting that contractile properties and neural activation might be responsible for the reduction in muscle strength in adult GHD patients

GH replacement therapy in GHD adults increases lean body mass (LBM), exercise capacity, muscle volume, muscle mass and maximum voluntary isometric muscle strength (20, 57, 60, 64-68). The changes in muscle mass and maximum voluntary isometric muscle strength has been shown to become apparent after approximately one year of therapy (60). However, dynamic muscle strength has not shown any obvious increase in response to GH treatment (65, 69).

The proportion of fast-twitch, type-2 muscle fibres is increased in hypophysectomised rats (70). However, the histology of muscles from GHD patients does not appear to differ from that of healthy adults (71) and it has not been possible to detect any changes in the proportions of muscle fibres in adult GHD patients receiving GH treatment (71, 72).

Lipolytic effects of GH

The lipolytic effects of GH have been known for decades. GH-induced lipolysis was first demonstrated in humans in 1959 (73). Lipolytic effects have also been demonstrated in GHD and acromegaly patients. GHD patients have increased total body fat and reduced body cell mass and extracellular water (ECW) (74, 75). GH treatment given to these patients improves the body composition (22, 59, 76). Furthermore, patients with untreated acromegaly have a marked decrease in adipose tissue mass compared with normal individuals (52) and surgical treatment results in an increase in the fractions of adipose tissue in the subcutaneous trunk and the intra-abdominal depots, while the fractions of adipose tissue in peripheral depots decrease (53).

Meals inhibit GH release, whereas fasting conditions amplify the pulsatile pattern of GH secretion (77), indicating that the main impact of GH is in the fasting state. Dose-dependent action by GH on the induction of lipolysis has been demonstrated, with an elevation of circulatory free fatty acids (FFAs) and glycerol and increased lipid oxidation rates (78). These effects occur despite increased insulin levels, indicating that relatively low doses of GH can overcome the lipogenic actions of insulin. GH stimulates lipolysis by activating hormone-sensitive lipase activity, with a subsequent increase in lipid oxidation (79).

Anti-natriuretic effects of GH

A sodium-retention effect with the simultaneous expansion of extracellular water (ECW) after GH administration was demonstrated in 1952 (80). Even though it is not the main focus of attention in a doping situation, the anti-natriuretic effect of GH is still of interest.

The sodium and water-retaining effect of GH is complex. To summarise, both GH and IGF-I are capable of causing fluid retention by stimulating Na^+K^+ATPase activity in the distal nephron (81). However, the stimulation of the renin-angiotensin-aldosterone system (RAAS) (82), the down-regulation of atrial natriuretic peptide (ANP) (83) and increased endothelial nitric oxide (NO) function have also been proposed as possible mechanisms (84).

The anti-natriuretic effects of GH explain the reduction in ECW that is found in adult patients with severe GHD and the marked increase in ECW found in acromegalic patients (52, 74). However, the exact mechanisms behind these effects are unknown. ECW is increased by as much as 25% in untreated acromegaly patients, an increase that normalises after successful treatment (85). After treatment, the excess ECW correlates with the GH concentrations (85). Further studies of acromegalic patients suggest a curve-linear dose-response relationship between GH concentrations and excess ECW (85, 86).

GH effects on bone

GH has a stimulating effect on both osteoblasts and osteoclasts and is thereby involved in the regulation of bone metabolism (87-89), leading to both bone formation and resorption (90, 91). The osteoclasts, osteoblasts and components of the bone matrix release several peptides and proteins into the circulation during bone resorption and formation, peptides and proteins that can be used as biochemical markers of bone metabolism.

In adult GHD patients, both normal (92) and reduced (93) serum levels of biochemical markers of bone turnover have been shown. Several studies of hypopituitary patients have shown that GH treatment accelerates bone turnover (90, 94, 95). Furthermore, it has been shown that biochemical markers of bone formation and bone resorption increase within a few weeks after the initiation of GH treatment in GHD adult patients (96).

Doping with GH

This misuse of GH is undesirable for both medical and ethical reasons. The lack of a test for detecting GH doping is unfortunate and the need to develop a reliable test is pressing.

Several issues make the development of a method for detecting GH doping complicated (97). It is, for example, not possible to distinguish exogenous recombinant GH from endogenous GH in a blood or urine test. Furthermore, GH secretion is influenced by many different factors such as exercise, food intake, sleep and stress (28). The lack of an official method to discover GH doping, might partly explain the strong position GH enjoys as a doping agent in elite sports.

The GH-2000 project was initiated by the International Olympic Committee (IOC) with the aim of developing a method for detecting GH doping among athletes. The project was funded by the European Union (EU) BioMed2 Research Programme, with additional support from industry and the IOC.

Prior to the start of the GH-2000 project, Wallace *et al.* studied the effects of exercise and supraphysiological GH administration on the GH/IGF-I axis and bone markers in 17 athletic adult males. To summarise, acute exercise increased all the molecular isoforms of GH, with 22kDa GH constituting the major isoform, with a peak at the end of acute exercise (98). The

proportion of non-22kDa isoforms increased after exercise, due in part to the slower disappearance rates of these isoforms. With supraphysiological GH administration, these exercised-stimulated endogenous GH isoforms were suppressed for up to four days (99). Moreover, all the components of the IGF-I ternary complex transiently increased with acute exercise and GH pre-treatment augmented these exercise-induced changes (100). Furthermore, acute exercise increased the serum concentrations of the bone and collagen markers, bone-specific alkaline phosphatase, carboxy-terminal cross-linked telopeptide of type I collagen (ICTP), carboxy-terminal propeptide of type I procollagen (PICP) and procollagen type III (P-III-P), while osteocalcin was unchanged. GH treatment resulted in an augmented response to exercise of the bone markers PICP and ICTP (101).

A forthcoming method to discover GH abuse will probably necessitate the use of specific markers of the GH/IGF-I axis and bone markers, with the prerequisite that these variables are more sensitive to exogenous GH administration than to exercise. As a result, it will be important closely to study how the levels of these variables are influenced by a maximum exercise test in comparison to rest and by other factors such as gender, age, fitness, type of sport, medication, menstrual status or illness.

GH and exercise

Physical training has been shown to change circulating levels of GH and, more inconsistently, IGF-I in normal subjects in relation to improvements in oxygen uptake and muscle strength (102-105).

It is well known that acute exercise above a certain intensity is one of the most potent stimulators of GH secretion and the magnitude of the GH response is closely related to the peak intensity of exercise, rather than to total work output (38, 39, 106). Exercise not only mediates the acute effects on GH secretion. It has been shown that one year of endurance training above the lactate threshold, increases the basal 24-h pulsatile GH release (40). Interestingly, subjects training below the lactate level did not show any change in the GH release, indicating that the training intensity may be important in regulating the GH-axis as well as fitness. This physiological GH increase in response to exercise and to other stimuli such as hypoglycemia makes it difficult to use measurements of GH itself in blood as a doping marker, as it would be difficult to discriminate a high exercise-derived endogenous GH level from that resulting from exogenous GH.

In addition, bone markers have been shown to respond to exercise and the effects of low-intensity endurance-type activity or brief high-intensity or resistance exercise have shown no acute change (107-109), increased markers (110, 111) or transient decreased markers (109). Furthermore, studies of high-intensity exercise showed no rise in PICP or ICTP in response to exercise (107, 109, 111). This could suggest that the duration of exercise is important in the response by bone markers to acute exercise.

Variability

Acute exercise above a certain intensity is one of the most potent stimulators of GH secretion. It is well known that elite athletes are able to train at much higher intensities than the normal population and that, during a training season, there are significant differences in training intensity, which might influence GH secretion. Many of the GH-related mediators, binding

proteins and markers do not exhibit the same fluctuations as GH and there is a real lack of knowledge about the seasonal stability of markers of the GH/IGF-I axis and circulating bone markers in athletes. A study of seasonal patterns of sleep stages and secretion of cortisol and GH during 24-hour periods in northern Norway revealed no difference in GH secretion as a function of season of the year (112). Another study reveals no circannual rhythm of plasma GH in pre-pubescent subjects (113).

There is some evidence that biological rhythms of bone turnover over long periods, such as circannual variation, exist (114-121). Woitge *et al.* have shown that seasonal variation contributes to the biological variability in bone turnover and needs to be taken into account when interpreting the results of bone marker measurements (121).

Supraphysiological doses of GH - effects on muscles, power, exercise capacity and body composition

GH plays a regulatory role in the maintenance of normal body composition through its well-known anabolic, lipolytic and antinatriuretic actions. These effects are easily demonstrated when GH-substitution therapy is initiated in patients with GHD, reversing muscle atrophy and decreasing central abdominal adiposity and dry skin, signs typically associated with GHD (20, 22, 122). The anabolic actions of GH include stimulated protein synthesis through the mobilisation of amino-acid transporters, which is reflected *in vivo* by an increase in the metabolic clearance rate of amino acids. IGF-I also directly stimulates protein synthesis, albeit to a lesser extent than GH, while insulin inhibits protein breakdown (123-127).

Even though GH has been regarded as an effective ergogenic drug among athletes since the 1980s, only a few controlled studies of the effectiveness of GH in relation to physical performance and the effects on body composition in athletes have been performed. These studies involving supraphysiological doses of GH have used somewhat lower doses than those reportedly used by GH abusers and have included only male subjects. A study of 16 young, healthy, adults revealed no differences between the GH or placebo group in terms of muscle size, strength or muscle protein synthesis after GH (40µg/kg/day) or placebo treatment for 12 weeks combined with heavy-resistance exercise. However, fat free mass (FFM) and total body water (TBW) increased in both groups but significantly more among the GH recipients (128). Another study of 22 power athletes assigned in a double-blind manner to either GH treatment (30µg/kg/day) or placebo for a period of six weeks revealed no difference between the groups in terms of maximum voluntary strength (biceps or quadriceps) and no change in body weight or body fat, but a remarkable increase in IGF-I was noted (129). Crist *et al*. found increased fat free weight and decreased body fat in eight well-trained adults when given 2.67 mg of GH 3 days/week daily for six weeks (130). Finally, a study of healthy, experienced male weight-lifters before and at the end of 14 days of subcutaneous GH administration (40 µg/kg/day) revealed no increase in the rate of muscle protein synthesis or reduced whole body protein breakdown, metabolic alterations that would promote muscle protein anabolism (131).
Some studies performed on elderly men show the same results. A study of healthy, sedentary men with low serum IGF-I levels who followed a 16-week progressive resistance-exercise program (75-90% max strength, 4 days/week) after random assignment to either a GH (12.5-24 µg/kg/day) or placebo group showed that resistance-exercise training improved muscle strength and anabolism, but these improvements were not enhanced when exercise was combined with daily GH administration (132). Further supportive findings of the lack of effect are found in elderly but not particularly GH-deficient men. Taffee *et al.* were unable to

see any increase in strength, muscle mass or fibre characteristics after GH treatment during a resistance-exercise training programme (133, 134).

GH effects on IGFBP-4 and IGFBP-5

IGFs are present in serum and other biological fluids bound to a family of structurally related proteins, the IGF-binding proteins (IGFBPs). The family of at least six IGFBPs, distinct from the IGF receptors, modulates the effects of IGFs in different tissues, including bone (135-141). IGFBP-4 was originally isolated from bone as an inhibitory IGFBP (142). IGFBP-5 is regarded as a stimulatory IGFBP in osteoblastic proliferation (143, 144). It has been shown that IGFs are important modulators of IGFBP levels in conditioned medium of several cell lines, including bone cells and IGFs have been described to decrease the level of IGFBP-4 and increase the level of IGFBP-5 in cultures of human bone cells and fibroblasts (145-151).

There are few clinical studies of GH and IGFBP-4 and IGFBP-5. However, GH treatment given to GHD patients has been shown clearly to increase serum levels of IGFBP-4 and IGFBP-5 (152, 153). Furthermore, treatment with GH secretagogues has been shown to produce an increase in serum levels of IGFBP-5 and a transient increase in serum IGFBP-4 (154).

IGFBP-4 and IGFBP-5 were not included in the original GH-2000 battery of potential doping markers investigated by Wallace *et al*. (100, 101). However, they might have a potential as markers of GH doping.

Aims of the thesis

The main objectives of this thesis were to study different aspects of specific GH dependent markers from the GH/IGF-I axis and bone markers in order to be used in the development of a test for detection of GH abuse in sports. Furthermore, different effects after administration of supraphysiological doses of GH were studied. The specific aims were:

I. To examine the response of the serum concentrations of specific GH dependent markers from the GH/IGF-I axis and bone markers in elite athletes of both genders from different sports to a maximum exercise test. The aim was also to present reference ranges for each marker in connection to the maximum exercise test.

II. To investigate the stability and variability of specific GH dependent markers from the GH/IGF-I axis and bone markers in elite athletes during one year and in connection to a competition.

III. To study the effects on body composition after one month's administration of supraphysiological doses of GH in physically active, healthy, young adults of both genders with normal GH/IGF-I axis using a placebo-controlled trial design.

IV. To evaluate the effects on exercise capacity after one month's administration of supraphysiological doses of GH in healthy young adults with normal GH/IGF-I axis.

V. To study the effects on IGFBP-4 and IGFBP-5 after one month's administration of supraphysiological doses of GH in healthy young adults with normal GH/IGF-I axis and to evaluate the possible use of IGFBP-4 and IGFBP-5 as potential markers of GH doping.

Subjects and methods

Subjects

Papers I+II

Paper I. One hundred and seventeen (117) elite athletes from Denmark, England, Italy, and Sweden (84 males and 33 females; mean age 25 years; range 18-53), competing in different sport categories were included in the study. The sport categories and the number of subjects were: alpine skiing (21), cross-country skiing (23), long-distance cycling (9), sprint cycling (3), decathlon (2), football (10), rowing (16), running (6), swimming (1), tennis (3), triathlon (8) and weight-lifting (15). All subjects were volunteers. The athletes were all at the national or the international level. One hundred and twelve (112) of the athletes were Caucasians, four were Blacks and one was Oriental.

Paper II. A total of 261 elite athletes (177 males and 84 females; mean age, 26 years; range, 17-53 years) from Denmark, England, Italy and Sweden, competing in eleven different sport categories were included in the study. The sport categories and the number of athletes were alpine skiing (26), cross-country skiing (28), long-distance cycling (12), sprint cycling (4), football (10), rowing (90), tennis (5), swimming (28), triathlon (8), track and field (32) and weight-lifting (18). The athletes were volunteers and were competing at a national or international level. Some of the athletes (117) included in this study were also included in *Paper I*.

Papers III-V

In *Papers III-V*, thirty healthy, physically active, volunteers (15 males and 15 females) mean age 25.9, range 18-35 (males: 27.4 years, range 18-35; and females: 24.8 years, range 21-30) participated.
The participants were recruited from military personnel and students at the Göteborg University. Inclusion criteria's were: age 18-40 years, regular exercise at least twice a week for at least the past 12 months. None of the participants was active in any organised sport competitions during the year of the study.

Study protocols

Paper I

In summary, sampling was performed in a standardised way during a maximum exercise test performed under laboratory conditions. Cannulation was done in a forearm vein 30 minutes before the test and a baseline sample was taken immediately before the start of the test. The following samples were taken at the end of the test and thereafter at +15, +30, +60, +90 and +120 minutes post-exercise.

Paper II

In summary, the athletes were followed during one year, with blood samples under different training conditions during the year. One to four samples were taken at rest and one sample after a competition.
In conjunction with every sampling occasion data was collected concerning age, ethnic origin, sport, level of sport, medication and inter-current illnesses. Also at each sampling situation specific details were registered such as time since last exercise.
The resting blood samples were taken at 1-4 occasions at an ideal three months interval over a 12 months training cycle.
The post-competition blood samples were taken at one post-competition occasion. The competition was on the national or international level and the samples were, in the majority of the cases, taken within one hour after the event, mostly within 15 minutes post exercise.

Papers III-V

The study was designed as a randomised, double-blind, placebo-controlled, parallel study with three groups (n=10, 5 males and 5 females in each group): Placebo (P), GH 0.1 IU/kg/day [0.033 mg/kg/day] (GH 0.1) and GH 0.2 IU/kg/day [0.067 mg/kg/day] (GH 0.2). The mean dose of GH was 3.9 mg (11.7 IU) per day for males and 3.0 mg (9.0 IU) per day for females. Half the final dose was given the first week and the dose was reduced by 50% in case of unacceptable side effects. IGF-I values were analysed on day 0 (baseline), day 21, 28 (end of treatment), 30, 33, 42 and 84.

Paper III

Body weight, body height, body mass index (BMI) and body composition (DXA, BIS) were measured at baseline and at end of treatment (day 28).

Paper IV

The performance at baseline and at the end of treatment (day 28) was evaluated in terms of computerised electrocardiography recordings during the bicycle exercise test. Concomitant sampling and analysis of breathing gases were performed. Systolic and diastolic blood pressures were measured at rest in the supine position and sitting on the bicycle before and after the test. Systolic pressure was measured each minute through out the test.

Paper V

During the GH-treatment period, blood samples of IGFBP-4 and IGFBP-5 were drawn at day 0 (baseline), 7, 14, 21 and 28 (end of treatment). Post-treatment samples were drawn at day 30, 33, 42 and 84 from baseline.

Ethical aspects

All the studies were approved by the Ethics committee at the Göteborg University and for *Papers III-V* by the Medical Products Agency in Sweden. All of the participants were volunteers and were given oral and written information about the studies and an informed consent was signed in accordance with the Declaration of Helsinki.

Methods

Standardised maximum exercise test

The athletes were tested in different ways according to country and type of sport.

Rowing test
Rowers were tested on a Concept II rowing ergometer (Concept II, Inc. Morrisville, VT). The protocol consisted of 4 times 5 min sub-maximal stages with a one minute break between the stages. After the final sub-maximal stage subjects were allowed a 10 minute rest, after which an "all out" test (6 minutes for males and 7 for females) was performed. The sub-maximal stages corresponded roughly to 55, 65, 75 and 85% of VO2-max, respectively. The highest VO_2 attained during the test was registered as the VO_{2max}-value. Heart rate was measured continuously by a heart rate monitor (Polar Sport tester, Kempele, Finland).

Cycle test (long distance)
Long distance cyclists were tested on a biking ergometer (Ciclo Training, Politecnica 80, Padova, Italy). The protocol consisted of 4 times 5 minutes sub-maximal stages as described in the rowing test. After a 10 minutes rest a 5 minutes "all out" test was performed. HR and VO2 were measured as previously described.

Cycle test (sprint)
Sprinters were tested on a biking ergometer (Monark, Sweden). The protocol consisted of 3 times 15 seconds "all out" sprinting separated by 15 minutes rest.

Weight lifting test
The weight-lifting tests were performed on a contact carpet (Newtest, Oulu, Finland). The protocol consisted of 5 times 3 maximal jumps on the carpet: 1) squat jump, 2) counter movement jump, 3) counter movement jump with 50% body weight overload, 4) counter movement jump with 100% body weight overload, 5) counter movement jump (12). Countermovement is a concentric dynamic muscle contraction that is preceded by an eccentric stretching of the muscle with the person starting from an upright position.

Treadmill
Cross country and alpine skiers were tested on a treadmill. There was an initial period of warming of about 10 min on a biking ergometer, with a workload of 200W and mean heart rate (HR) of 140-150 beats per minute (BPM) during the last two minutes of warming up. Calibration of airflow and volume before the test was made with a syringe pump. A facial mask was used to collect the expiratory gases for analyses. The test was performed on a treadmill (Spectra) with an elevation of 3 degrees from start. The speed was adjusted to the running capacity of the athlete with a HR of 160-170/min during 3-4 minutes. There was an increase in workload each minute (speed or elevation), starting after 4 minutes. A rise in HR

with 5-10 beats/min was aimed for each new level of workload. The number of increase in workload was 8-12 until VO2 reached a plateau. Respiratory quotient (RQ) and other parameters were followed during the test.

Cycle test (for non-cyclists)
Tennis players, football players, swimmers, decathlon athletes, triathletes and two Swedish cross-country skiers performed a cycle test. The test performed on a biking ergometer (Cardionix) followed the same principle as the running test. The athletes started with a workload of 200W for 4 minutes and thereafter a rise every minute monitored by HR. Approximate rise was 25W/minute. HR monitoring and analyses were made with the same equipment and in the same way as for the running test.

Body weight, body height and BMI
Body weight was measured in the morning to the nearest 0.1 kg using a Stathmos balance. Body height was measured to the nearest 0.5 cm using a wall-mounted stadiometer.
BMI was calculated from the formula: BMI=body weight/height² (kg/m²).

Body composition analysis

DXA (Paper III)

DXA was performed using a LUNAR DPX-L scanner (Scanexport Medical, Helsingborg, Sweden). The system uses a constant potential x-ray source and a K-edge filter to achieve a congruent beam of stable dual-energy radiation. Whole body scans were performed at the scan speed suggested by the system for each subject. Body fat, lean tissue mass, total body bone mineral content and density were analysed using software version 1.33.

BIS (Papers III and IV)

Body composition was also determined using bioelectrical impedance spectroscopy (BIS). In short, reactance and resistance were determined by a Xitron 4000B Bio-Impedance Spectrum Analyzer (Xitron Technologies, San Diego, CA, USA). Resistance and reactance were measured at 50 frequencies from 5kHz to 500kHz using Xitron Technologies BIS 4000 System utility version 1.00D from which resistance at frequencies zero and infinity were predicted (155). These predictions correspond to the extracellular resistance and the TBW resistance, respectively, and are combined with body weight, height and resistivity of extracellular and intracellular water, to calculate the total body water volume (TBW) and ECW. Intracellular water (ICW) was calculated as the TBW subtracted with the ECW (ICW=TBW-ECW).

Exercise electrocardiogram

Computerised bicycle exercise electrocardiograms were performed, using previously described technology developed at Sahlgrenska University Hospital (156, 157). This includes the consecutive averaging of 10-sec intervals of the electrocardiographic signal. The ST-level is measured automatically 60 msec after the end of the QRS-complex. The ST-heart rate slope was automatically measured from the last five minutes of exercise. The computer program was developed locally and run on a standard PC. A bicycle ergometer (RE 820/830, Rodby Innovation AB Södertälje, Sweden), with automatic work load increase was used.

Oxygen uptake (Paper IV)

A Sensor Medics ergospirometer Vmax 29c (Sensor Medics Corporation Yorba Linda USA) was used for gas exchange measurements. It measures tidal volume breath by breath using a flow sensor, an anemometer based on cooling a heated wire by the gas flow. The stated accuracy is ± 3%. The mass flow sensor was calibrated with a calibration syringe.
Oxygen tension in inspired (PIO_2) and mixed expired (PEO_2) gas was analyzed using a paramagnetic oxygen sensor. Carbon dioxide tension in inspired ($PICO_2$) and mixed expired ($PECO_2$) gas was analyzed using an infrared absorption sensor; both had a response time less than 130 msec and an accuracy of ±0.02% (158, 159).
The gas analysers were calibrated with two calibrated gases containing 16% O_2 and 4.0% CO_2, and 26.0% O_2 and 0.0% CO_2. Oxygen uptake (VO_2) and minute ventilation (VE) were calculated breath by breath, and we used the mean value for the last 30 sec (160).
The VO_2 at peak exercise (VO_2 max) was determined using a bicycle with increasing load. The test was terminated at the point of subjective exhaustion. The minute ventilation, VO_2, O_2 pulse (oxygen pulse=VO_2 per heart beat), carbon dioxide production, anaerobic threshold (by V slope method (161)), and RQ were determined.
VO_2 max (peak) was regarded as being achieved if the test met two of the following criteria: 1) RQ greater than 1.0, 2) heart rate ± 10 bpm of age-predicted maximum, and 3) plateau in oxygen uptake with increasing workload. The volunteers were asked to breathe through a mouthpiece connected to a Sensor Medics Vmax 29c metabolic computer. All the volunteers breathed room air.

Laboratory methods

Papers I-V

All analyses were performed in the laboratory of Per-Arne Lundberg at the Sahlgrenska University Hospital (*total GH, GH 22kDa, IGF-I, osteocalcin, PICP, ICTP and P-III-P*) and in the laboratory of Robert Baxter, Sydney, Australia (*IGFBP-2, IGFBP-3 and ALS*). All samples were stored in a freezer, –70 degree Celsius, at each centre and then shipped to the laboratories where the analyses were to be performed. All the samples in the study where analysed at the same time.

The serum concentration of *total GH* was determined by an immunoradiometric assay (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The serum concentration of *GH 22kDa* was determined by a fluoroimmunoassay (Wallac, Oy, Turku, Finland). The serum concentration of *insulin-like growth factor I (IGF-I)* was determined by a hydrochloric acid-ethanol extraction RIA, using authentic IGF-I for labeling (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The serum concentration of *osteocalcin* was measured by a double antibody RIA (International CIS, Gif-sur Yvette, France). The serum concentration of *carboxy-terminal propeptide of type I procollagen (PICP)* was measured by a RIA (Orion Diagnostica, Espoo, Finland). The serum concentration of the *carboxy-terminal cross-linked telopeptide of type I collagen (ICTP)* was measured with a RIA (Orion Diagnostica). The serum concentration of *procollagen type III (P-III-P)* was determined by a RIA (International CIS, Gif-sur Yvette, France). The serum concentration of *IGF-binding protein 2 (IGFBP-2)* was measured using in-house RIAs and polyclonal antibodies. The serum concentration of *IGF-binding protein 3 (IGFBP-3)* was measured using in-house RIAs and polyclonal antibodies. The serum concentration of the *acid-labile subunit (ALS)* was measured using in-

house RIAs and polyclonal antibodies. The specific CVs of the analyses are presented in the original papers.

Paper V

IGF-binding protein 4 (IGFBP-4) in serum was measured by a specific RIA using recombinant human IGFBP-4 expressed in *Escherichia coli* as antigen, tracer and standard as described previously (162). IGF-binding protein 5 (IGFBP-5) in serum was measured by a specific RIA using recombinant human IGFBP-5 as antigen, tracer and standard, as described previously (163).

Statistical methods

Paper I

All values are presented as mean ± the standard deviation (SD). The statistical analyses were done with non parametric tests in the Statview software package. Comparisons within groups were done with Wilcoxon Signed Rank test and comparisons between groups were done with Mann-Whitney U test. Correlations were tested with Spearman Correlation test. Results with $P<0.05$ were regarded as significant.

Paper II

All values are presented as mean ± SD.

Statistics concerning the variability of resting samples
For each individual and each variable the mean and SD were calculated. The variability within each individual was reflected by those standard deviations. Then the mean and SD were calculated for the means and for the standard deviations.

If the mean level and the variability have normal distributions the maximum value will be limited. Under the assumption of normality, the probability that the maximum of n individuals exceeds x is $(\Phi((x - \mu)/\sigma))^n$, where Φ is the standardised normal distribution function, μ the mean of the random variable and σ the standard deviation. Those probabilities were calculated with x equal to the observed maximum value of the corresponding variable. If the probabilities are high, that is more than 0.99, there is strong evidence that the variable does not have a normal distribution and that special reasons may cause an exceptionally high mean level or variability, respectively.

Statistics concerning resting samples versus post competition samples
A difference was calculated for each athlete: the mean from the resting samples (1-4) minus the corresponding post competition value. Fisher's test for pair comparisons was then used to investigate if the differences differed from zero.

Statistics concerning correlations
Correlations with the values for the different markers at the first resting sample were tested with the use of Pitman's test (164), a non parametric test. Results with $P<0.05$ were regarded as significant. Furthermore, to investigate correlations within the individual athletes regarding *time since last exercise* a regression coefficient was calculated for each athlete with x = *time since last exercise* and y = the different markers. Fisher's test for paired comparisons was then used to test if the regression coefficient differed from zero, that is if x and y correlates.

Paper III

Values are presented as mean with SD. Data from the two growth hormone treatment groups were pooled together, as both doses of growth hormone given were considered supraphysiological. Comparisons between basal and post-treatment values in the groups were carried out with paired t-test, with Bonferroni-adjustment for multiple comparisons. Changes are expressed as mean % changes. Treatment was also assessed by unpaired t-test in treatment group (n=20) compared to placebo group (n=10), and between treated male (n=10) and female subjects (n=10), with Bonferroni-adjustment for multiple comparisons. Correlations between changes in IGF-I-levels and in body composition were assessed by linear regression analysis. Results with $P<0.05$ were regarded as significant.

Paper IV

The data are presented as the mean ± SD for background variables and the mean ± standard error (SE) for the study measures. The significance of differences between treatment groups was evaluated by analysis of variance (ANOVA) for changes from baseline to 28-days-treatment values. *Post hoc* test (Bonferroni) was applied in the case of significant group differences. Pearson´s correlation coefficients were computed to evaluate relationships between changes in IGF-I, ICW or ECW and changes in measures of exercise capacity.

Paper V

Values are presented as the mean with SD unless otherwise stated. Changes are expressed as mean percentage changes. Data from the two GH treatment groups were pooled together, as both doses of growth hormone given were considered supraphysiological. Comparisons between women and men at baseline were carried out using unpaired t-tests. Analyses of differences in the response between the GH treatment and the placebo group were analyzed by one-way ANOVA for repeated measurements with study group as the independent variable. *Post hoc* analyses were performed using the Student-Newman-Keuls test. Correlations were assessed by Pearson's correlation analysis. Furthermore, linear regression models were used to investigate the independent predictive role of different covariates. A p-value of less than 0.05 was considered significant.

Summary of main results

Paper I

The hormonal responses to the maximum exercise test both among the GH/IGF-I axis hormones and the bone markers are shown in figures 1 and 2. Males and females are displayed separately.

GH/IGF-I axis hormones

The male and female athletes showed a rather uniform pattern in the serum concentrations of the different components in the GH/IGF-I axis with a peak value at the end of the test, followed by a subsequent decrease to baseline values within 30 to 60 minutes post exercise (Fig 1).

The time from end of exercise to the peak value of both total GH and GH 22kDa was shorter in females compared to males. Thus, the mean time (calculated as the mean time point for each individuals maximum concentrations) was for total GH +10.56 ±9.95 min (males) and +0.26 ±4.24 min (females), ($P<0.001$) and for GH 22kDa +10.12 ±10.15 (males) and –1.21 ±5.87 min (females), ($P<0.001$), respectively.

Bone markers

The serum concentrations of PICP, ICTP, and P-III-P showed in both males and females a uniform pattern for each marker with a peak at end of exercise followed by a decrease below baseline values within 120 min. The osteocalcin concentrations, however, showed no significant changes during the test (Fig 2).

Total minimal and maximal values

The minimal and maximal serum concentrations with the 10-90 percentile ranges of the markers in the GH/IGF-I axis and bone markers based on all of the samples performed in connection with the maximum exercise test were analysed and are shown in table 1.

Table 1. The minimal and maximal serum concentrations with the 10-90 percentile ranges of the markers in the GH/IGF-I axis and bone markers based on totally all the samples, at any timepoint, performed in 84 male and 33 female elite athletes in connection to a maximum exercise test.

	Males			Females		
	Min	Max	Range (10-90 percentiles)	Min	Max	Range (10-90 percentiles)
Total GH (mU/l)	0,0	203.4	0.3-51.2	0.6	202.4	2.0-84.4
GH22K (mU/l)	0,0	139.9	0.27-34.4	0.1	112.1	0.5-34.6
IGF-I (µg/l)	104	568	173-416	163	774	200-429
IGFBP3 (µg/ml)	2.6	6,0	3.2-4.7	3,0	6.5	3.5-5.5
ALS (nmol/l)	138	467	181-334	123	727	178-432
IGFBP2 (ng/ml)	114	325	139-296	42	365	65-315
Osteo (µg/l)	5,0	21.8	8.03-16.1	2.5	21.6	5.4-13.5
PICP (µg/l)	91.7	425	109-329	119	376	131-272
ICTP (µg/l)	2.2	9.2	3.0-6.3	1.8	7.3	2.5-5.4
P-III-P (kU/l)	0.16	1,00	0.37-0.70	0.23	1.07	0.39-0.78

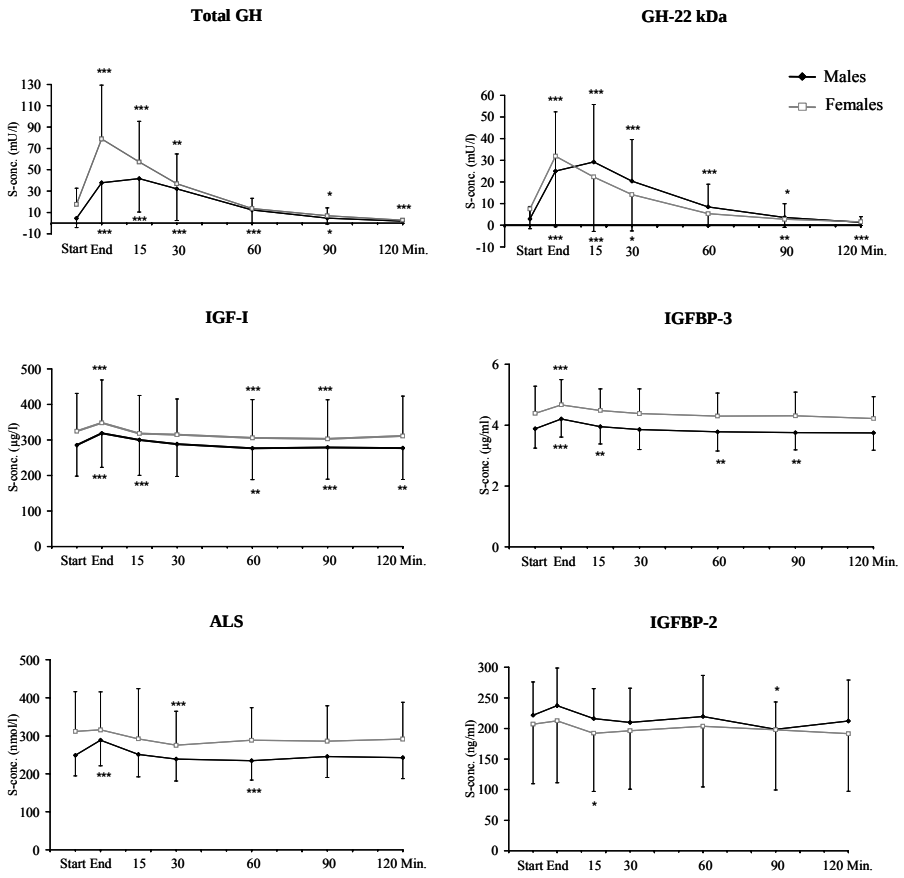

Figure 1. Serum-concentrations (mean±SD) of components in the GH/IGF-I axis in 84 male and 33 female elite athletes in connection to a maximum exercise test. *=p<0.05, **=p<0.01, ***=p<0.001 indicate changes compared to baseline.

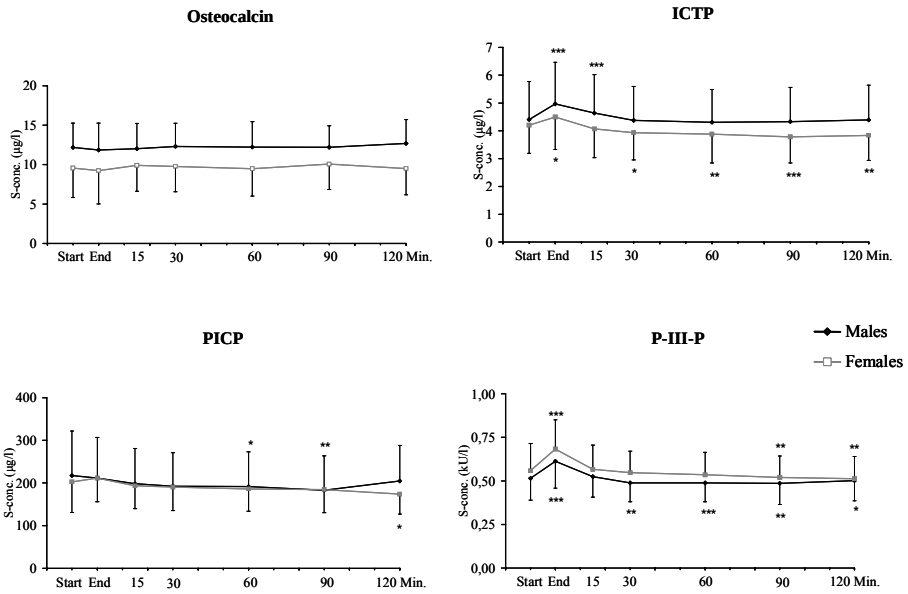

Figure 2. Serum-concentrations (mean±SD) of components in the bone markers in 84 male and 33 female elite athletes in connection to a maximum exercise test. *=p<0.05, **=p<0.01, ***=p<0.001 indicate changes compared to baseline.

Paper II

Male and female athletes were handled as separate groups. The athletes contributed with resting samples as follows: one sample (n=116), two samples (n=41), three samples (n=60) and four samples (n=44). Consequently, 145 of the 261 athletes included in the study contributed with at least two resting samplings. Post competition samples were taken from 74 of the athletes. All of the athletes that contributed with a post competition sample also contributed with at least one resting sample.

Resting values concentrations during the time course of the study

Samples were taken 1-4 times during one year, with an ideal interval of three months. In summary, signs of a high *inter*-individual variation were noticed for total GH, GH 22kDa, PICP and ICTP in males and for GH 22kDa and PICP in females. Signs for a high *intra*-individual variation were noticed for IGF-I and osteocalcin in males and for IGFBP-2, osteocalcin, PICP and ICTP in females (Table 2).

Post competition values

The post competition values including mean, min, max and ranges (10-90 percentiles) are shown in table 3. The mean time between end of competition and sampling was 13.2 ± 9.0 (range 3-44) minutes for males and for females 38.4 ± 59.4 (range 2-240) minutes.

Resting values versus post-competition values

In males, higher post competition values than resting values were seen for total GH (+1228%, $P<0.05$), GH 22kDa (+824%, $P<0.001$), IGFBP-3 (+8.3%, $P<0.001$), ALS (+11%, $P<0.01$), IGFBP-2 (+26%, $P<0.001$) and P-III-P (+11%, $P<0.01$) but lower post competition values for osteocalcin (-5.6%, $P<0.05$).
The female athletes showed higher post competition values than resting values for ALS (+34%, $P<0.01$) but lower values for osteocalcin (-15%, $P<0.001$) and ICTP (-19%, $P<0.01$).

Correlations to different variables

Age. Apart from GH 22kDa and PICP all resting samples of the markers correlated negatively with age.
Height. Positive correlations were found between height and IGF-I ($\rho=0.169$, $P<0.05$), IGFBP-2 ($\rho=0.208$, $P<0.05$), osteocalcin ($\rho=0.156$, $P<0.05$), ICTP ($\rho=0.191$, $P<0.05$) and P-III-P ($\rho=0.251$, $P<0.01$) in males and a positive correlation was found between height and GH 22kDa in females ($\rho=0.278$, $P<0.05$).
Weight. In males, a positive correlation was found between weight and the resting samples for P-III-P ($\rho=0.190$, $P<0.05$) and a negative correlation for IGFBP-3 ($\rho=-0.148$, $P<0.05$). In females a positive correlation was observed for GH 22kDa ($\rho=0.352$, $P<0.01$).
BMI. IGFBP-2 ($\rho=-0.159$, $P<0.05$) and PICP ($\rho=-0.180$, $P<0.05$) correlated negatively with BMI in males.

Table 2. Measurement of the variability in resting samples for 261 elite athletes (177 males and 84 females) competing in different sports.

	Population, inter individual						Intra individual					
	N	mean	SD	Min	Max	Prob	N	mean	SD	Min	Max	Prob
Males												
Total GH (mU/l)	76	2.9	5.2	0.0	23.9	**0.998**	28	5.2	7.3	0.0	24.6	0.890
GH22K (mU/l)	177	2.2	3.8	0.0	24.0	**1.000**	92	2.3	3.6	0.0	15.4	0.985
IGF-I (µg/l)	175	308	82	144	586	0.798	92	34.0	23.2	3.8	146.1	**1.000**
IGFBP3 (µg/ml)	177	4.2	0.6	2.9	6.5	0.979	93	0.46	0.29	0.0	1.3	0.838
ALS (nmol/l)	175	241	51	118	377	0.525	85	31.1	21.1	0.7	99.7	0.953
IGFBP2 (ng/ml)	158	215	79	41	486	0.971	80	41.0	30.1	0.7	142.7	0.971
Osteo (µg/l)	176	12.5	3.1	5.0	23.3	0.962	91	1.4	0.9	0.0	5.2	**0.999**
PICP (µg/l)	156	204	76	101	724	**1.000**	72	32.5	25.1	1.6	121.7	0.986
ICTP (µg/l)	176	4.5	1.5	2.1	10.9	**0.999**	93	0.5	0.3	0.0	1.7	0.987
P-III-P (kU/l)	176	0.56	0.13	0.27	0.94	0.455	93	0.06	0.04	0.0	0.2	0.978
Females												
Total GH (mU/l)	16	25.9	30.6	0.0	101.2	0.894	2	12.4	15.8	1.3	23.5	0.577
GH22K (mU/l)	84	8.8	10.6	0.1	52.5	**0.999**	51	5.0	5.4	0.0	21.8	0.948
IGF-I (µg/l)	84	349	103	155	683	0.952	51	40.8	28.0	2.6	121.1	0.900
IGFBP3 (µg/ml)	84	4.6	0.7	3.2	6.7	0.837	51	0.42	0.28	0.1	1.3	0.958
ALS (nmol/l)	83	297	80	169	549	0.937	50	41.0	29.6	2.8	114.3	0.719
IGFBP2 (ng/ml)	83	183	87	55	407	0.653	50	32.9	32.5	0.0	173.9	**1.000**
Osteo (µg/l)	81	9.9	3.0	2.9	17.4	0.602	46	1.6	1.7	0.3	11.2	**1.000**
PICP (µg/l)	82	179	53	91	373	**0.991**	48	26.5	23.4	0.1	113.4	**0.996**
ICTP (µg/l)	84	4.2	1.1	2.3	7.8	0.960	50	0.5	0.4	0.0	1.9	**0.997**
P-III-P (kU/l)	84	0.55	0.13	0.3	1.0	0.978	51	0.07	0.04	0.0	0.2	0.971

The mean is the mean value of the resting samples (1-4) for each athlete. The mean is caluclated from the values assessed, that is if the athlete only has 1 sample then the mean will be that value. Similarily, the SD is calculated from the values assessed and subsequently if an athlete has only 1 sample then that athlete will not have any SD. Min and Max are the mimimum and maximum mean values in any of the individual athletes. Probabilities above 0.99 are bolded and indicate strong evidence for a heavy right tail of the distribution of the variables compared to a normal distribution.

Table 3. Post competition values for 261 elite athletes (177 males and 84 females) competing in different sports.

	N	mean	SD	Min	Max	Range (10-90 percentiles)
Males						
Total GH (mU/l)	18	15.4	25.4	0.0	111.7	0.2 - 32.7
GH22K (mU/l)	53	18.2	19.3	0.0	76.8	0.6 - 48.8
IGF-I (µg/l)	53	314	93	118	546	184 - 427
IGFBP3 (µg/ml)	54	4.7	1.1	3.1	7.7	3.5 - 6.4
ALS (nmol/l)	47	250	69	151	552	172 - 329
IGFBP2 (ng/ml)	53	265	112	102	659	145 - 419
Osteo (µg/l)	52	12.7	2.9	6.6	19.7	9.0 - 16.2
PICP (µg/l)	33	216	104	96	682	121 - 290
ICTP (µg/l)	53	4.6	1.4	2.2	8.6	3.0 - 6.7
P-III-P (kU/l)	53	0.60	0.17	0.25	1.01	0.42 - 0.85
Females						
Total GH (mU/l)	5	30.3	23.0	9.7	66.7	9.7 - 66.7
GH22K (mU/l)	21	25.0	27.4	2.1	97.9	3.8 - 75.5
IGF-I (µg/l)	21	375	78	235	550	267 - 482
IGFBP3 (µg/ml)	21	5.0	0.6	4.2	6.6	4.3 - 5.8
ALS (nmol/l)	16	403	120	88	562	288 - 553
IGFBP2 (ng/ml)	21	173	93	61	470	76 - 263
Osteo (µg/l)	18	8.8	2.7	4.2	14.0	5.2 - 12.9
PICP (µg/l)	16	195	58	129	366	132 - 261
ICTP (µg/l)	21	3.7	0.8	2.5	5.4	2.9 - 4.8
P-III-P (kU/l)	21	0.57	0.12	0.40	0.83	0.42 - 0.73

Paper III-V

Side effects (Paper III-V)

All subjects completed the study, although 14/20 subjects on active treatment experienced side-effects with sweating and fluid retention, compared with 3/10 subjects in the placebo group. In two subjects on active treatment, the dose was reduced to half due to the fluid retention.

Paper III

There was no significant difference in changes in IGF-I levels or in body composition between the group with lower (0.1) *vs.* higher (0.2) dose of GH, and as both doses are considered supraphysiological, the results are presented as pooled groups.

Supraphysiological doses of GH resulted in significant increments in body weight, IGF-I levels, FFM, TBW and ECW compared to placebo. Percent changes are shown in figure 3 (IGF-I, body weight, fat free mass, body fat) and figure 4 (TBW, ECW, ICW).

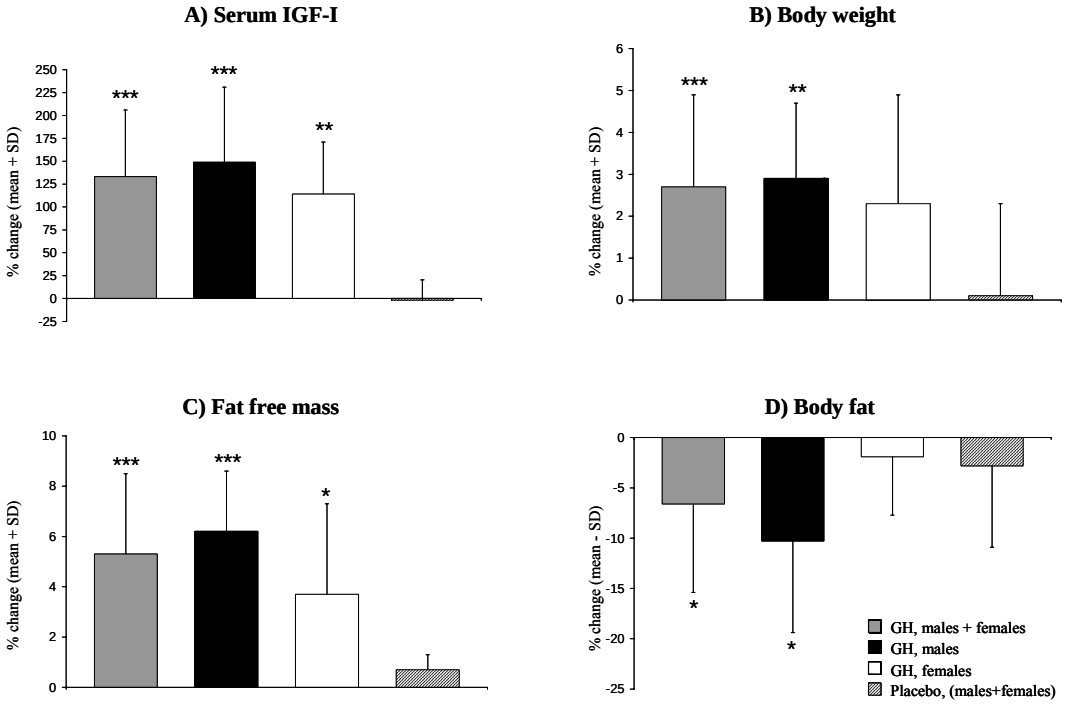

Figure 3. Percent change from baseline (mean ± SD) after one-month administration of supraphysiological doses of GH (n=20) or placebo (n=10) in 30 healthy individuals (15 males and 15 females) for serum IGF-I concentrations (A) and body composition measured by DXA: body weight, fat free mass and body fat (B, C and D). *, P<0.05; **, P<0.01; ***, P<0.001, indicates changes in mean values, baseline *vs.* one month

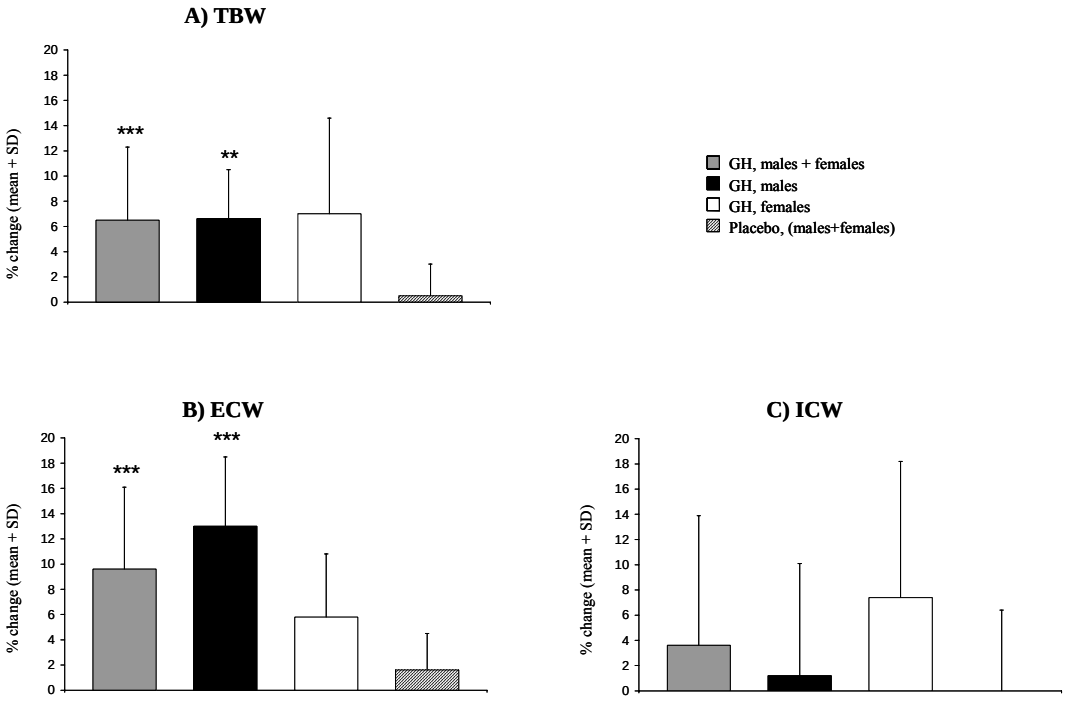

Figure 4. Percent change from baseline (mean + SD) after one-month administration of supraphysiological doses of GH (n=20) or placebo (n=10) in 30 healthy individuals (15 males and 15 females), for body water measured by BIS.
*, P<0.05; **, P<0.01; ***, P<0.001, indicates changes in mean values, baseline *vs.* one month

Pooled groups

In the pooled group on active GH treatment (n=20) IGF-I increased by 134% after one month (P<0.001). Body weight increased by 2.7% (P<0.001), with a 5.3% (P<0.001) increase in FFM by DXA, whereas body fat decreased by 6.6% (P<0.05). TBW, as measured by BIS, increased by 6.5% (P<0.001), with a sharp and significant 9.6% (P<0.001) rise in ECW but a smaller and non significant change by 3.6% detectable in the ICW.

The changes in body composition could also be detected in different body compartments. Thus, lean arm tissue (non-bone, none-fat tissue) increased by 9.2% (P<0.001), lean leg tissue increased by 6.8 % (P<0.001), whereas trunk fat decreased by 6.7% (P<0.05).

The quotient ECW/ICW changed, for the treatment group, from 0.95 at baseline to 1.00 after one month, which corresponds to a change in hydration in lean soft tissue of 0.776 vs 0.779, a difference of 0.4% (165). The measured change in FFM as estimated by DXA was 5.3% for the treatment group, that is at the most 0.4/5.3=0.075 (7.5%) of the change can be related to a shift in hydration.

Males

In the male subjects on active treatment (n=10) IGF-I concentrations increased by 149% (P<0.001). Body weight increased by 2.9% (P<0.01), body fat decreased by 10.3% (P<0.05), whereas FFM increased by 6.2% (P<0.001). TBW, as measured by BIS, increased by 6.6% (P<0.01), with a 13.0% (P<0.001) rise in ECW. The ICW showed a non-significant increase by 1.2%. Regional changes in body composition were also noted: arm lean tissue increased by 9.5% (P<0.01), leg lean tissue increased by 8.2% (P<0.01), whereas trunk fat decreased by 12.0% (P<0.001).

Females

IGF-I levels increased by 114% (P<0.01) for the female subjects on active treatment (n=10). No significant differences in basal or post treatment IGF-I levels were seen between females on oral contraceptives and females not using oral contraceptives (data not shown).

Body weight and body fat did not change significantly on supraphysiological GH treatment, but FFM increased by 3.7% (p<0.05). TBW, as measured by BIS, increased by 7.0% which was outside statistical significance after Bonferroni-adjustment for multiple comparisons (p=0.14). A nonsignificant increase of 5.8% (P=0.07) was detected in ECW and a non significant increase by 7.4% detected in ICW. Some of the regional changes in body composition were also in part noted: arm lean tissue increased by 8.6% (P=0.067 after Bonferroni adjustment), leg lean tissue increased by 4.8% (P<0.05); no changes were detectable in trunk fat.

Males vs. females

The increase in IGF-I levels was more pronounced in the male subjects compared with the females; thus, on the lower dose of growth hormone (GH 0.1) the five male subjects showed a mean increase of 149% (P<0.05), whereas the five female subjects had a non-significant increase of 76%. On the high dose (GH 0.2) the five male subjects had the same 149% increase (P<0.01) as male subjects on the lower dose and the female subjects had a similar

response with a rise in IGF-I of 157% (P<0.05). The increase in ECW was significantly higher in males compared to females (13.0% vs. 7.0%, P<0.05), while the changes in IGF-I, body weight, FFM, body fat, TBW and ICW were not.

Relationship between IGF-I and body composition

The changes in body composition in the pooled group with supraphysiological GH treatment were correlated to the changes in IGF-I levels; Pearson correlation coefficient for changes in IGF-I levels was 0.73 for FFM (P<0.01). No significant correlations were observed for body weight (Pearson correlation coefficient 0.47), body fat (-0.29), TBW (0.35), ECW (0.52) or ICW (0.06).

Paper IV

In summary, GH treatment caused an increase in weight (Table 4). This increase in weight could be explained by an increase in ECW, and neither correlated to VO_2 max nor power output. In contrast, individual changes in ICW correlated to VO_2 max ($r = 0.41$; $P < 0.05$) and power output ($r = 0.45$; $P = 0.02$), but changes in ICW were not attributable to GH treatment. Thus, ICW did not differ significantly between treatment groups (Table 4), and there was no significant relation to changes in IGF-I, whereas changes in ECW were strongly related to changes in IGF-I ($r = 0.66$; $P < 0.001$). No significant alteration was found in heart rate or blood pressure at rest. In the placebo group, IGF-I did not change during treatment. IGF-I rose by 67% in the low dose group and more markedly (135%) in the high dose GH group (Table 4).

We found no relationship between changes in IGF-I and changes in oxygen uptake or maximum achieved power output. Neither at rest nor during exercise was there a significant difference in the electrocardiographic ST level between treatment groups (Table 5). There was no significant difference between the groups in maximum heart rate response to treatment. We saw no effect of treatment on systolic blood pressure at maximum exercise, nor was there any difference between groups in O_2 pulse, carbon dioxide production, anaerobic threshold, or maximum expired volume.

Importantly, we found no effect by low or high dosages of GH on maximum achieved power output during exercise. Some individuals in each group displayed a slight increase in achieved power output (Fig 5). Furthermore, there was no effect on maximum oxygen uptake (Table 5); the individual response of weight-adjusted VO_2 is shown in figure 6. The individual gender response is illustrated in figures 5 and 6. No systematic difference in gender response was noted.

Table 4. Background variables

Variable	Occasion	Placebo (n = 10)	Low GH (n = 10)	High GH (n = 10)	*P* value (by ANOVA)
Height (m)	Baseline	174.0 ± 10.1	175.7 ± 8.3	171.9 ± 10.8	
	1 month	174.1 ± 10.0	175.7 ± 8.3	171.7 ± 10.9	
	Change	0.0 ± 0.5	0.0 ± 0.6	–0.2 ± 0.7	0.6605
Weight (kg)	Baseline	70.7 ± 17.1	72.5 ± 13.7	67.2 ± 10.5	
	1 month	71.0 ± 18.2	74.0 ± 14.8	69.0 ± 10.9	
	Change	0.3 ± 1.2	1.5 ± 1.4	1.8 ± 1.1[1]	0.0279
Heart rate (beats/min)	Baseline	72.2 ± 9.9	67.5 ± 11.6	72.9 ± 6.9	
	1 month	70.4 ± 8.2	68.1 ± 15.0	75.7 ± 9.3	
	Change	–1.8 ± 16.1	0.6 ± 10.1	2.8 ± 9.1	0.7029
SBP (mm Hg)	Baseline	122.8 ± 13.8	120.2 ± 17.2	116.8 ± 15.2	
	1 month	120.0 ± 7.2	118.4 ± 14.4	114.0 ± 13.0	
	Change	–2.8 ± 10.6	–1.8 ± 12.1	–2.8 ± 13.3	0.9773
DBP (mm Hg)	Baseline	70.8 ± 7.6	67.4 ± 9.9	73.0 ± 11.1	
	1 month	73.7 ± 9.4	73.4 ± 12.8	67.2 ± 11.2	
	Change	2.9 ± 8.8	6.0 ± 11.4	–5.8 ± 12.0	0.0573
IGF-I (μg/liter)	Baseline	301 ± 69	316 ± 103	304 ± 96	
	1 month	296 ± 75	678 ± 267	769 ± 218	
	Change	–5 ± 64	363 ± 220[1]	464 ± 152[1]	<0.0001
ECW (kg)	Baseline	18.5 ± 4.1	19.3 ± 4.9	18.0 ± 3.4	
	1 month	18.8 ± 4.5	20.7 ± 5.6	20.3 ± 4.2	
	Change	0.3 ± 0.6	1.4 ± 1.5	2.3 ± 1.1[1]	0.0054
ICW (kg)	Baseline	19.4 ± 5.3	19.6 ± 5.4	19.9 ± 5.1	
	1 month	19.4 ± 4.9	20.2 ± 6.0	20.7 ± 4.1	
	Change	0.0 ± 1.2	0.6 ± 1.8	0.8 ± 2.1	0.6556

Values are the mean ± SD. DBP, Diastolic blood pressure; SBP, systolic blood pressure.

[1] Significantly different from placebo.

Table 5 Measures of exercise reaction

Variable	Occasion	Placebo (n = 10)	Low GH (n = 10)	High GH (n = 10)	*P* value (by ANOVA)
STV5 rest (μV)	Baseline	84 ± 19	77 ± 16	91 ± 15	
	1 month	79 ± 19	85 ± 26	97 ± 15	
	Change	–5 ± 10	8 ± 27	6 ± 10	0.8547
STV5 max (μV)	Baseline	154 ± 38	143 ± 62	181 ± 58	
	1 month	122 ± 47	271 ± 145	277 ± 78	
	Change	–33 ± 34	129 ± 101	96 ± 32	0.1915
Power output (watts) max	Baseline	284 ± 22	272 ± 16	270 ± 22	
	1 month	292 ± 20	282 ± 19	282 ± 22	
	Change	8 ± 3	10 ± 5	12 ± 5	0.8392
SBP max (mm Hg)	Baseline	206 ± 8	206 ± 10	200 ± 11	
	1 month	206 ± 6	207 ± 8	206 ± 10	
	Change	0 ± 4	1 ± 4	6 ± 5	0.5925
Heart rate max (beats/min)	Baseline	185 ± 3	180 ± 4	178 ± 4	
	1 month	184 ± 3	184 ± 4	181 ± 4	
	Change	–1 ± 2	4 ± 2	3 ± 1	0.0844
VO_2 max (liter/min)	Baseline	3.22 ± 0.30	3.12 ± 0.25	3.10 ± 0.33	
	1 month	3.20 ± 0.27	3.19 ± 0.27	3.12 ± 0.27	
	Change	–0.03 ± 0.07	0.07 ± 0.08	0.02 ± 0.12	0.7586
VO_2 max (ml/kg·min)	Baseline	45.2 ± 1.6	42.8 ± 1.6	44.8 ± 3.4	
	1 month	45.2 ± 2.1	42.8 ± 1.6	44.8 ± 2.2	
	Change	–0.1 ± 1.1	0.0 ± 1.6	0.0 ± 1.0	0.9984
O_2 pulse max (ml/beat)	Baseline	17.5 ± 1.5	17.4 ± 1.4	16.8 ± 1.7	
	1 month	17.4 ± 1.3	17.3 ± 1.4	16.6 ± 1.3	
	Change	–0.0 ± 0.3	–0.1 ± 0.5	–0.17 ± 0.7	0.9834
VCO_2 max (liter/min)	Baseline	3.54 ± 0.31	3.40 ± 0.26	3.42 ± 0.36	
	1 month	3.50 ± 0.25	3.47 ± 0.28	3.36 ± 0.27	
	Change	–0.05 ± 0.11	0.06 ± 0.10	–0.06 ± 0.15	0.7307
VO_2 at AT (liter/min)	Baseline	1.49 ± 0.12	1.53 ± 0.18	1.68 ± 0.22	
	1 month	1.51 ± 0.12	1.76 ± 0.24	1.59 ± 0.11	
	Change	0.17 ± 0.20	0.23 ± 0.12	–0.09 ± 0.19	0.2310
VE max (liter/min)	Baseline	116 ± 12	114 ± 6	119 ± 12	
	1 month	123 ± 9	119 ± 8	116 ± 11	
	Change	7 ± 5	6 ± 4	–3 ± 4	0.2776

Values are the mean ± SE. AT, Anaerobic threshold; SBP, systolic blood pressure; VE, volume expired (minute ventilation).

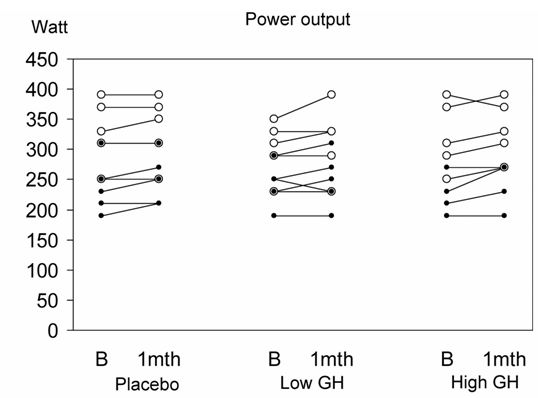

Figure 5. Maximum power output at baseline (B) and after one month of treatment (1 mth) in the placebo group and in the low dose GH group (0.033 mg/kg·d or 0.1 IU/kg·d) and the high dose GH group (0.067 mg/kg·d or 0.2 IU/kg·d). °, Males; •, females.

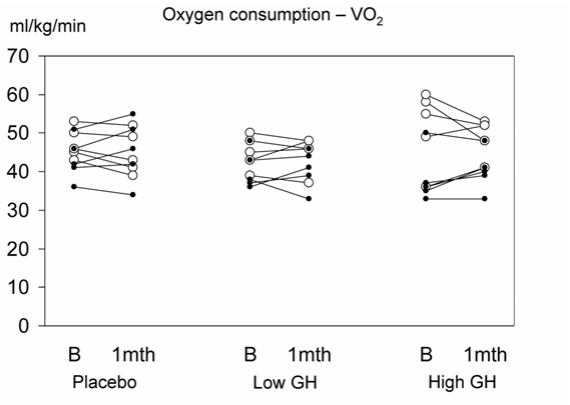

Figure 6. VO2 max at baseline (B) and after one month of treatment (1 mth) in the same three study groups as those described in Figure 5 (for symbols, see Figure. 5).

Paper V

Effect of age and gender on IGFBP-4 and IGFBP-5

The baseline levels of IGFBP-4 were higher, while those of IGFBP-5 were lower in women than in men (Fig 7). IGFBP-5 levels were positively correlated to age ($r=0.433$, $P<0.05$) and age was a positive predictor of IGFBP-5 levels also after adjustment for gender ($\beta=0.325$, $P<0.05$). No correlation, however, was found between age and baseline IGFBP-4 levels.

Effect of GH treatment

As in *Paper III*, the GH 0.1 and GH 0.2 groups are pooled as both are regarded supraphysiological. GH treatment increased the IGFBP-4, IGFBP-5 and IGF-I levels *vs.* placebo at all the time points investigated during treatment (Fig 8).
The effect on serum IGFBP-5 and IGF-I levels *vs.* placebo remained for 2 days in the post-treatment period for IGFBP-5. No effect was seen on serum IGFBP-4 levels after the end of treatment. So, for both IGFBP-4 and IGFBP-5, the GH-effect could be detected after two weeks of treatment with a maximum effect seen after 3-4 weeks' treatment. IGF-I displayed a similar pattern (Fig 8).

Serum IGF-I as a mediator of the stimulatory effect of GH on serum levels of IGFBP-4 and IGFBP-5

Univariate correlations demonstrated that the percentage changes from baseline values for the levels of IGFBP-4 and IGFBP-5 at day 28 were well correlated to the percentage change in IGF-I levels (IGFBP-4; $r=0.508$, $P<0.01$: IGFBP-5; $r=0.499$, $P<0.01$).

To investigate whether the effects of GH treatment on the percentage change in IGFBP-4 and/or IGFBP-5 were mediated via affected serum IGF-I levels, a linear regression model including GH treatment, age and gender with or without the percentage change in IGF-I levels as covariates was used. GH treatment predicted the change in both IGFBP-4 and IGFBP-5, in regression models not including the percentage change in IGF-I. However, GH treatment was not able to significantly predict the IGFBP-4 and IGFBP-5 changes, when IGF-I changes were included as covariates in the regression model (Table 6).

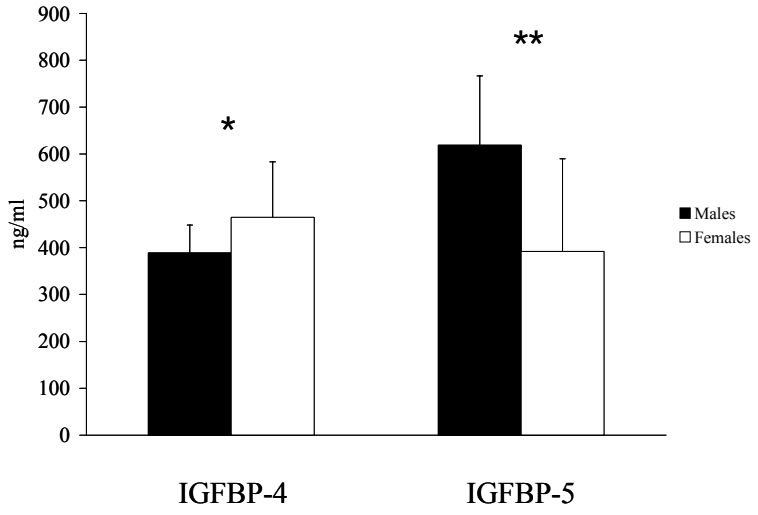

Figure 7. Baseline serum levels for IGFBP-4 and IGFBP-5 in men and women. *, p<0.05 and **, p<0.01, indicate differences between men vs women

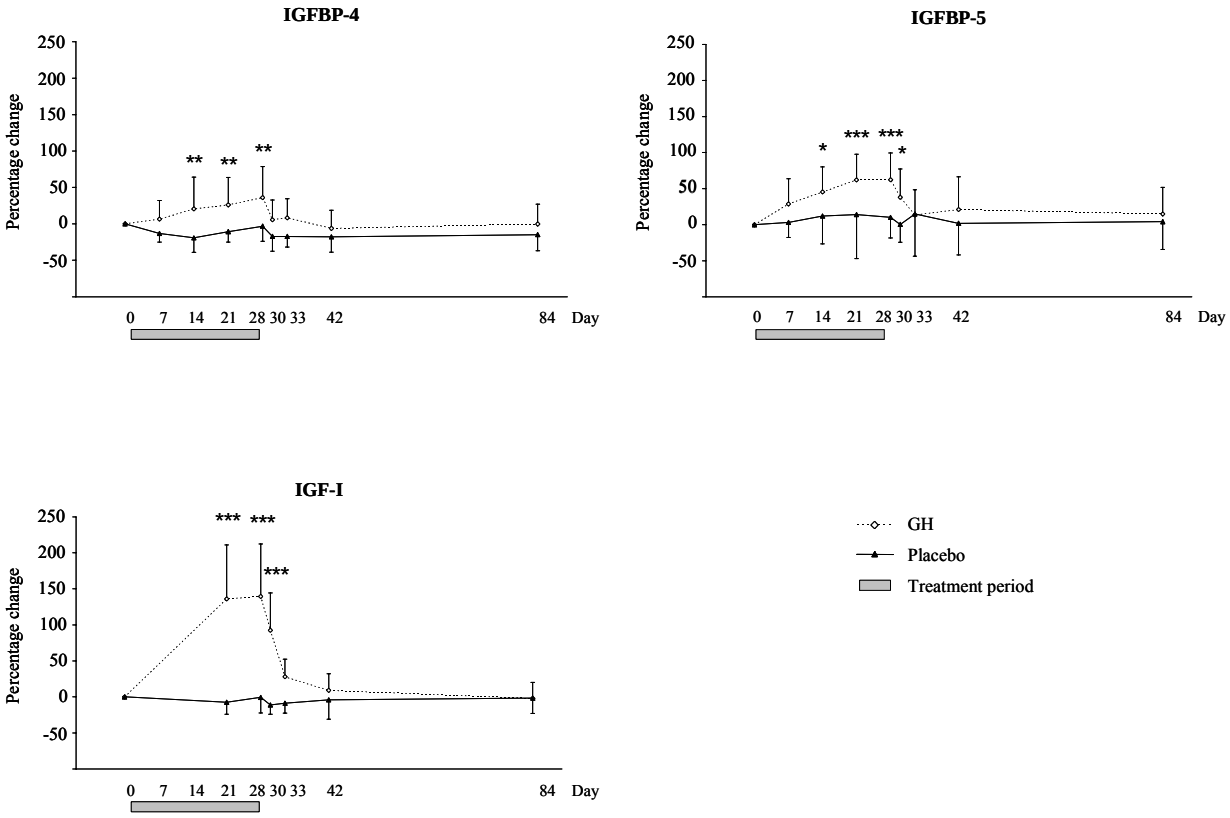

Figure 8. Percentage change in IGFBP-4, IGFBP-5 and IGF-I levels at different time points during and post treatment with GH (pooled group) or placebo. *P<0.05; **P<0.01; ***P<0.001, indicate difference between the GH and the placebo group. Statistics analysed by one-way ANOVA for repeated measurements with study group as the independent variable. Post hoc analyses were performed using the Student-Newman-Keuls test.

Table 6. The predictive role of GH treatment for changes in IGFBP-4 and IGFBP-5 levels (percentage change from baseline). Linear regression including GH treatment (no=0, yes=1), age and gender, with or without percentage change in IGF-I as covariates.

	Adjusted for gender and age		Adjusted for gender, age and percentage change in IGF-I	
	β	P	β	P
IGFBP-4	0.46	0.014	0.14	0.62
IGFBP-5	0.56	0.001	0.34	0.17

General discussion

Paper I

Maximum exercise test

The study comprising almost 120 elite athletes at national or international level, describes the hormonal profiles in the GH/IGF-I axis and among bone markers in response to a maximum exercise test. To summarise, the hormonal response in the GH/IGF-I axis displayed a fairly uniform pattern including a peak value directly post-exercise and a successive decrease to baseline values within 30-60 minutes post-exercise, although this pattern was less obvious among the bone markers. Interestingly, the peak value for the total GH and GH 22kDa concentrations occurred significantly earlier in the female athletes compared with the males. The reference ranges for components in the GH/IGF-I axis and among bone markers in each gender presented in this paper could be of considerable use in the future development of a test for the detection of GH abuse in sports.

The increase in GH levels in the early post-exercise phase of the maximum exercise test is in accordance with previous findings (38, 39, 166, 167). The neuroendocrine mechanisms of exercise-induced GH release are still incompletely understood. As a result, it is not yet obvious whether the GH increase is due to increased GHRH stimulation or decreased somatostatin stimulation, or a combination of these (28). The exact roles of stimulating α-adrenergic, inhibiting $ß_2$-adrenergic and stimulating cholinergic pathways on the secretion of somatostatin and GHRH remain to be determined, although it seems that cholinergic agents stimulate GH secretion, via suppression of somatostatin release (49, 50).

We noted that the GH peak took place within 15 minutes post-exercise. Previous studies have shown that the GH release induced by exercise is delayed until 15 minutes into exercise (168, 169) and that it peaks by the end of exercise (168, 169) or shortly afterwards (170). Furthermore, the GH release is related to the intensity of the exercise (18-20), thereby rendering a higher GH release from high-intensity anaerobic work compared with low-intensity aerobic work, even if they are equal in duration and total work effort (171). Finally, training in itself increases GH secretion (40).

The exercise-induced GH increase was noted in both genders, although the maximum GH peak value was seen directly post-exercise in the females, compared with approximately 15 minutes post-exercise among the males. This is in accordance with a previous study involving constant-load aerobic exercise (172), where females attained their GH peak significantly earlier than men (24 *vs*. 32 minutes after start of exercise). The present study shows that this phenomenon is valid also in anaerobic conditions among elite athletes. Previous studies of elite athletes have revealed no major gender differences in VO2-max-induced GH release, although some authors have noted that females have higher GH levels pre-exercise and that these levels do not return to baseline within one hour, although the actual response pattern did not differ (168, 172, 173).

The increase in serum total IGF-I concentrations in the direct post-exercise period, with a subsequent decrease to baseline levels, was noted in both genders and has been observed in

other trials (174, 175). Furthermore, we noted the same pattern of response to exercise by the remaining components in the 150kDa ternary complex; IGFBP-3 and ALS. The maximum response was observed directly after exercise, in comparison with the maximum GH response, which was sustained until 15-30 minutes post-exercise. The exact mechanism for the parallel changes in IGF-I, IGFBP-3 and ALS in response to exercise is not known, but reasonable exercise, with a subsequent GH increase and possibly under the influence of proteases, causes changes such as the dissociation of the ternary complex according to the theory presented by Wallace *et al.* (100).

In both genders, the bone and collagen markers P-III-P and ICTP, like the components of the ternary complex described above, displayed a peak level directly post-exercise, followed by a subsequent decrease to baseline levels within two hours. The increase in osteocalcin levels was much less obvious, with only a minor increase at the two-hour value. Previous studies have shown divergent results for bone markers in response to exercise (107-111). Long-term exercise training from one to 18 months has shown increases in the concentrations of osteocalcin and other markers of bone formation, with a slight decline in the levels during the first four weeks (176-178). The mechanism for the direct post-exercise increase in P-III-P and ICTP concentrations is probably multifactorial. Metabolic acidosis with high lactate levels is known to stimulate osteoclastic formation and thus increase ICTP concentrations (179) and, furthermore, long-term training, including mechanical factors from the exercise performance itself, causing micro-damage to bone and muscles, with leakage to the blood, might also contribute.

The present study also presents the actual reference ranges for each marker in the GH/IGF-I axis and among the bone markers during different time courses i.e. baseline, end of exercise and at +30 min post-exercise. Furthermore, we also present the "all-time high and low", including the 10-90 percentile range values recorded at any time point, in connection with the maximum test, thereby giving us the overall description of the total ranges in connection with the test, including baseline. The variables in the ternary complex and most of the bone markers correlated negatively with age and this will have implications when attempting definitely to define reference ranges. Even though some variables in the ternary complex had some correlation to weight and BMI at some time points this does not appear to have any practical consequences.

Most of the markers had a maximum value at about the end of exercise in the maximum exercise test, with a gradual decrease to baseline values within 30-60 minutes. We therefore, recommend that, in a future GH-doping control situation, blood samples should be taken not earlier than 30 minutes after the end of exercise to avoid confusion with this physiological post-exercise increase.

We cannot exclude the possibility that some of the changes might be due to the effect of hemoconcentration in response to the exercise itself, with a decrease in plasma volume (PV). In this study the PV changes were estimated by measuring serum albumin concentrations *(data not shown)*, which revealed a PV decrease estimated at about 20%, which is roughly in accordance with previous studies, where PV reductions from 4-15% were noted after moderate submaximum exercise and after total exhaustion, respectively (180).

The reference ranges we have presented for each marker are described according to the actual laboratory results that were obtained on the actual sampling occasion, regardless of whether

or not there was a significant hemoconcentration and in that sense they are comparable to the results obtained in the sampling situation of a doping test.

Paper II

The main message from this longitudinal study of elite athletes is that we have assessed the markers of the GH/IGF-I axis and bone markers that show strong evidence of high *inter*- or *intra*-individual variation. This is based on analyses of resting samples with the emphasis on the right tail of the distribution in relation to a normal distribution. The results may be of value in the work of developing a method for detecting GH doping, as the markers with high variability should be used with caution in a test for GH doping.

This study also provides ranges for markers of the GH/IGF-I axis and bone markers that could be used in the development of a GH-doping test, both for out-of-competition tests (resting values) and for normal doping tests in connection with a competition (post-competition values).

Even though it was performed on a large number (261) of elite athletes in four countries, the homogeneity of the practical performance of the protocol was high.

Resting values

In males, resting values of total GH, GH 22kDa, PICP and ICTP showed strong evidence for a high *inter*-individual variation, while IGF-I and osteocalcin values showed evidence of high *intra*-individual variation. In females, evidence of high *inter*-individual variation was noticed for GH 22 kDa and PICP and high *intra*-individual variation for IGFBP-2, osteocalcin, PICP and ICTP.

The high variability of GH is not surprising, as the GH secretion is influenced by many factors and this is one reason for the problems associated with developing a test method for GH doping (28).

Nor is the high variability of some of the bone markers surprising (121, 181, 182). The reason for this appears to be multifactorial. An osteocalcin assay is sensitive to lipaemia and hemolysis, both of which reduce the measured concentrations (182), although in our study samples with macroscopically apparent lipaemia and hemolysis were excluded. Moreover, both the renal and hepatic clearance of the bone markers might affect the concentrations. There is also a seasonal variation, giving higher values in osteocalcin, for example, in the winter (121). After fractures the bone markers increase and remain high for six months (183, 184). Furthermore, the bone markers have been reported to be higher in the luteal phase of the menstrual cycle, although we did not observe this in our study (185). However, the concentrations of IGF-I, ICTP and P-III-P have been shown to be stable in male and female athletes during a 6-month period (186).

The results with high variability (*inter*- or *intra*-individually), provide strong evidence that some markers have a skewed distribution with a heavy right tail in relation to the normal distribution and that special factors may cause an exceptionally mean level or variability respectively. There may be natural explanations for extreme values of these markers and we

have to be careful if we use such markers to identify extreme individuals in a doping test. It is possible to discuss whether a high *intra*-individual variability within well- defined cut-off levels might initially pose a problem in a longitudinal situation with several measurements for each individual. However, a doping test is a cross-sectional situation and a high *intra*-individual variability is not necessarily a huge problem, as long as the variable is *inter*-individually stable and the cut of levels for the variable are well defined within the population. There is, however, an ongoing discussion about introducing medical passports, 'blood passes', and in such a situation, where repeated tests are included, high *intra*-individual variability will be a problem.

Resting values versus post competition values

In males, significantly higher post-competition values were seen for most of the markers (total GH, GH 22kDa, IGFBP-3, ALS, IGFBP-2 and P-III-P) but with lower post-competition values for osteocalcin. In females, significantly higher post-competition values were noted for ALS and significantly lower values for osteocalcin and ICTP. This gender difference could be explained by the shorter time from the end of competition to sampling in males compared with females (13 *vs*. 38 minutes respectively).

The results are mostly in accordance with the results in *Paper I*, where the athletes were tested in connection with a maximum exercise test. One possible explanation for the somewhat different results might be that a pre-test (baseline) was used as the resting sample in the maximum exercise test, but the resting samples in this study are the mean values for all the resting samples (1-4 in each athlete) and were not taken in connection with the competition. Furthermore, we felt that the degree of exhaustion appeared to be lower among some of the athletes in the post-competition phase compared with the phase after the maximum exercise, as all the athletes were pushed to their maximum physical performance capacity in the maximum exercise test. This is not necessarily the case in the post-competition tests that were taken from a wide spectrum of sports ranging from extreme endurance to extremely explosive types of sport. Consequently, there might not be any differences at all between resting and post-competition samples for some sports and pronounced differences for other sports, thereby producing a far more complex situation than in a maximum exercise test.

Paper III-V

Side-effects

As many as 70% of the participants receiving active treatment with GH complained of muscle pain and/or swollen hands and feet, which caused a reduction in the dose in two participants. Five subjects complained of increased sweating, which was probably secondary to the GH treatment.

The fluid retention symptoms, caused by the antinatriuretic effect of GH, are naturally secondary to the high dose that was given with a short run-in period. These problems are now seldom seen in the treatment of GHD patients, where the GH dose is lower and, furthermore, slowly and individually titrated. The finding that fluid-retention symptoms occurred in the majority of individuals on active GH treatment might indicate a limit in the use of GH as a doping agent. Finally, the risk of long-lasting acromegalic signs and symptoms following the long-term use of GH as a doping agent must be considered.

IGF-I

The increase in IGF-I concentration to the acromegalic range after only four weeks of supraphysiologic GH doses underlines the potential risks of acromegaly in cases of long-term GH doping. This fear of long-term acromegalic symptoms is often expressed among athletes and might in fact have a preventive effect when it comes to the use of GH as a doping agent. The increase in IGF-I was seen in both genders, but was more pronounced in men. This is also in line with previous reported findings including those from the GH-2000 project, where it was concluded that male subjects are more responsive than females to exogenous GH (187-189). These differences could perhaps be explained by a higher 'susceptibility' to GH in male subjects compared with females. This higher 'GH susceptibility' in male subjects might be secondary to the permissive role of androgens in GH-stimulated IGF-I production (190, 191). In addition, oral oestrogens have a known inhibitory effect on hepatic IGF-I production (192). Furthermore, normal GH production in women exceeds that in men by 30%, which might further explain the differences in IGF-I concentrations, as the GH doses in the study were based on kg body weight in both genders, giving a relatively higher dose in men. This choice of dosage was in accordance with the practice in the treatment of GHD patients at the time of the initiation of the study. Finally, the lack of difference in IGF-I levels between the two supraphysiological GH doses (0.1 and 0.2) appears to indicate that a plateau effect is reached with the lower dose and that a further increase in the GH dose has no additional effect on IGF-I concentrations.

We found no differences in IGF-I concentrations between women using oral contraceptives (OCs) and those not using OCs at baseline or after treatment. This contrasts to the findings in a previous two-week study, in which the effects of GH on IGF-I concentrations were smaller in female subjects using OCs than in those not using OCs (188). However, the total GH doses, duration of the studies, OC types and timing of GH treatment *vs.* the phase in OC use differ between studies.

Paper III

To summarise, this study clearly shows that supraphysiological doses of GH given for only one month cause dramatic changes in both IGF-I levels and body composition in healthy active individuals. To our knowledge, this is the first placebo-controlled trial to show the effects of supraphysiological GH doses on body composition and IGF-I levels in physically active, healthy individuals of both genders. The GH doses, equal to doses used in GH doping situations, gave rise to IGF-I levels in the acromegalic range, a 6.6% decrease in body fat and a 9.6% increase in ECW. As no significant increase in ICW was found, the observed increase in FFM of 5.3% is attributed to the ECW increase, thus indicating limited anabolic effects by the supraphysiological GH doses. The role of GH as an effective anabolic doping agent is questionable. Changes were noted in both genders, even though they were more prominent in the men. Fluid-retention symptoms occurred in a majority of individuals on active GH treatment, which might further indicate a limit in the use of GH as a single doping agent.

Body composition

There are few previous studies of the effect of supraphysiological GH doses on body composition and physical training. Moreover, these studies used somewhat lower GH doses, other body composition methods and included exclusively male subjects. Crist *et al*. found

increased fat free weight and reduced body fat as determined by underwater weighting in eight well-trained adults when given 2.67 mg of GH 3 days/week for six weeks (130). In a 12-week study by Yarasheski *et al*., which included 16 men, the subjects were randomly assigned to either a resistance-training group with GH treatment (40μg/kg/day) or to a group with placebo and resistance training (128). FFM (measured by hydrodensiometry) and TBW (determined by using the dilution of deuterium oxide in body fluid) increased in both groups but significantly more among the GH recipients. Deyssig *et al*. found no change in body weight or body fat (determined by skinfold thickness) in 22 healthy, nonobese male subjects in a double-blind controlled study, receiving GH (30μg/kg/day) or placebo for six weeks, but a remarkable increase in IGF-I was noted (129).

Body composition methods

In this study, we used whole body DXA and BIS for the assessment of body composition.Whole body DXA has been used extensively during the last decade for body composition purposes because it is fast, precise and fairly accurate in the direct assessment of body fat and FFM (BMC + lean tissue) (193, 194). BIS is an indirect convenient method for estimating body water compartments. At low frequencies the cell membranes are impermeable to an electrical current, which means that the resistance is more directly correlated to the ECW. The resistance at higher frequencies is correlated to both intra- and extracellular volume, which make up TBW (155). BIS has been validated as a convenient method for indirect determination of ECW and TBW in healthy individuals, by simultaneous determination of ECW and TBW using dilution techniques, predominantly bromide space and deuterium oxide respectively (195). BIS has also been tested in situations with disturbed water balance, such as experimentally induced hyperhydration and dehydration (155), as well as in different clinical settings, such as on surgical patients (196), in chronic obstructive pulmonary disease (197) and in HIV infection (198). The use of BIS as a reliable means of measuring changes in ECW secondary to growth hormone replacement therapy in growth hormone-deficient adults has also been demonstrated (199).

General changes in body composition

The changes in body composition with an increase in body weight, FFM, TBW and ECW combined with a decrease in body fat, are all changes that are consistent with the well-known effects of GH. These effects have been well described in GH-deficient patients after several months of GH treatment, with mean GH doses of 0.3-0.5 mg/day (122). This study shows that in healthy, non-GH-deficient adults taking supraphysiological GH doses, these changes in body composition can already be seen after one month of treatment.

The supraphysiological GH doses caused profound fluid-retention effects. There was a mean increase in TBW and ECW of 7-10% after one month compared with baseline; the ECW increase was significantly higher in men. No significant changes in ICW could be detected.

It is possible to speculate about whether the observed change in FFM as estimated by DXA is due to shifts in body fluids. However, the ECW/ICW quotient change during the treatment corresponds to a change in hydration in lean soft tissue of only 0.4% (165), whereas the measured change in FFM was 5.3%. We therefore conclude that the observed change in FFM is real.

The effect of GH on sodium and water retention has been known for a long time and several mechanisms have been proposed to explain it (200). Studies by Jens Möller *et al.* have shown that two weeks of supraphysiological GH doses administered to healthy men resulted in an increase in ECW, but not in plasma volume or in ICW, with a concomitant decrease in atrial natriuretic peptide (ANP) (83). Furthermore, lack of dose dependence or a possible plateau effect with increasing GH doses has been observed (201), and also a decrease in 24-h urinary sodium excretion, indicating a the GH-induced activation of the renin-angiotensin-aldosterone system (82). Furthermore, in acromegalic patients there is an increase in both ECW and PV, which normalises after the successful treatment of acromegalic disease (202).

The complex theory of fluid retention holds that both GH and IGF-I stimulate sodium retention at the tubular level, via stimulation of Na^+K^+ATPase activity (81), suggesting that IGF-I levels play a major part. Furthermore, a hypothesis has been put forward that a major effect of GH on body fluid homeostasis is mediated via positive inotropic action of IGF-I on the heart, leading to a decrease in ANP release. This in turn results in the stimulation of the RAAS (203). Finally, the role of nitric oxide as a mediator of IGF-I function in the liver has to be considered, as well as the effect of insulin and prostaglandins (203).

The observed increase in IGF-I concentration then explains the effect of supraphysiological GH doses on TBW and ECW and the observed fluid-retention symptoms. These effects appear to be mostly extracellular, as no change in ICW was noted with the methods used here. The lack of significant change in ICW is in accordance with the findings of Möller *et al.*, who stated that that the fluid volume retained by GH is distributed mainly in the extracellullar space (201). Although, as stated previously, the ICW is not measured directly, we are confident of the accuracy of the ICW findings. This is because the BIS-method used has been validated as a reliable method for measuring TBW, ECW and ICW, in dehydrated, normal and hyperhydrated states (155).

Supraphysiological doses of GH increase FFM but, with no detectable increase in ICW, this increase is most probably secondary to ECW increase. This implies that supraphysiological doses of GH have no distinct anabolic effects on muscle mass. The observed increase in regional lean arm and leg mass of 7-9% by DXA is interpreted as mostly an expansion of ECW.

This is contradictory to the otherwise well-described anabolic actions of GH in directly stimulating protein synthesis; this effect is suggested to be perhaps at least as powerful as that of testosterone. The protein synthesis is most probably mediated through the mobilisation of amino acid transporters in the cell membranes, as with insulin and glucose transporters. GH also has a 'partitioning action', diverting nutrients to protein synthesis. GH also stimulates IGF-I gene expression in all tissues, especially in the liver, and the IGF-I that is produced directly increases protein synthesis. Finally, the anabolic effect of GH is potentiated by the insulin effect of inhibiting protein degradation. In this manner GH, IGF-I and insulin together work in an anabolic direction (7).

However, studies investigating the effects of supraphysiological GH doses on whole body protein synthesis have produced conflicting results. Yarasheski *et al*. demonstrated no increase in muscle protein synthesis in experienced weight-lifters. Measurements were taken in seven young, experienced weight-lifters before and after 14 days of GH administration (0.040 mg/kg/day) by using a constant intravenous infusion of [13C]leucine (131). On the other hand, Healy *et al.* showed, in a randomised placebo-controlled study of 11 endurance-

trained male athletes, that GH administration (six subjects received GH 0.067 mg/kg/day and five placebo) for four weeks has a net anabolic effect on whole body protein metabolism at rest and after exercise, measured by whole body leucine turnover, using a five-hour infusion of [13C]leucine (204).

In addition, body fat was influenced by the high GH doses. Body fat decreased by about 6% after one month, mainly in men, and the decrease was especially noticeable in trunk fat. These findings are in agreement with several previous studies in which GH treatment reduced fat mass in GHD patients (122). However, the exact mechanisms of the lipolysis are complex and not fully understood. The lipolytic effect of GH is described in a study by Niels Möller *et al*., which showed an increase in serum FFA and ketone bodies after a single GH dose; a twofold increase in baseline values and peak values were observed after two to three hours (205). Niels Möller *et al*. have also suggested that adipose tissue is a prime target for GH (206). The fat mass reduction thus appears to be secondary to the lipolytic action and, furthermore, this lipolysis preferably occurs in the upper body. GH may also initiate hormone-sensitive lipase activity in adipose tissue and thereby reduce FFA deposits in adipose tissue (206).

GH doping and body composition

Among GH abusers at gyms, GH doses in the range of 6-9 mg/day, three to four days a week are used, at least when taken in a single-dose regimen. However, in combination with AAS, GH doses of 1-2 mg/day are taken. GH is often administered in six- to 12-week cycles, but continuous use for six to 12 months is also seen (7, 9, 207-209). The doses used in this study (0.033 mg/kg/day and 0.067 mg/kg/day) are comparable to the doses used in a doping situation. It therefore appears that GH in these supraphysiological ranges mostly exerts anti-natriuretic effects causing fluid retention but no detectable anabolic effects. The reason for this is not obvious, but it is possible to speculate that one month's treatment is too short to demonstrate anabolic effects of GH (78, 210), although this time period seems long enough according to knowledge from GH treatment in GHD patients (211). It is also possible to speculate that the given doses are actually too low to induce anabolic effects in four weeks, although the otherwise pronounced effects observed, in the case of IGF-I and body composition indicate a truly supraphysiological dose.

The fact that most abusers probably use GH in combination with AAS and that this combination possibly has an anabolic effect, due to synergistic actions between the two agents, should also be taken into account. Athletes find the noted water-retention symptoms negative and they appear to hamper their ability to exercise, thereby limiting the potential of GH as a powerful doping agent.

A reduction in fat mass, especially centrally located fat mass, is a desirable GH effect in a doping situation, especially in body-building competitions where a minimum of visible body fat is rewarded.

Paper IV

Endurance exercise capacity

In this study, short-term (one month), supraphysiological GH administration did not cause any increase in maximum power output or maximum oxygen uptake in well-trained individuals.

There is no published evidence that GH leads to an improvement in aerobic exercise capacity in non-GH-deficient subjects (212). On the contrary, Lange *et al.* (213) suggested that a single relevant GH dose in combination with bicycling exaggerate plasma lactate and may be associated with a reduction in aerobic exercise capacity. A study of acute GH administration before exercise demonstrated no effect on total work, caloric expenditure, blood lactate, or perceived exertion, but lower exercise oxygen consumption (214).

As expected, we found increased levels of IGF-I in our treated groups, but we found no correlation between IGF-I and VO_2 or maximum achieved power output. This agrees with the lack of correlation between peak weight corrected VO_2 and endogenous IGF-I or IGFBP-3 demonstrated by Eliakim *et al.* (103) in normal male subjects. Although plasma IGF-I levels not invariably reflect IGF-I bioactivity, they are related to peak VO_2 (104, 105). However, these studies did not evaluate the effect of supraphysiological levels of GH and IGF-I on VO_2 and the way they affect maximum power. Instead, VO_2 was more of a correlate for fitness and because GH increases during exertion, it is likely to be related to VO_2 (215). A correlation between endogenous GH and VO_2 in healthy young adults has been ascribed to the degree of fitness (216), but age appears to be a concomitant factor for relationships to the GH-IGF-I axis (129, 217). The achieved peak VO_2 has been found to be more closely related to the 24-h integrated serum GH concentration in male than in female subjects (216), whereas no gender difference was found in its age-dependent relation to a single measure of IGF-I (217). During exercise, the stimulation of GH secretion appears to be greater in women than in men (215). In contrast, we previously demonstrated in the current study population that, in terms of IGFBP-3, male subjects are more responsive than females to exogenous GH (187). Although the study lacks statistical power to test gender differences, we did not note any tendency towards this with regard to maximum aerobic exercise capacity in response to supraphysiological GH treatment.

In the current study we did not evaluate every aspects of physical performance, *e.g.* maximum voluntary muscle strength and flexibility. However, it has been shown that in healthy subjects, even in highly trained power athletes, muscle strength does not improve after GH administration (128, 129, 131). Supraphysiological doses of GH may also suppress the exercise-stimulated endogenous circulating levels of GH (99) and produce equivocal effects on the already enhanced muscle protein synthesis induced by the training itself (131, 204). In contrast, replacement therapy in GHD improves reduced ventilatory function and oxygen uptake (218, 219). Furthermore, a beneficial effect of GH replacement has been noted on exercise capacity and perceived maximum power output (64, 67, 219), changes normally related to an increase in muscle mass and lean body mass (57, 64, 67). However, an increase in muscle protein synthesis may not necessarily cause an increase in muscle strength (220). Moreover, it has been demonstrated that GHD adults benefit from resistance training even without replacement therapy (221, 222). Effects other than muscular, from administered GH as well as from training, may be important for work performance. In addition to its direct effects on cardiac and pulmonary muscles in GHD, GH replacement is known to have profound effects on fuel metabolism (223), erythropoiesis, and total blood volume (224), but studies of these effects in healthy, trained subjects are lacking. Previous findings by Deyssig *et al.* (129) do not suggest a muscle strength performance-enhancing effect by low-dose GH administration in already trained adults. Probably due to the comparatively low dose, they achieved no total body weight gain after GH treatment, in contrast to our results. However, the weight gain after GH administration in our study was caused at least in part by an increase in ECW and not muscle mass. This could explain why we found no evidence of a positive effect

on maximum aerobic exercise capacity in the present study, despite the use of a high GH dosage. Small changes in ICW, independent of GH treatment, might be due to different physical exercise habits; some individuals in the various groups may have made an effort to improve their test results. One indication of this is the approximately 10-watt higher power output in each group on the second occasion.

To summarise, we observed no beneficial effects of GH administration on aerobic exercise capacity in active healthy subjects, only water retention. This indicates that only untoward effects can be anticipated from GH abuse (225, 226). Instead, we suggest that a normal level of GH is required for maximum muscle performance to be achieved. The improvement produced by GH substitution in GH deficiency (64) and the comparatively poor exercise performance in acromegalics (6) support this theory.

Paper V

IGFBP-4 and IGFBP-5

To summarise, this study shows that one month's treatment with supraphysiological doses of GH in physically active, healthy, young adults increases the levels of IGFBP-4 and IGFBP-5. The effect is seen during treatment and for up to two days after the end of treatment. Some of the effect of GH on IGFBP-4 and IGFBP-5 appears to be IGF-I dependent and does not, at the present time, support the use of these markers in a forthcoming method to detect GH doping.

The higher levels of baseline IGFBP-4 and lower levels of baseline IGFBP-5 in women compared with men and the positive association between age and IGFBP-5 in the present study contrast with previous studies. In one of the few previous clinical studies, no gender difference in IGFBP-4 levels was found in healthy men and women. However, compared with our study, the age ranges (0-78 vs 18-35 years) were wider and the subjects were not as physically active (227). Furthermore, a study of GHD patients found no gender differences or age correlations in the baseline levels of IGFBP-4 and IGFBP-5 (153).

To our knowledge, this is the first study to show the effects of supraphysiological GH treatment on IGFBP-4 and IGFBP-5 in healthy young adults, resulting in increased levels of both markers in the group receiving active GH treatment vs. the placebo treatment group from day 14 until the end of treatment (day 28) and for IGFBP-5 for another two days (day 30). This finding is largely in accordance with previous clinical studies of healthy subjects and of GHD patients on GH treatment, demonstrating that IGFBP-4 and IGFBP-5 levels are influenced by the GH/IGF-I axis (152, 153, 228, 229).

There are few studies of the effects on IGFBP-4 and IGFBP-5 of GH treatment given to healthy individuals. In a study of healthy, elderly women and men, IGFBP-4 levels were unaffected after 26 weeks of GH treatment, whereas IGFBP-5 increased by 20% in men after GH. The weekly GH dose in that study was, however, approximately only one-sixth of the dose per kg and week used in our study (229).

However, van Doorn *et al*. found no significant effect (basal versus treatment) by the IGFBP-4 concentrations in GHD patients on GH treatment. Furthermore, they stated that GH status does not seriously affect circulating levels of IGFBP-4, as the mean levels of IGFBP-4 in both

(partially) GH-deficient patients and untreated acromegalic patients were within the normal range (227).

After the inclusion of serum IGF-I as a covariate in the linear regression analysis, the associations between GH treatment and the IGFBP-4 and IGFBP-5 levels were not significant. This finding indicates that at least some of the GH effect on IGFBP-4 and IGFBP-5 is IGF-I mediated and this is in accordance with previous papers, where it has been stated that at least some of the GH-mediated effects on IGFBP-4 and IGFBP-5 concentrations are mediated by IGF-I (153, 154, 230-234).

However, the present study, which comprised relatively few subjects, cannot exclude the possibility that some of the effects on serum levels might be mediated by direct effects of GH, as has been shown in some previous studies. A difference in the response of IGFBPs to IGF-I and GH infusion in hypophysectomised rats has been described in a study demonstrating that IGF-I therapy is not able in every respect to replace GH therapy (235). The authors state that differences in the response of IGFBPs might be due to direct effects of GH, including effects of locally produced IGF-I (235). Furthermore, a stimulatory effect by both GH and IGF-I on the expression of IGFBP-5 in primary rat osteoblast-enriched cultures has been described (236).

This study, although comprising relatively few subjects, does not support the hypothesis that IGFBP-4 and IGFBP-5 can be used as IGF-I independent markers in a forthcoming method for detecting GH doping and further studies with, a larger number of participants are needed to investigate the potential use of IGFBP-4 and IGFBP-5 as markers of GH doping.

GH doping

GH doping - current and future aspects

The use of GH as a doping agent is widespread and doping with GH has become an increasing problem in sports and among young people at ordinary gyms during the last 10–15 years (8, 9, 237). The actual use of GH as a doping agent is not known, but 5% of male American high-school students have been reported to have used it (8). The GH abusers primarily aim to benefit from the potential anabolic effects of GH, mostly in combination with AAS, in order to increase muscle mass and muscle power. It has also been popular among female athletes, who wish to avoid the androgenic side-effects of anabolic steroids. However, our own interviews with hormone abusers (to be published elsewhere) indicate a more differentiated pattern of GH doping, revealing that its effect is preferentially on muscle volume, and not on muscle strength, thereby making it more popular among body-builders than among weight-lifters.

Although, previous reports from GH-abusing athletes uniformly describe the positive effects of GH doping on muscle volume and strength (237), the effectiveness of GH as a doping agent has been questioned during the last few years. There is a lack of scientific evidence that GH in supraphysiological doses has additional effects on muscle exercise performance than those obtained from optimised training and diet itself (128, 129, 131, 134, 238-240). These data have initiated speculations that the reputation of GH as an effective doping agent is highly exaggerated, at least when taken alone. However, in elite athletes, even a small increase in muscle power or exercise performance might make the difference between a gold

or silver medal. Furthermore, there is a great deal of counterfeit, inactive GH present on the black market, complicating the real picture of the effectiveness of GH as a doping agent.

There are risks involved with GH abuse. Several potential risk factors have been suggested by observations in GH-supplemented and acromegalic patients including carpal tunnel syndrome and pitting edema (241), myocardial hypertrophy and cancer (242, 243). Since GH needs to be injected there is also a risk of hepatitis and HIV if the abusers share syringes. Finally, cadaver-derived GH is still present on the black market and abusers using this may acquire the fatal Creutzfelt-Jacobs Disease (9, 244).

Our findings are in line with those of previous studies. We found no evidence of any GH effect on muscle mass or physical performance. The results in *Paper III* show an increase in ECW but no effect on ICW after GH treatment, indicating that there is no effect on muscle mass, and *Paper IV* shows no effect on physical exercise capacity after one month of GH treatment.

Taken as a whole, there is no evidence of any anabolic effect on muscles or positive effects on physical performance, as a result of GH administration alone or combined with exercise (238-240).

It has been claimed that one main function of GH is its stimulating effect on collagen synthesis and that this might have positive consequences for athletes (245, 246). In a review paper, Doessing & Kjaer conclude that supraphysiological doses of GH do not appear to increase the synthesis of myofibrillar protein, but that it is possible that a supraphysiological GH level has an effect on connective tissue (245). It is known that the GH/IGF-I level is associated with pathological changes in connective tissue in patients with clinical conditions involving a change in GH activity (247-252). Furthermore, studies of healthy subjects treated with GH have revealed an increase in levels of whole body collagen synthesis (101, 253), indicating that there is a stimulatory effect on connective tissue in normal healthy subjects.

Tendons heal more rapidly in rats treated with GH (254) and anecdotal reports have suggested that GH prevent tendon and muscle ruptures, especially if combined with AAS (255). By protecting the myotendinous junction regarded as a ‘weak link in the chain’ in athletes training at high intensity and in those with fast growing muscles due to heavy resistance training and/or AAS abuse, GH could therefore enable training at even higher intensity with shorter recovery periods.

A study of the effect on physical performance of GH and testosterone was recently presented at the Endocrine Society meeting in Toronto in 2007. The study by Ho *et al.* which has not yet been published, revealed that the short-term (8 weeks) use of either GH or testosterone alone in recreational athletes did not significantly improve physical performance. However, the combination of GH and testosterone significantly increased anaerobic work capacity in men (256). This study further supports the view that the use of GH as a single doping agent can be questioned but that it can be effective if combined with AAS.

Test method

Regardless of whether or not GH doping is effective, there is a huge need for the development of a test method to detect GH doping. The problem of developing a reliable method has been described in several papers and work on different approaches is ongoing (7, 97, 209, 257).

Isoform method

There are currently two main approaches to developing a method for detecting GH doping. The first is an *isoform* method, developed by the *Strasburger* group, based on the knowledge that normal, endogenous GH exists in a variety of isoforms. In the pituitary, the most abundant isoform is 22kDa GH, while other isoforms (non-22kDa GH) are present in varying amounts (258). Recombinant GH, on the other hand, is solely made up of the 22kDa isoform. Wallace *et al.* have shown that all the measured isoforms of GH increased during and peaked at the end of acute exercise, with 22kDa GH constituting the major isoform in serum during exercise. They also found that the proportion of non-22kDa isoforms increased after exercise, due in part to slower disappearance rates of 20kDa GH and perhaps other non-22kDa GH isoforms (99). Furthermore, it has been shown that supraphysiological doses of GH in trained adult males suppressed exercise-stimulated endogenous circulating isoforms of GH for up to four days and that the clearest separation of treatment groups required the simultaneous presence of high exogenous 22kDa GH and suppressed 20kDa or non-22kDa GH concentrations (98).

Consequently, even if it is not possible directly to distinguish exogenous recombinant GH from endogenous GH in a blood or urine test, the method developed by the *Strasburger* group, which was used in the Summer Olympics (2004) in Athens and in the Winter Olympics (2006) in Turin, is based on the change in the normal ratio of 22/non-22kDa GH isoforms after the administration of exogenous GH. One disadvantage with this isoform-based method is, however, that it is only able to detect GH administration up to 24 hours after the last administration and therefore, out-of-competition testing will be crucial.

GH marker-based method

The other approach to detecting GH misuse is to use longer-lasting GH-dependent markers. This approach, used by the GH-2000 group, involves markers that are more sensitive to exogenous GH substitution than exercise-induced GH increase. Several papers from GH 2000 have been presented (187, 204, 253, 259-261) and the results from the GH-2000 group have resulted in mathematical, statistical formulae that have been presented as a potential test for detecting GH doping. The formulae presented for males and females are:

Males = -6.586+2.905*p3p+2.100*igf-101.737/age

Females = -8.459+2.454*p3p+2.195*igf-73.666/age

where marker levels are recorded on a logarithmic scale writing ‘igf=log(IGF-I)’ and ‘p3p=log(P-III-P)’. The larger the value found, the more likely it is that a subject has administered GH (261).

These markers were selected to construct a formula which produced optimal discrimination between the GH (GH 0.1 IU/kg/day [0.033 mg/kg/day] (GH 0.1) and GH 0.2 IU/kg/day [0.067 mg/kg/day] (GH 0.2) and placebo groups used in GH-2000. Furthermore, adjustments were made to account for the fall in IGF-I and P-III-P with age and the change in distribution seen in elite athletes. However, a threshold value for the marker score needs to be set. A result above this would be deemed to be positive but the threshold value also needs to minimise the risk of a false positive result. A threshold value of, for example, 3.7 will be exceeded by

chance in around 1 in 10,000 observations and would allow a detection rate of up to 86% in men and 60% in women abusing GH at the doses used in the study (261).

This method has then been further validated in a study stating that the test proposed by the GH-2000 study group can be used to detect subjects receiving exogenous GH (262).

However, some questions still remain before an accurate, reliable method is found. The test proposed by the GH-2000 study group requires further evaluation and discussion before it can be finally accepted as a doping test. Any test to detect drug abuse in sport must minimise the risk of a false positive result. The risk of a false positive result can be adjusted by the threshold values set for the formula. A specificity of 1:10,000 has preliminary been suggested as being sufficient for a legally enforceable doping test (261).

General conclusions

The response of markers of the GH/IGF-I axis and bone markers to a maximum exercise test shows a rather uniform pattern with peak concentrations immediately after exercise, followed by a subsequent decrease to baseline levels. The time to peak value for GH is significantly shorter for females compared with males (*Paper I*).

Some of the markers show strong evidence of high *inter*- or *intra*-individual variability in resting samples during one year and we conclude that we have to be careful if we use these markers in a forthcoming doping test for GH (*Paper II*)

The reference ranges presented of the markers in connection to a maximum exercise test, at rest and after a competition might be of considerable use in the future development of a test for the detection of GH-abuse in sports (*Papers I+II*).

Treatment with supraphysiological doses of GH during one month given to healthy individuals results in a decrease in body fat, an increase in ECW but no visible effect in ICW, indicating limited anabolic effect on muscles. Furthermore, no improvements in power output or oxygen uptake in a bicycle exercise test before and after treatment were observed (*Papers III+IV*).

Serum levels of IGFBP-4 and IGFBP-5 are increased by treatment with supraphysiological doses of GH but there is no obvious role for IGFBP-4 or IGFBP-5 as potential markers of GH doping (*Paper V*).

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