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The GH-IGF-1 Axis in Postmenopausal Women with Abdominal Obesity

Celina Franco

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Celina Franco

To my family

Learning is not a shore undertaken to acquire knowledge, it is in itself something enjoyable, something that brings satisfaction to oneself and can benefit others.

Chogye Trichen Rimpoche

Abstract

Abdominal obesity is associated with blunted growth hormone (GH) secretion. GH treatment in abdominally obese men reduces visceral adipose tissue (VAT) and improves the metabolic profile.

The aim of this study was to investigate alterations in the GH-IGF-1 axis in postmenopausal women and to study the effects of GH treatment on abdominal obesity, insulin resistance and other metabolic alterations associated with the metabolic syndrome. Gender differences in the response to GH treatment were also investigated.

Forty abdominally obese women participated in a randomized, placebo controlled study. The 12-month GH treatment reduced VAT, increased thigh muscle area and reduced total and LDL-cholesterol as compared with placebo. Insulin sensitivity was increased at 12 months as compared with baseline values within the GH-treated group.

There was a reduction in serum CRP and IL-6 concentrations after six and 12 months in the GH-treated women as compared with placebo. The reduction in CRP and IL-6 was associated with a reduction in VAT and hepatic fat content, as well as an increase in serum IGF-1 levels. No significant effect was seen on markers of endothelial dysfunction: sE-selectin, VCAM-1, ICAM-1 or MMP-9. These findings suggest that GH exerts an attenuating effect on the state of chronic inflammation associated with the metabolic syndrome.

A comparative study of postmenopausal women and middle-age men with abdominal obesity demonstrated that GH reduced VAT and increased thigh muscle mass more markedly in men as compared with women.

In a cross-sectional study of postmenopausal abdominally obese women an independent, negative association between pulsatile GH secretion and intermuscular AT, and between basal GH secretion and VAT was shown. These findings suggest that the interactions between fat mass and the somatotropic axis are depot-dependent.

In conclusion, these studies have shown that GH intervention improves the cardiovascular risk profile in abdominally obese postmenopausal women and that the interaction between fat mass and GH secretion seem to be depot-dependent. Men are more responsive to the lipolytic action of GH in VAT than women. Low GH secretion may have a role in the metabolic abnormalities associated with the metabolic syndrome.

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List of papers

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

I.   Franco C, Brandberg J, Lönn L, Andersson B, Bengtsson B-Å, Johannsson G.
Growth Hormone treatment Reduces Abdominal Visceral Fat in Postmenopausal. Women with Abdominal Obesity: a 12-month Placebo-controlled Trial
J Clin Endocrinol Metab 2005 Mar;90(3):1466-74

II.   Franco C, Andersson B, Lönn L, Bengtsson B-Å, Svensson J and Johannsson G.
Growth Hormone Reduces Inflammation in Postmenopausal Women with Abdominal Obesity: a 12-Month Placebo Controlled Trial
Submitted to JCEM 11 January 2007

III.   Franco C, Koranyi J, Brandberg J, Lönn L, Bengtsson B-Å, Svensson J and Johannsson G.
Gender Differences in the Response to one Year of GH Treatment in Abdominally Obese Men and Women
Manuscript in production

IV.   Franco C, Veldhuis JD, Iranmanesh A, Brandberg J, Lönn L, Andersson B, Bengtsson B-Å, Svensson J and Johannsson G.
Increased Thigh Intermuscular Fat is Associated with Decreased Growth Hormone Secretion in Postmenopausal Women with Abdominal Obesity
European Journal of Endocrinology 2006; 155 261-268

Contents

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Abbreviations

ALS   Acide-labile subunit
ApEn   Approximate entropy
BF   Body fat
BMI   Body mass index
BW   Body weight
CRP   C-reactive protein
CVD   Cardiovascular disease
CT   Computed tomography
DM   Diabetes mellitus
DXA   Dual energy X-ray absortiometry
FFA   Free fatty acid
FFM   Fat-free mass
GDR   Glucose disposal rate
GH   Growth hormone
GHBP   Growth hormone binding protein
GHD   Growth hormone deficiency
GHR   Growth hormone receptor
GHRH   Growth hormone releasing peptide
HbA1c   Hemoglobin A1c
HDL   High-density lipoprotein
HOMA-IR Homeostasis model assessment of the insulin resistance index
HU   Hounsfield units
ICAM-1   Intercellular molecule-1
IDF   International Diabetes Federation
IGF-1   Insulin like growth factor 1
IGFBP   IGF-binding protein
IL-6   Interleukin-6
IMAT   Intermuscular adipose tissue
LBM   Lean body mass
LPL   Lipoprotein lipase
L4   The fourth lumbar vertebra
MMP-9   Matrix metalloproteinase-9
NAFLD   Non-alcoholic fatty liver disease
NCEP´s ATP III
            The National Cholesterol Education Programme's Adult Treatment Panel III
NHANES III
            The National Health and Nutrition Examination Survey of 1999-2000
NS   No significant
OGTT   Oral glucose tolerance test
PAI-1   Plasminogen activator inhibitor 1
SAT   Subcutaneous adipose tissue

SSA   Serum amyloid polypeptide A
TBK   Total body potassium
VAT   Visceral adipose tissue
VCAM-1 Vascular adhesion molecule-1
WHO   World Health Organization

Introduction

Historical background

Abdominal obesity is a major risk factor for CVD, type 2 DM and stroke (1-3). The clustering of abdominal obesity, insulin resistance, dyslipidemia and hypertension is now well known as the metabolic syndrome (4, 5). In 1947, Vague postulated that the obesity phenotype characterised by upper body adiposity was most commonly associated with type 2 DM and CVD (6). However, when Reaven introduced the term "Syndrome X" (7) in 1988 to describe the existence of a constellation of metabolic abnormalities, with insulin resistance as the main pathophysiological mechanism, abdominal obesity was not included. In the last two decades, efforts have been made to reach a consensus on the definition of the metabolic syndrome in order to facilitate new strategies for its prevention and treatment. For example, the WHO (8) definition in 1998 proposed insulin resistance as the underlying etiological factor (9), while the NHANES III survey of 1999-2000 and the NCEP's ATP III definition (Table 1) suggested abdominal obesity as a major risk factor associated with or leading to the clustering of metabolic perturbations such as insulin resistance, dyslipidemia, hypertension and a prothrombotic and proinflammatory state (10). The recent consensus definition of the metabolic syndrome by the IDF (Table 1)(11) in 2005 defines abdominal obesity as the central feature of the syndrome but differs from the NCEP ATP III definition by proposing broader criteria for waist circumference, HDL cholesterol and fasting plasma glucose.

Definitions of the Metabolic Syndrome

	NCEP ATP III / 2001	IDF / 2005
Abdominal obesity (Waist circumference)	>88 cm (women) or 102 cm (men)	≥ 80 cm (women) or ≥ 94 cm (men)
Triglycerides	≥ 1.7 mmol/l	≥ 1.7 mmol/l
HDL-cholesterol	< 1.3 mmol/l (women) or < 1.0 mmol/l (men)	< 1.29 mmol/l (women) or < 1.03 mmol/l (men)
Blood pressure	≥ 130/85 mm Hg	≥ 130/85 mm Hg
FPG	> 6.1 mmol/l	≥ 5.6 mmol/l

Table 1

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The prevalence of the metabolic syndrome is steadily increasing at global level (9, 10). In contrast to previous reports suggesting that the syndrome tends to be more common in men than in women (12), the condition may be equally prevalent in both sexes (13), with an equal risk of developing type 2 DM. According to the Framingham Heart Study, the metabolic syndrome is a stronger denominator among women who develop cerebrovascular disease than among men (14), but this finding was not confirmed in another study (15).

Regional fat distribution and GH secretion

Increased abdominal obesity is associated with blunted GH secretion, but it remains unclear whether this hyposomatotropism is a consequence rather than a causal factor of central fat accumulation (16). However, as the reduced mean GH concentrations did not reverse after weight loss in abdominally obese women, it was proposed that blunted GH secretion is a primary defect that predisposes subjects to accumulate intra-abdominal fat (17).

There is consistent evidence that abdominal obesity is associated with blunted lipolysis during fasting, suggesting a defective lipolytic response to fasting in these subjects that may contribute to the augmentation of fat accumulation (18). In postmenopausal women with abdominal obesity, the lipolytic response to a 20-hour fast was reduced and the increment in GH secretion in response to fasting was markedly attenuated as compared with normal weight controls, suggesting that blunted GH secretion during fasting may help to preserve fat mass.

On the other hand, fat infiltration of the liver (19) and skeletal muscle (20) is linked with insulin resistance and other metabolic perturbations characterising the metabolic syndrome. NAFLD includes a broad spectrum of hepatic conditions from fatty liver to non-alcoholic steatohepatitis (21) that are associated with impaired insulin sensitivity (19) and a state of sub-clinical inflammation (22-24). It has therefore been suggested that NAFLD should be a feature of the metabolic syndrome (25). Furthermore, impaired lipid oxidation in skeletal muscle is linked to insulin resistance, muscle fat accumulation and the development of visceral adiposity (26). When the studies presented in this thesis were performed, it was not known whether fat accumulation in skeletal muscles and in the liver is associated with blunted GH secretion.

The physiology of the GH-IGF-1 axis

A wide spectrum of physiological processes, including somatic growth and development, carbohydrate and lipid metabolism and liver function are regulated by GH action (27).

GH is produced in the pituitary gland as a 22 kDa polypeptide (28). It is released in a pulsatile manner, with the vast majority of GH secretion occurring during sleep. Hypothalamic GHRH and ghrelin, mainly produced in the stomach and to some degree also in the hypotahalmus (29), stimulate GH synthesis and secretion, whereas somatostatin inhibits GH release (30). Furthermore, peripheral feedback mechanisms, such as IGF-1, glucose and other pituitary hormones, exert a modulatory effect on GH secretion.

Most circulating GH is bound to a high-affinity GH-binding protein produced by proteolytic cleavage of the extracellular domain of the GHR. The GHR is a member of the cytokine receptor superfamily (31). The extracellular domain of the GHR dimerizes upon GH binding (32, 33). Following dimerisation, a variety of signal transduction cascades are activated, catalysed by the phosphorylation of cellular polypeptides by the Janus kinase 2 (JAK2) and the induction of signal transducers and activators of transcription (STAT) proteins that lead to the stimulation of target gene activation (34). Some of the GH effects are indirect, mediated by hepatic IGF-1, while others result from the direct effect of GH on target tissues. In spite of this, there is still no clear separation of the autocrine and paracrine effects of locally produced IGF-1 from the endocrine effect of hepatic IGF-1.

In humans, gender, age, exercise, body composition and nutritional status are important modulators of GH secretion (35-37). Fertile women have higher GH secretion than men, with increasing age and adiposity GH secretion decreases and with increased physical fitness GH secretion is increased.

The GH-IGF-1 axis in women with abdominal obesity

In healthy, non-obese men and women, intra-abdominal fat mass showed a strong negative exponential relationship with mean 24-hour serum GH concentrations, which was independent of age, gender and physical fitness (38). This indicates that, for each increment in intra-abdominal fat mass, there is a more than a linear reduction in mean 24-hour GH concentrations. In addition, high VAT accumulation was found to be associated with both reduced basal and pulsatile GH secretion and a loss of regularity of GH release in healthy premenopausal women (17). For reasons not totally understood, VAT appears to accelerate the removal of GH from plasma (17, 39, 40). With increased adiposity, GH secretion thus declines, with a reduction in the mass of GH secreted per burst but without any major impact on GH secretory burst frequency (41).

The regularity of the pattern of GH release has been determined by using a randomness score: the ApEn ratio (42). High ApEn ratios approaching 1.0 denote greater secretory irregularity, whereas smaller ApEn values imply greater regularity (Figure 2) (17). A positive association between VAT and ApEn ratio has been reported, suggesting the loss of regularity of GH secretion with increasing VAT (17, 41), as it has also been reported in obese women with polycystic ovary syndrome (PCOS) (43).

A study including both healthy men and women demonstrated that abdominal visceral fat and fasting insulin were important predictors of reduced 24-hour GH concentrations, independent of age and gender, while serum IGF-I was a consistent predictor of 24-hour GH concentrations in young but not older subjects (44).

The interaction of sex steroids and GH in postmenopausal abdominally obese women

Oestrogen and androgen exert divergent modulatory effects on the metabolic actions of GH (45). Gender-related differences in body composition could therefore be mediated by the interaction

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of sex steroids and GH (45). Furthermore, women with severe GHD have a less marked response to GH replacement than GHD men in terms of changes in serum IGF-I concentration and body composition (46-48). This reduced responsiveness is particularly seen in GHD women who are receiving oral oestrogen therapy.

Healthy men have more FFM and less total BF than healthy women (49, 50) and men tend to accumulate more abdominal fat, while women have a larger proportion of fat in the lower body (51). Furthermore, measurements using CT scans have shown that the proportion of VAT in relation to total body fat is larger in men of all ages (52, 53). Premenopausal women have a greater proportion of subcutaneous fat than men, whereas, in parallel with the marked reduction in oestrogen levels in postmenopausal women, there is increasing VAT accumulation in both lean and obese postmenopausal women (54). These gender differences in body composition suggest an influence of sex steroids on the amount and distribution of lean and fat tissue in healthy individuals.

Hyposomatotropism and cardiovascular risk

Adult patients with hypopituitarism exhibit a similar clustering of cardiovascular risk factors as those present in the metabolic syndrome (55). The most central findings in both these conditions are abdominal obesity, insulin resistance, high triglycerides and low HDL-cholesterol concentrations and hypertension. In addition, both conditions have elevated serum levels of pro-inflammatory markers, premature atherosclerosis, endothelial dysfunction and increased mortality from cardiovascular diseases (55). The similarities between these two syndromes and the fact that GH replacement therapy improves most of these features in adult GHD patients suggests that blunted GH secretion may be of importance for the metabolic aberrations observed in both these conditions.

The efficacy of GH treatment in adult GHD patients with hypopituiarism has been widely studied. In contrast, at the time we started our studies, there were only a few trials that had investigated the effect of GH treatment in individuals with abdominal obesity, simple obesity and the metabolic syndrome. Pedersen *et al.* showed that treatment with GH for five weeks in obese women was followed by a reduction in body fat mass (56). One early trial included middle-aged men with abdominal obesity (57). In this nine-month, randomised, placebo-controlled study, there was a reduction in both visceral and subcutaneous abdominal fat. Insulin sensitivity, as assessed by the hyperinsulinemic euglycemic glucose clamp technique, diastolic blood pressure and total cholesterol were all improved. A 12-week, double blind, placebo-controlled study including newly diagnosed type 2 DM patients, who received a low dose of GH treatment combined with caloric restriction, resulted in reduced visceral fat and improved muscle mass and insulin sensitivity after 12 weeks as compared with placebo (58). However, at the time the studies presented in this thesis were initiated, there were no long-term data on the effect of GH treatment in women with abdominal obesity.

Aims of the thesis

The general aim of this study was to investigate alterations in the GH-IGF-1 axis in postmenopausal women with the main features of the metabolic syndrome and its relationship with abdominal obesity, insulin resistance and other metabolic alterations associated with the syndrome. The association between endogenous GH secretion and body composition and metabolism as well as gender differences in response to GH treatment were also investigated. The specific aims of the papers were as follows.

I. To study the effect of 12 months of GH treatment on insulin sensitivity, body composition and other metabolic variables in women with abdominal obesity

II. To study the effect of 12 months of GH treatment on circulating levels of inflammatory markers and vascular adhesion molecules in postmenopausal women with abdominal obesity

III. To study gender-related differences in the response to 12 months of GH treatment in men and women with abdominal obesity

IV. To study the association between spontaneous GH secretion and regional fat distribution in women with abdominal obesity

Subjects and Methods

Subjects

The participants in all the papers were recruited through advertisements in a local newspaper. Of the 607 women who responded to the advertisement, 145 were screened during the autumn of 2000 (Fig. 3). Forty women were found to be eligible for inclusion. The participants in

Papers I and II are the same cohort of 40 women with a mean age of 57.3 years (range 51 to 63 years) who were recruited and randomised to either GH or placebo groups. Both study groups were well matched at baseline in terms of age, BMI, WHR, smoking habits, alcohol consumption and antihypertensive treatment.
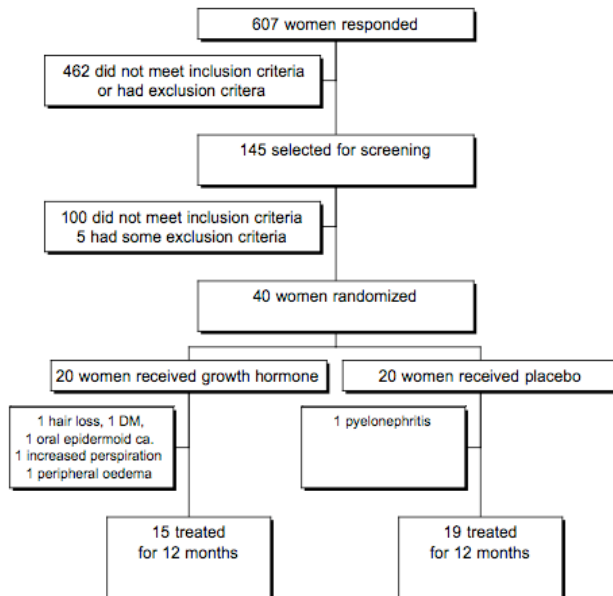
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                  ┌──────────────────────────────┐
                  │     607 women responded       │
                  └──────────────────────────────┘
   ┌──────────────────────────────────┐
   │ 462 did not meet inclusion criteria │
   │      or had exclusion critera       │
   └──────────────────────────────────┘
                  ┌──────────────────────────────┐
                  │   145 selected for screening   │
                  └──────────────────────────────┘
   ┌──────────────────────────────────┐
   │ 100 did not meet inclusion criteria │
   │      5 had some exclusion criteria   │
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                  │     40 women randomized        │
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   ┌──────────────────────────────┐   ┌──────────────────────────────┐
   │ 20 women received growth hormone│   │ 20 women received placebo     │
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 │ 1 hair loss, 1 DM,       │          │ 1 pyelonephritis         │
 │ 1 oral epidermoid ca.    │          │                          │
 │ 1 increased perspiration │          │                          │
 │ 1 peripheral oedema      │          │                          │
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      │ 15 treated       │                  │ 19 treated       │
      │ for 12 months    │                  │ for 12 months    │
      └─────────────────┘                  └─────────────────┘
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Figure 3. *Study design (Papers I and II)*

Fifteen of 20 postmenopausal abdominally obese women from the 12-month GH vs placebo controlled trial entered the study. Fifteen men matched for age, BMI and anthropometric measures were recruited for comparison

from an open metabolic study at our research group (no published data) (Paper III).

The first 20 women recruited during the autumn of 2000 from the cohort of 40 participated in Paper IV. In this study, all

the women had normal fasting glucose and HbA1c. One individual was diagnosed with glucose intolerance and another with DM type 2 using OGTT. The patient with DM was excluded from all the statistical analyses in Paper IV and in Papers I and II.

The criteria for inclusion in all the studies were age 50-65 years, a body mass index of 25-35 kg/m^2, a waist-to-hip ratio of > 0.85 for women and > 0.95 for men (Paper III) and a sagittal diameter of > 21.0 cm for women and > 22.0 for men (Paper III) and a serum IGF-I concentration of between -1 and -2 SD score. The criteria for exclusion were DM, CVD, claudicatio intermittens, stroke, any malignancy and any other hormone treatment. The women were all menopausal and had not received oestrogen therapy for at least one year before entering the studies.

Ethical aspects

Informed consent was obtained from each patient before entry into the study. The study was approved by the Ethics Committee at the University of Göteborg and by the Medical Products Agency, Uppsala, Sweden.

Methods

Study protocols

Papers I and II are based on a 12-month, randomised, double-blind, parallel group trial in abdominally obese, postmenopausal women (Figure 3). After a one-month run-in period in which concomitant medications were optimised, the women were randomised to receive treatment with recombinant human GH or placebo. The computerised randomisation was performed by the Sahlgrenska hospital pharmacy. Measurements of the studied variables were performed at baseline and after six and 12 months of treatment.

Paper III was a parallel group study in which abdominally obese men and women received GH treatment for 12 months. Assessments of body composition, insulin sensitivity and other metabolic indexes were estimated at baseline and after 12 months (Figure 4).

Paper IV was a cross-sectional study. Participants attended the outpatient clinic three times within the space of one month, with at least one week between each visit (Figure 5).

In Papers I-III, laboratory examinations including safety assessments were performed at the start, one, two, three, six, nine and 12 months and one month after discontinuing treatment.
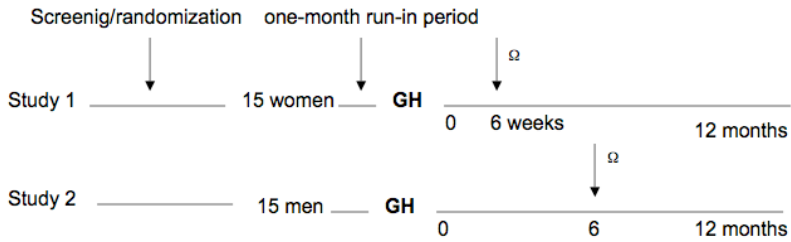
Study design

Figure 4. *This trial was designed to compare effects of GH between middle-age men and women with abdominal obesity. Participants from the studies 1 and 2 were recruited by advertisements in a local newspaper. A cohort of 15 postmenopausal abdominally obese women that had participated in a larger metabolic study (Study 1; n = 40 women) were eligible to participate in this study. Fifteen men with comparable age and anthropometric measures were recruited from an open metabolic study (Study 2; n = 33 men) at our research group (no published data), for comparison. After one-month run-in period in which concomitant medications were optimized, participants received GH treatment during 12 months. Ω: the up-titrating phase of the GH dose in women and in men.*
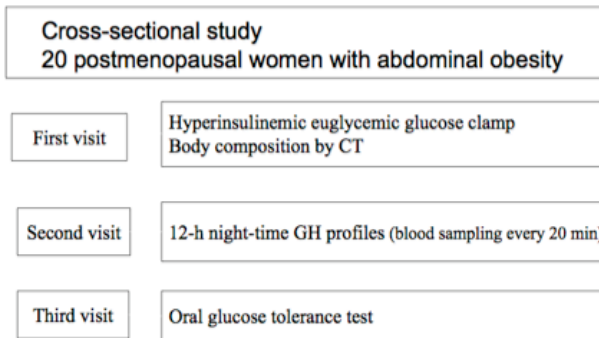
Design

Figure 5

Treatment

In Papers I and II, GH (Genotropin®, Pfizer, Stockholm, Sweden) vs. placebo was administered subcutaneously before bedtime. The treatment regimen was initially formulated in international units per day, IU/day, and subsequently converted to mg/day. The initial dose of GH was 0.13 mg/day (0.4 IU/day), which was then increased to 0.27 mg/day (0.8 IU/day) after two weeks, 0.4 mg/day (1.2 IU/day) after four weeks, 0.53 mg/day (1.6 IU/day) after five weeks and, after six weeks, to the target dose of 0.67 mg/day (2.0 IU/day). In Paper III, the up-titration of the GH dose differed somewhat in men and women. In the female group, during a period of six weeks, the initial dose of GH 0.13 mg/day was gradually up-titrated to the maintenance dose of 0.51 mg/day according to a pre-determined schedule. The male group received a starting GH dose of 0.13 mg/day and then, during the following six months, the dose was titrated under the guidance of serum IGF-1 in order to achieve a serum IGF-1 concentration within 1-2 IGF-1 standard score, until the maintenance dose of 0.47 mg/day was reached. After the titration phase, and in the absence of side-effects, the aim was to keep the GH dose stable in both genders until the end of the study. Symptoms and signs of adverse effects were carefully monitored on each visit. The dose was reduced by half in the event of fluid-related side-effects. Oral and written instructions about administration and dose were given. Compliance was assessed by counting the returned empty vials and expressing that number as a percentage of the vials needed for the treatment period (Papers I-III).

Insulin sensitivity measures

Hyperinsulinemic euglycemic clamp
The hyperinsulinemic euglycemic glucose clamp technique is considered to be the gold standard method for assessing insulin sensitivity. This method was performed as described by DeFronzo in 1979 (59). After an overnight fast, an iv catheter was placed in an antecubital vein for the infusion of insulin (0.12 UI/kg/min) and 20% glucose. A second catheter was placed in the contralateral arm for arterialised blood. The plasma insulin level was maintained between 150-250 mU/l, in order to suppress endogenous hepatic glucose production. Blood glucose was monitored every 10 minutes during the insulin infusion and, during the last 30 minutes, every five minutes. Euglycemia was maintained (5.5 mmol/l) by infusing 20% glucose in variable amounts. The glucose disposal rate (GDR) was measured for 20 minutes in steady-state conditions, which were reached after 100 minutes.

In Paper I, GDR was estimated on the basis of BW. As the variability and reproducibility of this method have been demonstrated to be around 15%, the adjustment of GDR for FFM was performed in Paper III, as previously recommended (60).

OGTT

All the subjects performed an OGTT before the start, at six and 12 months respectively and one month after treatment in Paper I. A standard dose of 75 g of glucose was administered and fasting blood samples were obtained at baseline and every 30 minutes for two hours. The definition criteria for normal, impaired glucose tolerance and DM were based on the American Diabetes Association (ADA) recommendations

(61). In order to eliminate any type of interference, OGTT assessments were performed one week after the hyperinsulinemic euglycemic clamp.

HOMA-IR

The homeostasis model assessment of the insulin resistance index (HOMA-IR) was estimated using the formula: HOMA-IR = (fasting insulin x glucose)/ 22.5, previously validated against a hyperinsulinemic euglycemic clamp for insulin resistance (62, 63).

Night-time GH profiles

In Paper IV, 12-hour GH profiles (8 pm-8 am, blood sampling every 20 min) were used to appraise GH secretory status. Deconvolution analysis, a statistical reconstruction of GH secretion (Figure 1), was used to quantitate pulsatile and basal GH secretion and other features of GH secretion such as pulse mass, mean interval, half-life and peak frequency (64).
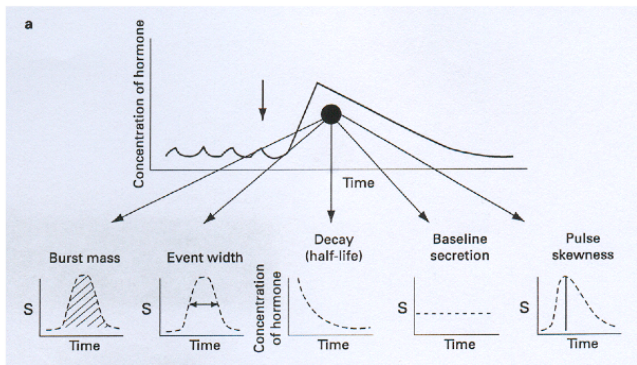

Figure 1. *Deconvolution analysis applied to an observed hormone concentration peak (top center). Deconvolution analysis quantitates the mass (bottom, left-to-right) and width (duration, min) of the underlying secretory burst; exponential decay (elimination) of secreted molecules; baseline (non pulsatile) secretion; and waveform shape.*
With permission of Prof. J.D. Veldhuis. J. Endocrinol. Invest. 26: 799-813, 2003.

A randomness score (ApEn ratio) was applied to quantify the pattern regularity of GH release (Figure 2) (42). ApEn ratios approaching 1.0 denote greater secretory irregularity, whereas smaller ApEn values imply greater regularity (17). The analysis was conducted blind to the time-series assignments
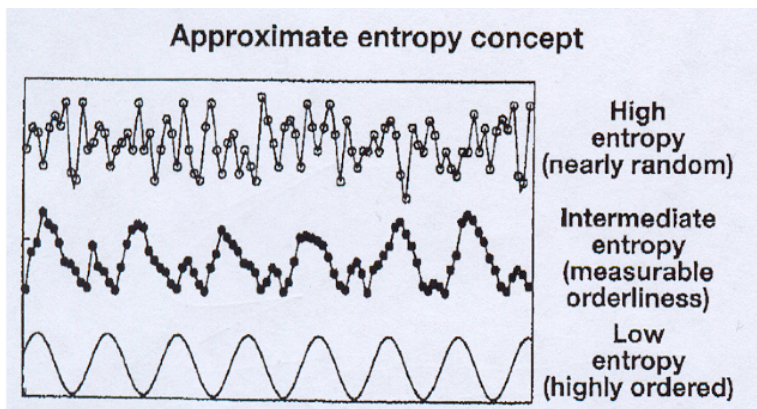
Figure 2. *Estimate of GH secretion regularity pattern by the ApEn statistic. With permission of Prof. J.D. Veldhuis. J. Endocrinol. Invest. 26: 799-813, 2003.*

Biochemical assays

Blood samples were drawn in the morning after an overnight fast and, after processing the vials with the specimens, the samples were frozen at -80°C.

Serum IGF-1, IGFBP-1, IGFBP-3 and GHBP

In all the papers, the serum concentration of IGF-I was determined by a hydrochloric acid ethanol extraction RIA using authentic IGF-I for labelling (Nichols Institute Diagnostics, San Juan Capistrano, Ca) with a within-assay CV of 2.2% and 4.2% at serum concentrations of 125 µg/L and 345 µg/L respectively. The SD score for IGF-I was calculated from the predicted IGF-I values, adjusted for age and gender values obtained from the normal population (65).

The IGF-binding protein 3 (IGFBP-3) concentration in serum was determined by RIA (Nichols Institute Diagnostics) with a total CV of 6.2% and 5.7% at serum concentrations of 2.05 mg/L and 3.49 mg/L respectively. The IGF-binding protein 1 (IGFBP-1) was determined by ELISA (Immunotech, Marseille, France), CV 12.8% (Papers I and IV).

GH assay

Concentrations were analysed using a highly sensitive chemiluminescence assay with a lower detectability limit of 0.002 µg/L (at 2 SD above the assay blank) and 0.005 µg/L (at 3 SD above the assay blank) (Paper IV) (66).

Lipids

Serum total cholesterol and triglyceride (TG) concentrations were determined with enzymatic methods (Thermo Clinical Labsystems, Espoo, Finland). The within-assay CV for total cholesterol and TG determinations was 2.2% and 2.3% respectively. High-density lipoprotein (HDL) cholesterol was determined after the precipitation of apolipoprotein (apo) B-containing lipoproteins with magnesium sulphate and dextran sulphate (Thermo Clinical Lab Systems), CV 1.9%. The LDL-cholesterol concentration was calculated according to Friedeval's formula (67).

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Serum LDL cholesterol was estimated if serum TG values were < 4.3 mmol/L. Apolipoprotein B (apoB) and apolipoprotein A-I (apoA-I) were determined by immunoprecipitation enhanced by polyethylene glycol at 340 nm (Thermo Clinical Labsystems), CV 3.2% and 5.9% respectively. Lipoprotein (a) was measured by an immunoturbidinemic test (DiaSys Diagnostic Systems Gmbh&Co, Holzheim, Germany), CV 6.7%. All the analyses were performed on a Konelab 20 autoanalyser (Thermo Clinical Labsystems) (Papers I and III).

Inflammatory markers and markers of endothelial dysfunction (Paper II)

CRP, IL-6 and SSA in serum were used as biomarkers of systemic inflammation. CRP was measured using a (latex) highly sensitive immunoturbidimetric assay with a detection limit of 0.03 mg/l (Tina-quant, Roche Diagnostics, Indianapolis, USA) determined on a Roche/Hitachi analyser. The intra-assay CVs were 1.34% at 0.55 mg/l and 0.28% at 12.36 mg/l, while the inter-assay CVs were 5.7% at 0.52 mg/ml and 2.51% at 10.98 mg/ml. IL-6 concentrations were measured using a high-sensitivity enzyme-linked immunoassay (ELISA) kit (R&D Systems, Inc., Abingdon, UK). The intra-assay CVs were 5.9% at 2.73 pg/ml and 3.8% at 7.94 pg/ml. Serum SAA was determined by a highly sensitive latex agglutination assay using particle-enhanced immunonephelometry on the Behring Nephelometer II (BNII) (Dade Behring, Marburg, Germany). The intra-assay CVs were 4.3 to 6.2, while the total CVs were between 5.4 and 6.4%.

Endothelial dysfunction was studied by determinations in serum of soluble E-selectin (sE-selectin), VCAM-1 and ICAM-1 concentrations measured using quantitative sandwich ELISA (R&D Systems, Inc., Minneapolis, MN, USA, catalogue no. BBE 2B for sE-selectin, catalogue no. BBE 3 for sVCAM-1 and catalogue no. BBE 1 B for sICAM-1) and MMP-9 measured by ELISA (Amersham Biosciences, Uppsala, Sweden, product code no. RPN2614).

Adipocytokines

Serum concentrations of the adipocytokines leptin and adiponectin were estimated in Paper III. Serum leptin and adiponectin were determined by RIA according to the instructions from the manufacturer (Linco Research, Inc. Missouri, USA, catalogue no. HL-81AK for leptin and catalogue no. HADP-61HK for adiponectin).

Insulin, glucose, HbA1c, C-peptide and FFA

Serum insulin was determined by RIA (Pharmacia, Uppsala, Sweden). Plasma glucose was measured using the Gluco-quant method (Roche/Hitachi, Mannheim, Germany). Haemoglobin A1c was determined by high-pressure liquid chromatography (Walters, Millipore AB, Sweden) and C-peptide was determined by an immunoenzymetric method (Dako Diagnostics Ltd, Dakopatt AB). FFA levels were determined using an enzymatic colorimetric method (NEFAC; Waco, Neuss, Germany).

Body composition

Total body potassium

TBK was measured by counting the emission of 1.46 MeV γ radiation from the naturally occurring [40]K isotope in a highly sensitive 3 π whole-body counter

with a coefficient of variation (CV) of 2.2%. Potassium is mainly located in FFM. Based on previous comparative studies using CT and K-40, FFM was estimated by assuming a potassium content of 62 mmol/kg FFM in women (68) (all papers) and 64.7 mmol/kg FFM in men (69) (Paper III). Total body fat BF was then calculated as BW - FFM.

Computed tomography

Regional body fat and muscle mass were assessed using CT as cross-sectional areas obtained at two different levels: at L4, for abdominal SAT and VAT (Figure 6A), and in the right thigh for SAT and IMAT (Figure 6B). This method provides a visual image related to the density of different tissues. The tissue areas are determined with the following intervals: air, gas and lungs: -1001 to -191 HU,

adipose tissue: -190 to -30 HU; all other soft tissues and organs: -29 to 151 HU; skeleton: 152 to 2500 HU (the scanners are calibrated to measure air as -1000 HU and water as 0 HU) (70). Tissue areas were quantified with the subject in a recumbent position with a General Electric High Speed Advantage CT system (HAS), version RP2, GE Medical Systems, Milwaukee, Wisconsin, USA. Using a variant of the five-level model (71), three scans were obtained from each participant (Figure 6C). Scan 1 was obtained in the thigh region one cm below the gluteal fold, scan 2 at the L4 level and scan 3 at the mid-liver level. In the case of scan 1, the tissue areas of the right leg are reported. The effective radiation dose equivalent per examination was < 0.8 msv using a dose reduction protocol (72).
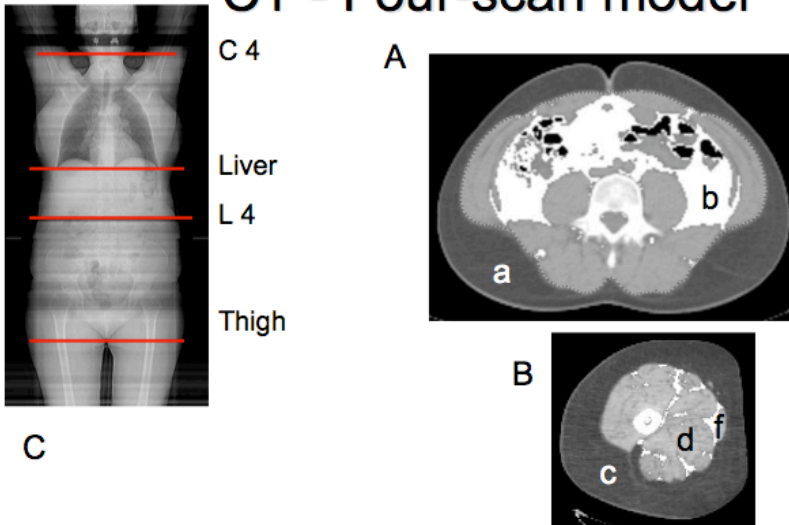
Figure 6. A: *The cross-sectional area obtained at L4 shows the abdominal SAT (a) and VAT (b).* B: *The cross-sectional area obtained in the thigh region 1 cm below the gluteal fold, shows the thigh SAT (c), thigh muscle mass (d) and thigh IMAT (f) which is the adipose tissue between the muscles bundles and within the boundary of the muscle fascia.* C: *The cross-sectional area anatomical levels.*

The IMAT regions were manually corrected and distinguished by the operator when necessary. The attenuation of the liver and spleen was determined within three circular regions of interest (ROI) located in the dorsal aspect of each organ. Attempts were made to avoid vessels, artifacts and non-homogeneous areas. Hepatic fat content was determined by measuring liver attenuation (absolute values). Cut-off values for the diagnosis of a fatty liver were considered to be a liver attenuation of ≤ 30 Hounsfield units (HU) or a liver/spleen ratio of < 1. As the absolute values of liver attenuation yielded information equivalent to a liver/spleen ratio of < 1, the latter estimation was only used in Paper I. We determined the mean muscle attenuation in HU in the right thigh. *In vivo* measurements using CT have shown that skeletal muscle attenuation is inversely associated with muscle lipid content (73).

Physical activity and quality of life questionnaires

Physical activity was studied by assessing indices of habitual physical activity at work, during sport and during leisure time using a questionnaire developed by Baecke et al. (74). Quality of life was assessed using the Psychological General Well-Being (PGWB) index, which includes an overall score and six sub-scores (anxiety, depression, well-being, self-control, health and vitality), described elsewhere (75).

Statistical methods

All the descriptive statistical results are presented as the mean (SEM). The results in Papers I and II have been analysed on an intention-to-treat basis with the exception of the sub-group analysis of GDR and weight, which included only subjects who completed one year of treatment (Paper I). Between-group treatment effects were analysed using a two-way ANOVA for repeated measurements (at baseline, six and 12 months) or an unpaired t-test of the percentage change (Papers I, II and III). Within-group treatment effects were estimated by one-way ANOVA, Papers I, II, or a paired t-test, Paper III. All the variables were tested for normality using the Kolmogorov-Smirnov test. Variables with non-normal distribution were logarithmically transformed before analysis (Papers I, II and IV), or when necessary, non-parametric tests were applied (Paper I). An unpaired t-test was used for baseline between-group analyses (Papers I, II and III). In Paper III, mixed model methods were use to estimate the fixed effects over time and to model the underlying covariance structure of the

data. Fixed effect estimates become more accurate using this method because additional information is obtained from the random effect error stratum (76). A non-parametric test (Spearman's rank test) was applied to analyses of categorical data (Paper I). Correlation analyses were performed using Pearson's linear regression coefficient (Papers I and IV) or Spearman's R (Paper III). In Paper IV, Pearson's linear regression coefficient was used to determine whether associations existed between any of the regional fat depots and any measure of basal or pulsatile GH secretion and other metabolic variables. Two multiple regression models and forward stepwise analysis were applied in Paper IV. The first model was used to determine which fat depots were predictors of GH secretion. The second model tested whether markers of insulin sensitivity and other metabolic factors might explain/contribute to the association between GH secretion and fat depots. A two-tailed probability value equal to or less than 0.05 was considered significant in all papers.

Main results

GH- vs. placebo-treated women in Papers I and II were well matched at study entry in terms of age, BMI, waist circumference, sagittal diameter, WHR, smoking habits, alcohol consumption and antihypertensive treatment (Table 2). In Paper III, female and male participants were comparable in terms of age, BMI, waist circumference and waist to hip (W/H) ratio. In all the papers, all the women had low serum estradiol concentrations and were not receiving oestrogen replacement (data not shown).

Table 2. Baseline clinical characteristics of the postmenopausal abdominally obese women treated with GH / placebo for 12 months

Characteristics	GH	Placebo
Number of women	20	20
Age (mean and range) yr	58.2 (51-63)	56.5 (51-63)
Waist (cm)	104 (1.4)	102 (1.6)
Sagittal diameter (cm)	25.8(0.34)	25.0(0.45)
W/H ratio	0.93(0.01)	0.94(0.012)
BMI (kg/m^2)	30.6 (0.7)	30.0 (0.8)
Smokers	5	5
Antihypertensive treatment[a]	4	4
ACE inhibitors/ AT II antagonists	2	2

[a]β-Blockers, angiotensin I-converting enzyme (ACE) inhibitors, Ca-antagonists and angiotensin II (AT-II) antagonists. P value = NS by unpaired t test.

GH dose, serum IGF-I and IGF-I SD score

The mean maintenance dose of GH was 0.51 (0.05) mg/day for women (Papers I-III) and 0.47 mg/day for men (Paper III) (p = 0.5 men vs. women in Paper III). In Papers I and II, the baseline mean serum IGF-1 concentration in the GH- vs. placebo-treated women was 105 ± 31 and 121 ± 24 µg/L respectively; p = 0.1. Serum IGF-1 increased after six months in response to GH treatment, with no further change at 12 months (Figure 7) (Papers I-III). In Paper III, there were no baseline differences in serum IGF-1 level between men and women and, after 12 months, the levels and the increase in serum IGF-1 concentration were similar in both groups. After 12 months, the IGF-1 SD score in men was 2.9 ± 1.2, while it was 2.3 ± 1.5 in women (Figure 8) (Paper III).

Insulin sensitivity and glucose metabolism

Between-group analysis did not reveal any difference in GDR after one year of

GH/placebo treatment (Paper I). Within-group analysis showed a slight decrease in GDR values after six months of GH treatment, followed by a significant increase after 12 months within the GH-treated group, whereas there was no change in the placebo group. In a sub-group analysis, women were divided into groups depending on whether they had a baseline GDR value above or below the median for the whole group (8.4 mg/kg.min). The increase in GDR between baseline and 12 months was more marked in the GH-treated women with baseline values below the median for the group. A similar pattern was not seen in the placebo group.
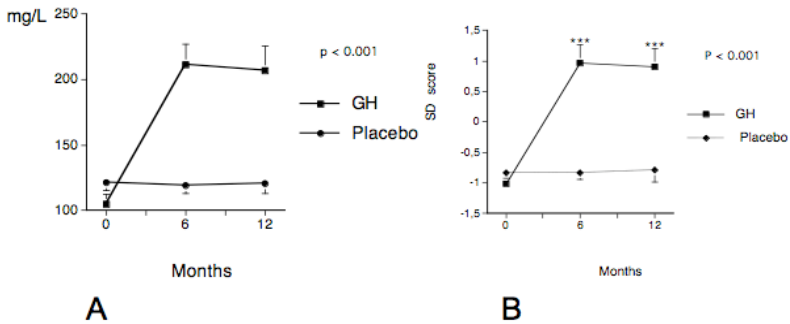

Figure 7. *Effect of GH-treatment on IGF-1 (A) , and expressed as IGF-1 score SD, adjusted for age and gender (B) in 40 postmenopausal women receiving GH or placebo for 12 months. P < 0.001 represents overall treatment effect analyzed using one-way ANOVA.   ***P < 0.001 as compared with baseline.*
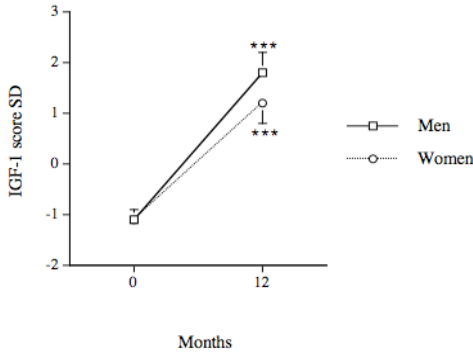
Figure 8. *IGF-1 score SD at baseline and after 12 months of GH treatment.* ***$P < 0.001$ *by paired t test. Analysis of delta % values between the groups by unpaired t test was not significant.*

In Paper I, GDR was corrected for BW (Figure 9a). In additional analyses, GDR corrected for FFM (Figure 9b) by K^{40} showed an approximately similar result as GDR corrected for BW. There was no difference in delta % GDR between men and women using K^{40} (Paper III).
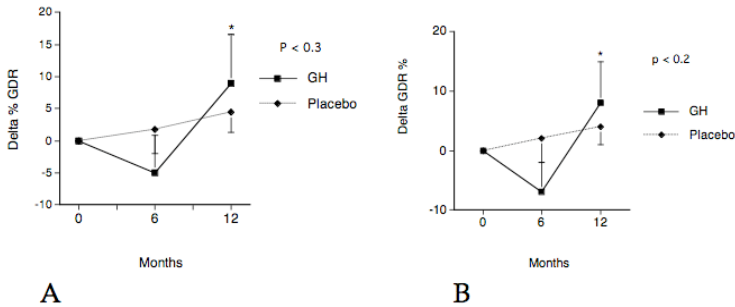

A

B

Figure 9. *Insulin sensitivity expressed as percent change in GDR corrected for weight (A), and corrected for FFM (B), in postmenopausal women with abdominal obesity receiving GH/placebo-treatment for 12 months.*

P = no significant: overall effect between groups using one-way ANOVA. *$P < 0,05$ as compared with baseline.*

An inverse relationship between baseline HOMA-IR and GDR corrected for BW was found in Paper I (r = -0.34; p < 0.033). Retrospective adjustment of GDR corrected for FFM showed a higher inverse association: r = -0.52; p < 0.001. HOMA-IR and fasting insulin levels increased within the GH-treated group at 12 months and were unchanged in the placebo group (Paper 1). Between-group analysis in Paper I did not reveal significant changes in fasting plasma glucose, two-hour glucose values after an oral glucose load or blood HbA1c. No differences were observed in the glucose area under the curve during the OGTT. A wide variability in two-hour glucose after an OGTT was observed in both groups at all measurements. The baseline two-hour glucose after an OGTT revealed impaired glucose tolerance (IGT) in two individuals in each group. After six months, there were four subjects with IGT in both groups, whereas two subjects in the GH group and one subject in the placebo group had diabetic values. After one year, two GH-treated subjects normalised their two-hour glucose, while two new subjects presented with IGT. In the placebo group, three subjects remained glucose intolerant.

Body composition

Mean BW increased similarly in both the GH/placebo groups (within-group analysis < 0.05 for both). In the women who completed one year of treatment, seven of 15 women in the GH-treated group and 11 of 19 women in the placebo group gained more than one kilogram, while the remaining women were regarded as weight stable (Paper 1). No differences were found between the GH/placebo-treated women in terms of FFM and BF assessed by K^{40} (Paper 1).

A reduction in VAT (Figures 10, 11a and Table 3) and an increase in thigh muscle area (Figure 11b) were determined by CT scan in response to one year of GH treatment (Paper I). No differences were observed in abdominal SAT. While VAT decreased after 12 months in the GH-treated women, an increase occurred in the placebo group, p > 0.01, resulting in a significant between-group difference.
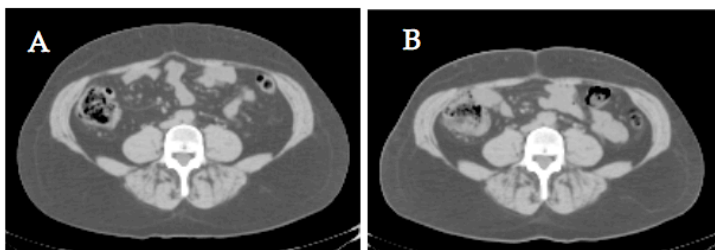

Figure 10. A: *Cross-sectional area at L4 illustrating the VAT area before and* B: *after (B)12 moths of GH in one postmenopausal women with abdominal obesity.*

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Table 3. Assessment of body composition by K[40] and CT scan of the postmenopausal abdominally obese women treated with GH / placebo for 12 months

Variable	Group	Baseline	6 months	12 months	P value*
Weight (kg)	GH	86.0(2.4)	86.1(2.6)	87.2(2.5)[a]	0.9
	Placebo	80.9(2.2)	80.7(2.3)	81.8(2.3)[a]	
Total BF (kg)	GH	37.4(1.9)	37.1(2.1)	38.9(2.0)	0.9
	Placebo	34.0(1.8)	33.1(1.8)	35.0(1.7)	
FFM (kg)	GH	48.7(1.3)	48.9(1.2)	48.2(1.1)	0.9
	Placebo	46.9(1.1)	47.6(1.2)	46.8(1.2)	
Thigh muscle area (cm^2)	GH	110.4(2.7)		113.0(2.5)[c]	0.002
	Placebo	110.9(3.4)		110.7(3.2)	
Abdominal SAT area (cm^2)	GH	430.2(20.2)		432.0(22.3)	0.8
	Placebo	400.9(20.8)		400.5(22.0)	
VAT area (cm^2)	GH	177.2(8.7)		170.6(10.0)	0.003
	Placebo	161.0(7.9)		172.0(8.9)[b]	
Liver attenuation (HU)	GH	49.0(2.3)		51.1(2.2)	0.6
	Placebo	51.0(2.9)		51.2(2.5)	

All the values are expressed as the mean (SEM). BF and FFM estimated by TBK. * P values represent overall treatment effect analysed using one-way ANOVA.
a $P < 0.05$ (at the given time point vs. baseline value).
b $P < 0.01$ (at the given time point vs. baseline value).
c $P < 0.001$ (at the given time point vs. baseline value).

Correlation analysis in Paper I: a positive linear correlation between the percentage change in GDR and liver fat content, expressed as increased liver attenuation (r = 0.65, p < 0.01), and GDR and liver/spleen attenuation ratio (r = 0.60; p < 0.001) was only found in the GH-treated women. The increase in IGF-I was associated with the reduction in VAT in the GH-treated women, r -0.53; p < 0.02.

Sub-group analysis based on BW (stable weight/weight loss vs. weight gain) showed a reduction in VAT and in hepatic fat content expressed as an increase in liver attenuation and an improvement in GDR, particularly among the GH-treated women who had a stable weight or experienced a weight reduction throughout the study period (Paper I).

Serum lipids metabolism

Total cholesterol and LDL cholesterol decreased by 10% after six months in the GH-treated group compared with the placebo group (Paper I) (Table 4). This effect was less sustained at 12 months, showing a reduction of 5% for both. A transient increase in TG and a decrease in HDL-cholesterol concentrations were observed after six months of treatment within the GH-treated group, but there were no between-group differences in these variables. ApoA-I, apoB, Lp (a) and the apoB/apoA-I ratio remained unaffected by GH treatment (Paper I). In Paper III, a reduction in serum LDL-cholesterol level from 4.3 (0.2) to 3.9 (0.2) mmol/L occurred within the female group (p < 0.05 vs. baseline but p = N.S. vs. men), after 12 months. Other

circulating lipid levels were not significantly changed by treatment.

Table 4. Measurements of total cholesterol, LDL cholesterol, HDL cholesterol, TG, apoB, apoA1, Lp (a), and Apob/ApoA-1 in postmenopausal abdominally obese women treated with GH/placebo for 12 months

Variable	Treatment	start	6 months	12 months	P value*
Total cholesterol	GH	6.31(0.15)	5.82(0.18)[b]	6.09(0.16)	0.05
mmol/L	Placebo	6.34(0.26)	6.30(0.23)	6.21(0.24)	
LDL cholesterol	GH	4.33(0.16)	3.87(0.18)[a]	4.13(0.17)	<0.05
mmol/L	Placebo	4.39(0.24)	4.29(0.20)	4.21(0.23)	
HDL cholesterol	GH	1.31(0.06)	1.23(0.06)[a]	1.31(0.05)	0.6
mmol/L	Placebo	1.27(0.08)	1.24(0.08)	1.27(0.07)	
Tryglicerides	GH	1.49(0.12)	1.71(0.19)[a]	1.55(0.15)	0.8
mmol/L	Placebo	1.49(0.10)	1.74(0.24)	1.61(0.14)	
ApoB	GH	1.13(0.03)	1.07(0.04)	1.10(0.04)	0.1
g/L	Placebo	1.16(0.06)	1.18(0.06)	1.13(0.06)	
ApoA-I	GH	1.44(0.04)	1.35(0.04)[a]	1.38(0.03)[a]	0.4
g/L	Placebo	1.41(0.05)	1.38(0.04)	1.37(0.04)	
Lp(a)	GH	0.28(0.04)	0.30(0.05)	0.30(0.05)	0.7
g/L	Placebo	0.42(0.07)	0.43(0.07)	0.42(0.07)	
Apo B/Apo A-I	GH	0.8(0.03)	0.8(0.04)	0.8(0.04)	0.3
	Placebo	0.8(0.05)	0.9(0.06)	0.8(0.05)	

All the values are expressed as the mean (SEM). *P values represent overall treatment effect analyzed using one-way ANOVA.
a $P < 0.05$ (at the given time point vs. baseline value).
b $P < 0.01$ (at the given time point vs. baseline value).

Markers of inflammation and endothelial dysfunction

In Paper II, GH treatment reduced serum levels of CRP and IL-6 as compared with placebo (p = 0.03 and p = 0.05 respectively (Figure 12a and 12b and Table 5). Within-group analysis revealed a reduction in serum IL-6 and CRP levels in the GH-treated group after six months, with a further reduction in IL-6 concentrations after 12 months (p < 0.01 vs. baseline). There was no further reduction in serum CRP level between six and 12 months in the GH-treated group.

No between-group differences in serum concentrations of SAA, E-selectin, VCAM-1, ICAM-1 or MMP-9 were observed after six and 12 months of treatment
In the GH-treated women, the reduction in serum CRP level was positively associated with the reduction in VAT (r = 0.7; p < 0.001 (Figure 13a) and abdominal SAT (r = 0.6; p < 0.01) and negatively associated with the reduction in liver fat content (r = -0.5; p < 0.05 (Figure 13b) (Paper II). The reduction in serum IL-6 concentrations correlated positively with the reduction in VAT (r = 0.5; p < 0.05). No correlations were found

between the percentage changes in markers of inflammation or endothelial dysfunction and changes in IMAT or skeletal muscle attenuation.

In the entire study population, baseline regression analysis revealed an inverse correlation between serum concentrations of CRP and IL-6 and GDR (r = -0.4; p < 0.01 for both). No correlation was found between the changes in serum levels of CRP and IL-6 or changes in markers of endothelial dysfunction and GDR (data no shown).
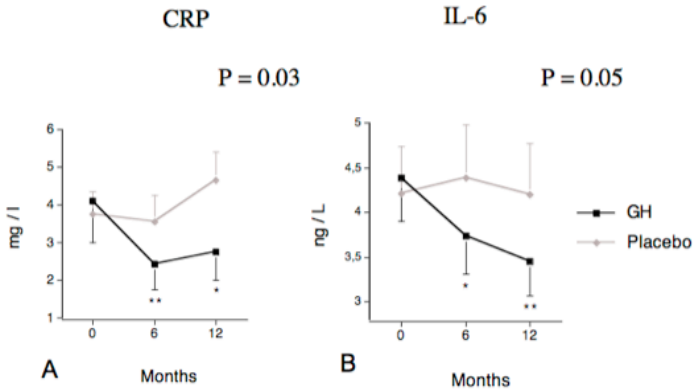

Figure 12. *Effect of GH/placebo treatment on CRP (A) and IL-6 (B) expressed as mean and SEM, after 6 and 12 months.*
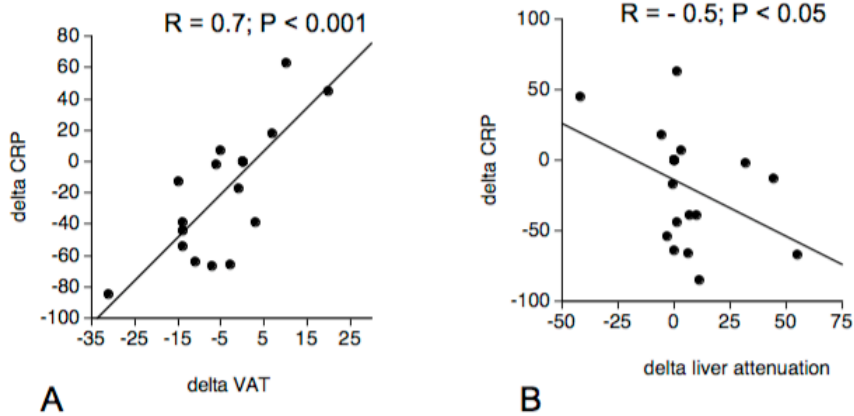
Figure 13. *Correlations between the percent change in serum CRP concentration and the percen in VAT (A) and in liver attenuation (B) in the GH treated group, after 12 months.*

Table 5. Measurements of inflammatory markers in postmenopa usal abdominally obe se women treated with GH/placebo for 12 months.

Variable	Treatment	Start		6 months		12 months		p-value*
CRP	GH	4.3	(1.1)	2.5	(0.7)[b]	3.0	(0.8)[a]	0.03
mg/L	Placebo	3.8	(0.6)	3.6	(0.7)	4.7	(1.1)	
IL-6	GH	4.4	(0.5)	3.6	(0.4)[a]	3.3	(0.3)[b]	0.05
ng/L	Placebo	3.4	(0.3)	3.5	(0.4)	3.5	(0.4)	
SAA	GH	5.3	(0.8)	4.1	(0.6)[a]	4.5	(0.7)	0.1
mg/L	Placebo	5.8	(0.8)	5.3	(0.9)	7.9	(1.9)	
E-selectin	GH	52.9	(5.1)	53.1	(5.7)	53.8	(6.4)	0.6
µg/L	Placebo	55.8	(3.6)	52.7	(3.9)	53.8	(4.2)	
VCAM-1	GH	596.1	(39.5)	621.3	(34.5)	601.5	(31.3)	0.5
µg/L	Placebo	581.8	(32.7)	581.6	(29.1)	580.2	(29.9)	
ICAM-1	GH	249.5	(13.9)	250.9	(12.6)	253.7	(15.5)	0.3
µg/L	Placebo	254.9	(10.6)	253.4	(11.5)	244.0	(10.2)	
MMP-9	GH	207.3	(25)	202.8	(29)	193.7	(29)	0.6
µg/L	Placebo	235.3	(26)	244.1	(31)	223.7	(33)	

All the values are expressed as the mean and SEM.
* p-value represents overall treatment effect analysed using one-way ANOVA
a p < 0.05 (at the given time point vs. baseline value)
b p < 0.01 (at the given time point vs. baseline value)

Changes in serum concentrations of CRP and IL-6 correlated inversely with the rise in serum IGF-1 concentration (r = -0.6; p < 0.01, and r = -0.5; p < 0.05, respectively in Paper III) in the GH-treated group, while no correlations were found in the placebo-treated group. The change in serum IGF-1 correlated positively with the change in liver attenuation (r = 0.7; p < 0.01) and inversely with the change in VAT (r = 0.5; p = 0.02), while no relationship was found between the changes in serum IGF-1 and total BF.

Gender differences

At baseline, the abdominally obese women had higher total BF, abdominal and thigh SAT areas and a less FFM and thigh muscle area than the abdominally obese men (Table 6) (Paper III).

Between-group analysis: by applying the mixed model estimators (Paper III), an 18% reduction in VAT was shown in men compared with 5% in women, p < 0.0001 (Figure 14a). An increase in thigh muscle mass area was seen in men (4%) vs. a 3% increase in women, p < 0.0001 (Table 2 and Figure 14b). FFM increased in both groups, 4.6% in women vs. 3% in men, p = 0.0001. No between-group difference was observed in the response to GH treatment in terms of abdominal SAT, liver fat content, thigh IMAT and thigh muscle fat content (data not shown).

Table 6. Baseline clinical features in men (n=15) and women (n=15) with abdominal obesity and reduced insulin sensitivity

Variable	Men	Women	P value[§]
Age (years)	58 (51-64)	58 (51-63)	0.8
BMI (kg/m^2)	29.6 (0.6)	30.7 (0.9)	0.3
Waist (cm)	107 (1)	103 (2)	0.1
Sagittal diameter	25.8 (0.3)	25.5 (0.4)	0.6
Smoking[◆]	2	2	
Antihypertensive treatment[◆][*]	2	3	
Weight (kg)	92.8 (2)	85.7 (3)	0.6
Total BF (kg)	27.9 (2)	38.9 (2)	0.001
FFM (kg)	64.9 (1)	46.8 (1)	0.0001
Abdominal SAT (cm^2)	293.3 (21.0)	442.2 (25.3)	0.0001
VAT (cm^2)	195.4 (13.4)	167.3 (8.9)	0.09
Liver attenuation (HU)	45.9 (4.4)	48.7 (2.8)	0.4
Thigh IMAT (cm^2)	7.6 (0.6)	8.2 (0.6)	0.5
Thigh SAT (cm^2)	101.3 (6)	193.0 (10.5)	0.0001
Thigh muscle attenuation (HU)	42.7 (0.8)	39.7 (1.1)	0.05
Thigh muscle mass (cm^2)	159.1 (4.2)	109.6 (3.4)	0.0001

All the values are expressed as the mean and SEM or range. N = 30 (men:15, women:15).
§ P value by unpaired t test, between groups at baseline.
◆number of men and women.
* b-blockers, ACE inhibitors, AT-II antagonist

Within-group analysis: a 12% reduction in the thigh IMAT area was only observed in the male group (p < 0.05 vs. baseline).
In Paper III, baseline GDR, FFA and leptin values were higher in women than in men, while there were no baseline differences in fasting glucose, serum lipids and plasma insulin (Table 7). After 12 months, a reduction in serum LDL-cholesterol level from 4.3 (0.2) to 3.9 (0.2) mmol/L was seen in the female group (p = NS vs. men). Other circulating lipid levels were not significantly changed by treatment. Women responded with a more marked increase in serum fasting insulin concentration than men (Table 7). No difference in the response of adipokines or other metabolic indices was seen between the groups (Table 7).
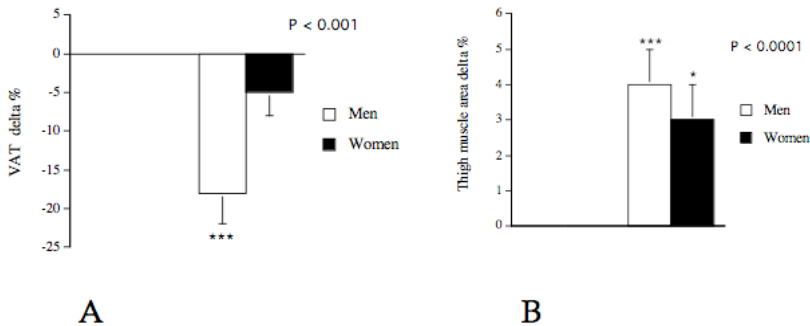
Figure 14. A: *Reduction in VAT expressed as delta % between the groups, P < 0.001.*
B: *Increase in thigh muscle mass area expressed as delta % between the groups, P < 0.0001*

GH secretion and regional fat distribution

Indices of GH secretion estimated by 12-h GH profiles in 19 postmenopausal women with abdominal obesity are illustrated in Table 8.

In Paper IV, both basal and pulsatile GH secretion, as estimated using 12-hour night-time GH profiles, correlated negatively with VAT and thigh IMAT but not with mean thigh muscle attenuation or hepatic fat content when applying simple regression analysis. There was no correlation between ApEn and regional AT depots.

VAT area correlated positively with thigh IMAT ($r = 0.7$; $p < 0.0001$) and inversely with thigh mean muscle attenuation ($r = -0.5$; $p < 0.04$). An inverse correlation was also found between mean thigh muscle attenuation and thigh IMAT ($r = -0.7$; $p < 0.002$).

By entering basal GH secretion as a dependent variable and VAT, thigh IMAT, age and GHBP as independent variables in a forward stepwise regression analysis, a negative correlation was observed between basal GH secretion and VAT (Figure 15a) (*B* coefficient = -0.77, $p < 0.001$) but not with thigh IMAT. In the regression equation, VAT and age explained 57% of the variability in basal GH secretion (R square = 0.57; overall p-value = 0.002). By using pulsatile GH secretion as the dependent variable, an inverse correlation between pulsatile GH secretion and IMAT was demonstrated (Figure 15b) (*B* coefficient = -0.67; $p < 0.01$. R square = 0.45; overall p-value = 0.002), whereas the relationship with VAT became non-significant. In the same multiple regression model, neither age nor GHBP showed any correlation with basal or pulsatile GH secretion.

In the second multiple regression model, entering log basal GH secretion as the dependent variable, VAT was the only independent predictor of basal GH secretion, while IGFBP-1, triglycerides, GDR, fasting insulin and fasting glucose lost significance. In the same model,

using pulsatile GH secretion as the dependent variable, thigh IMAT was the only independent predictor of pulsatile GH secretion, whereas VAT and metabolic risk factors had no predictive value.

Table 8. GH secretion indexes in postmenopausal women with abdominal obesity

Total GH secretion (basal + pulsatile)	15.8 ± 2.50
Basal GH secretion (basal/min x sampling duration)	1.80 ± 0.26
Pulsatile GH secretion N of peaks x mean area)	11.6 (1.9 - 36.3)
Area (pulse mass)	1.55 (0.27 - 5.40)
Number of peaks	7 ± 0.3
Mean interval	108 ± 5.49
Half life (min)	18.6 ± 0.5
ApEn*	0.5 (0.2 - 0.7)
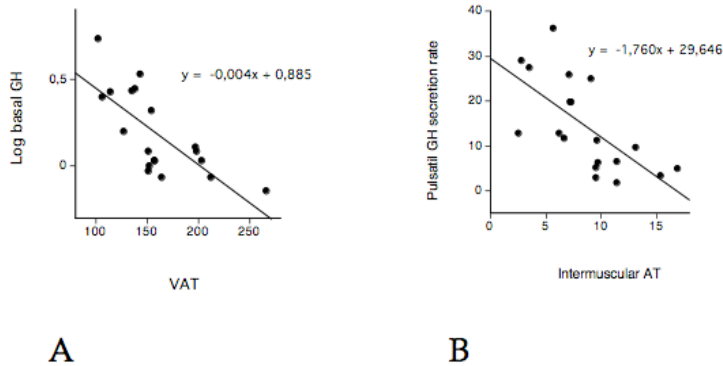
Figure 15. A: *Association between log basal GH secretion (µg/L/min x sampling duration) and VAT (cm²). Persson correlation coefficient:* $r = -0.7$; $P = 0.001$. B: *Association between pulsatile growth hormone (GH) secretion (number of peaks x mean area) and thigh IMAT (cm²). Pearson correlation coefficient:* $r = -0.7$; $P < 0.002$.

Physical activity and quality of life assessments

Baseline physical activity determined using a questionnaire was similar in both groups and remained unchanged during the study. The Psychological General Well-Being (PGWB) test did not reveal any difference in quality of life between the groups at any time (Paper I).

Side-effects and compliance

In Paper I, twelve women in the GH-treated group experienced side-effects related to fluid retention (arthralgia, joint stiffness or peripheral edema). These side-effects appeared during the first four weeks of treatment and were all considered to be of mild to moderate severity. In 11 subjects, dose adjustments were required, while in one subject the symptoms subsided spontaneously after nine months. Two of the 11 subjects presenting signs of fluid retention had an IGF-1 score of > 2 SD at the end of the trial.

Five drop-outs occurred in the GH-treated group, four of which could be potentially attributed to the GH treatment (Figure 3). Type 2 DM was diagnosed in one woman by OGTT at baseline. Since the baseline OGTT was performed three weeks after inclusion, the diagnosis of DM was delayed and the patient was excluded from further treatment at six months. In retrospect, DM was already present during the OGTT at the first visit. However, these data were not available until one week after the test was performed. One woman was withdrawn after four months of treatment due to the diagnosis of an epidermoid tumour in the oral cavity. The lesion was already

present several months before the start of the trial, but the diagnosis was first established by biopsy two months later. One subject left the trial after three months of persistent swelling and numbness in spite of dose adjustments. One subject complained of profuse perspiration one month after the start of treatment and was excluded from the trial after two months when oestrogen treatment was commenced, while one subject decided to discontinue treatment after three months as she experienced increased hair loss.

Dose adjustments were made in one of the five who discontinued GH treatment. In the placebo group, two women complained of slight peripheral oedema and dose adjustments were made in one of them who decided to discontinue treatment after six months because of recurrent pyelonephritis. Compliance with treatment was 97.4% in the GH-treated group and 95% in the placebo group.

Discussion

Twelve-month GH treatment showed a reduction in intra-abdominal fat and an increase in muscle mass and a decline in total and LDL cholesterol concentrations in postmenopausal women with abdominal obesity (Paper I). Insulin sensitivity was improved within the GH treated women (Paper I). GH treatment reduced subclinical inflammation but did not affect endothelial dysfunction (Paper II). The comparative study of 12-month GH treatment between 15 abdominally obese men and women (Paper III) showed that men are more responsive than women in terms of body composition, exhibiting a more marked reduction in VAT and increase in muscle mass, without no effect on insulin sensitivity. In paper IV, the 12-h GH profiles showed that the association between GH secretion and body fat is depot dependent.

The target dose of GH was selected on the basis of previous reports that suggest that a daily dose of GH of about 0.6 mg/day would match the physiological GH production in middle-aged women (77). An increase in the mean IGF-1 SD score to 1 SD after six months, with no further changes after 12 months, indicated that the given dose was within the physiological range (for women in Papers I-III). However, dose adjustments were necessary in 11 of the GH-treated women, suggesting that a dose lower than the selected target dose might have been suitable for some of the women. In Paper III, the mean IGF-1 score rose to almost 2 SD, indicating that the GH dose in men was slightly over the physiological range (78). Serum IGF-1 values increased, however, similarly in both genders in response to an approximately similar GH dose. Therefore, the present analyses of gender differences in the responsiveness to GH treatment were not to any major extent, affected by between-group differences in GH dosing or in serum IGF-I levels.

Effect of GH treatment on insulin sensitivity (Paper I)

The primary efficacy variable in the intervention study was the change in GDR, an established method for assessing insulin sensitivity. In Paper I, there was no between-group difference in GDR at baseline or during treatment. However, the GH-treated women who had the lowest baseline insulin sensitivity values demonstrated the best improvement in insulin sensitivity as compared with GH-treated women with greater insulin sensitivity at baseline. The possibility of a regression towards the mean is unlikely as this was not seen in the placebo group, indicating that subjects with the most severe insulin resistance respond best to treatment. After an initial deterioration, the glucose metabolism remained unaffected as compared with baseline in terms of fasting plasma glucose, serum insulin and HbA1c levels.

In contrast to insulin sensitivity, glucose tolerance did not show any improvement in the GH-treated women as compared with placebo. This apparent discordance may be explained by the fact that insulin sensitivity estimated by the euglycemic insulin clamp in paper 1 represents the whole-body insulin sensitivity (hepatic and peripheral), as in this study was not

used labeled glucose to quantify the individuals contributions of hepatic and muscle insulin sensitivity. On the other hand, glucose tolerance estimated by OGTT predominantly reflects the grade of disturbances in the peripheral (primarily muscle) insulin-mediated glucose metabolism (79). In addition, OGTT also provides an index of defective insulin secretion **(80).**

Effect of GH treatment on body composition (Paper I)

By using CT scan, a reduction in VAT and an increased amount of thigh muscle mass were demonstrated in the GH-treated women. In contrast to a similar study including middle-aged men with abdominal obesity who received GH treatment for nine months (57), no changes in abdominal or thigh subcutaneous AT were found in this study (Paper I), suggesting that postmenopausal women are less responsive to the lipolytic effect of GH in the subcutaneous fat depots. It is known that young women with GHD due to a pituitary disease require higher doses of GH than men at a similar age to achieve a comparable serum IGF-I response, which is associated with the level of estradiol (81, 82).

In addition, data comparing in vitro abdominal and gluteal SAT metabolism suggest that the menopausal status is associated with changes in adipose tissue metabolism that predispose to lower lipolysis and higher activity by lipoprotein lipase LPL, in abdominal and gluteal SAT (83). Furthermore, in Paper III, men showed more pronounced responsiveness to GH treatment in terms of reduced intra-abdominal fat which supports the hypothesis that women are less responsive to the lipolytic effect of

GH. However, since the women in these studies were all postmenopausal and were not receiving oestrogen replacement, it is most likely that the gender differences in response to GH treatment could be related to the synergistic action of endogenous testosterone on GH rather than to oestrogen.

There are conflicting results when it comes to whether GH reduces fat mass through direct effects on the adipocyte, making the adipocyte more responsive to catecholamine, or through an inhibitory effect on LPL (84, 85). We did not examine cellular fat metabolism, but our results suggest major responsiveness by VAT compared with subcutaneous AT, which is in agreement with previous data in GH-deficient subjects (86, 87), promoting more favourable peripheral fat distribution in terms of the cardiovascular risk (88). The intrinsic metabolic charactheristics of VAT as well as its higher blood flow that drains directly in the portal vein system, are essential for the highly lipolytic activity of VAT. A higher β-adrenergic receptor density, lower $\propto_2$-adrenoreceptor inhibition (12) and a higher density of glucocorticoid and androgen receptors which in turn modulates LPL activity (89) does VAT more responsive to stimuli than abdominal and gluteal SAT.

Lipid metabolism (Paper I)

A reduction in serum total cholesterol and LDL cholesterol was observed in the GH-treated women, although the reduction in LDL cholesterol was more marked after the first six months (10%) compared with that after 12 months (5%). In some studies dealing with GH-deficient patients receiving GH replacement therapy, a transient reduction in total cholesterol, LDL cholesterol and the LDL-

cholesterol/HDL ratio, the total cholesterol/HDL ratio and an increase in lipoprotein (a) have been reported (47, 90). In contrast to these data, no significant changes in lipoprotein (a) or total apo B were observed in Paper 1. One plausible explanation is that the target dose of GH in these women was considerably lower than that used in previous trials in GHD adults. The mechanism behind the reduction in total- and LDL cholesterol may be an effect of enhanced hepatic LDL receptor activity (91).

The tendency towards an improvement in insulin sensitivity, the increased muscle mass and the reduction in VAT in Paper I are less likely to be explained by caloric restriction or increased exercise, as the participants did not receive any dietary intervention and did not report any change in physical activity as determined by a questionnaire. This placebo-controlled trial demonstrated beneficial metabolic effects by GH treatment in women with abdominal obesity and particularly in those women who were more insulin resistant. The trend toward an improvement in insulin sensitivity was associated with reduced hepatic fat content.

In contrast to other AT depots, VAT has a direct connection to the liver through the portal vein. Visceral obesity probably increases the delivery of fatty acids to the liver, contributing to hepatic fat accumulation. GH treatment, with its strong lipolytic action on VAT, might therefore induce or aggravate non alcoholic fatty liver disease. Our data, however, suggest that 12 months of treatment reduces the hepatic fat content as a result of reduced VAT and/or an increase in the output of fat from the liver by enhanced VLDL production and

secretion (92) or increased biliary lipid output (93). These data therefore support the hypothesis that the improvement in insulin sensitivity exhibited in the GH-treated women might be mediated at least in part by the reduction in hepatic fat content.

Effect of GH treatment on sub-clinical inflammation (Paper II)

There were reductions in serum CRP and IL-6 concentrations after six and 12 months of GH treatment as compared with placebo, whereas there was no significant treatment effect in sE-selectin, SAA or markers of endothelial dysfunction (VCAM-1, ICAM-1 or MMP-9) (Paper II).

These findings suggest that GH exerts an attenuating effect on the state of chronic inflammation associated with the metabolic syndrome. In support of this hypothesis, there is evidence that women with severe GH deficiency due to hypothalamic-pituitary disease, otherwise receiving conventional hormonal replacement, have increased CRP and IL-6 concentrations (94). Furthermore, GH replacement in men with hypopituitarism was found to reduce CRP and IL-6 levels (90). Several possible mechanisms by which GH might exert an effect on the inflammatory process have been suggested, including a direct effect by GH or an indirect effect mediated by IGF-1 on the immune system (95), endothelial cells (96) or changes mediated by the improved body composition (94).

There was a positive relationship between baseline serum CRP and IL-6 concentrations and intra-abdominal fat and hepatic fat content – the latter expressed by an inverse relationship

between CRP and liver attenuation, whereas no associations were found between either CRP or IL-6 and total BF. In addition, the reduction in CRP and IL-6 was positively associated with the reduction in VAT and hepatic fat content. These findings support the hypothesis that intra-abdominal fat accumulation and hepatic fat content, more than adiposity per se, play a central role in generating the pro-inflammatory state associated with the metabolic syndrome.

Intra-abdominal fat mass is particularly metabolically active, exhibiting not only high lipolytic activity with increased FFA flux into the circulation that leads to insulin resistance but also an increase in the release of IL-6 and other cytokines (10). In addition, NAFLD (a variety of conditions from fatty liver to non-alcoholic steatohepatitis) has been associated with the metabolic syndrome (21), as well as with the increased hepatic production of cytokines and CRP. On these bases, the reduction in serum CRP and IL-6 concentrations in Paper II may have been mediated by the lipolytic effect of GH and its reduction in visceral fat mass and hepatic fat content. A reduction in hepatic fat content may thus contribute to a reduction in the production of CRP by the hepatocytes, production which is mainly induced by IL-6 (97, 98).

One other possible mechanism might be that the observed effects are mediated by the GH-induced increase in IGF-1. This is supported by the inverse association between the increase in serum IGF-I and the reduction in serum CRP and IL-6 concentrations. On the other hand, the increase in serum IGF concentration was associated with a reduction in the hepatic fat content and visceral fat mass. Since hepatic tissue express very low levels of

IGF-1 receptors a plausible explanation is that IGF-1 exert an indirect effect on inflammation through improvement of insulin sensitivity by stimulating glucose uptake in the skeletal muscle (99). Taken together, these data suggest that the reduction in serum concentrations of CRP and IL-6 occurred as an effect of the treatment-induced reduction in VAT and hepatic fat content.

No association was found between serum markers of endothelial dysfunction and intra-abdominal fat mass in this population of postmenopausal women with abdominal obesity. This is in line with previous reports suggesting that circulating vascular adhesion molecules are associated with total BF rather than with regional adipose tissue distribution (100-102).

Although it is not known whether GH treatment has any primary effect on the vascular endothelium, there is evidence to suggest that GH can affect atherosclerosis in GH-deficient adults, with a reduction in early atherosclerotic plaques in the carotid arteries (103). This may be due to an effect mediated through IGF-I (104) and/or nitric oxide (105). We have not specifically studied atherosclerotic processes, but serum markers reflecting atherosclerosis were not significantly affected in this placebo-controlled trial, arguing against direct effects of GH treatment on the vascular wall. The present results, however, suggest that important risk factors for atherosclerosis and its progress, such as improvements in body composition, serum lipid pattern (106) and the reduction of sub-clinical inflammation, are significantly affected by GH treatment.

The reduction in serum levels of inflammatory markers by GH treatment was associated with a reduction in abdominal obesity and hepatic fat content, thereby suggesting an overall reduction in the risk of CVD. In spite of this, it remains to be determined whether prolonged GH treatment can reduce the progress of atherosclerosis in postmenopausal women.

Factors that could possibly have influenced the inflammatory process during this study were similar in both study groups. Firstly, there was no weight loss in either of the study groups. Secondly, smoking habits and, thirdly, concomitant medical therapy were similar in both groups.

Gender differences in responsiveness to GH treatment (Paper III)

A more pronounced response to GH treatment was seen in men than in women in terms of reduced intra-abdominal fat and increased muscle mass. The increase in FFM was, however, more pronounced in women. In addition, a reduction in thigh muscle fat content (expressed as an increase in thigh muscle attenuation) was only found in men.

Muscle volume increased more in men whereas the FFM increased more in women. The differences were small and not conclusive. In contrast to previous trials except in one, the serum IGF-I response was similar in GHD men and women (107) the anabolic action of GH treatment has been more marked in men (108).This is in line with previous studies of GH-deficient men that have demonstrated that testosterone enhances the effect of GH on serum IGF-1 level, resting energy expenditure, fat oxidation,

protein metabolism and extracellular water (45). On the other hand, oestrogen modulates GH action by several mechanisms that are independent of GH secretion (109). Before menopause, oestrogen promotes a peripheral pattern of fat distribution with less intra-abdominal fat and a greater accumulation of subcutaneous fat, particularly in the hip (110). Oral, but not transdermal, oestrogen downregulates serum IGF-1 levels through a first-pass effect on the liver. This reduced response in serum IGF-I levels may result in a less marked response to GH in lean body mass (109).

Since all the women were postmenopausal and were not receiving oestrogen replacement, it was expected that they would be just as responsive to GH as the male cohort. However, the women included in this study turned out to be less responsive to GH, as this treatment produced only a slight increase in thigh muscle mass and induced a non-significant 5% reduction in intra-abdominal fat. In addition, women exhibited a mild weight gain that could be explained in part by an increase in extracellular water volume and increased FFM. The present results therefore show that, in abdominal obesity, as previously shown in adult GHD, women have a lower responsiveness to GH than men in terms of changes in body composition.

In adult GHD, non-physiological replacement with sex steroids has been indicated as one possible explanation for gender differences in the response to GH replacement (45). In the present study, the observed gender differences in the response to GH cannot be explained by the action of oestrogen, as none of the women was receiving oestrogen therapy. It is therefore most likely that a

synergistic effect of the endogenously produced testosterone on GH action explains the greater response in the male group. The possibility cannot be excluded that women may be less responsive to GH than men due to other factors that have not yet been explored.

Insulin sensitivity remained unchanged in Paper III and this may be partially explained by weight gain in the women or by the absence of weight loss in the men. The weight gain in women may antagonise the lipolytic effect of GH and augment insulin resistance by diminishing the reduction of abdominal fat in particular. This hypothesis is supported by a study of diabetic patients that showed an improvement in insulin sensitivity after weight loss by combining GH treatment with a hypocaloric regimen (58). This suggests that GH may have an additive effect on the metabolic action of caloric restriction.

In Paper III, women had higher circulating FFA concentrations compared with men. There are previous observations that abdominal subcutaneous fat is the major source of FFA release in the postabsorptive state in obese women (111). The higher systemic FFA concentrations in women in this study may therefore have contributed to increase insulin resistance, particularly in skeletal muscles, and to attenuate the anabolic effect of GH.

There is experimental evidence that the GH receptor expression in liver and the growth plate is increased by testosterone and decreased by estradiol (112). Therefore, it has been suggested that this mechanism could explain gender differences in IGF-1 response to GH treatment in GH deficient adults (48).

Leptin and other adipokines are associated with insulin resistance (113). The women in Paper III exhibited higher leptin levels that men. This is consistent with a previous report (114) that suggested that the subcutaneous fat depot is the major source of leptin in women due to the combination of a mass effect and a higher secretion rate in SAT than in VAT. In GH deficient adults, GH replacement reduced serum leptin levels, possibly due to the gradual reduction in total body fat (115) (116) (117). In paper III, serum leptin concentrations were not affected by treatment either in women or in men. On the other hand, an unchanged amount of SAT in this study may explain the unchanged leptin levels. It is unclear whether the baseline between-group difference in leptin contributed to, or only was dependent of, the gender related variations in body fat distribution observed in this study.

It has been suggested that adiponectin may enhance insulin sensitivity by several metabolic pathways such as the inhibition of hepatic glucose production (118) and/or by increased muscle glucose transport and fatty acid oxidation in muscles (119). However, adiponectin remained unchanged after 1 year of GH treatment, and so was insulin sensitivity.

One limitation that should be taken into account in Paper III is that the up-titration of the GH dose differed between the two study groups. In women, the dose of GH was up-titrated during a period of six weeks, whereas in men the dose of GH was not fully up-titrated until after six months. However, women received a similar or even slightly higher dose of GH than men. After the titration phase was completed, the GH dose remained stable in both genders until the end of the study.

It is therefore unlikely that the differences in the titration of the GH dose contributed to the observed gender differences.

The response to GH treatment was more marked in men in terms of body composition. Sex steroids may therefore contribute to modulate the GH effect.

GH secretion and regional fat distribution (Paper IV)

Twelve-hour GH profiles with sampling every 20 minutes and a highly sensitive chemiluminiscence GH assay were used to appraise GH secretory status in Paper IV. The use of night-time GH profiles ensured that mean values reflected the secretion when maximum GH release occurred. The usefulness of night-time GH profiles for deconvolution and ApEn analysis has previously been validated with sampling every 10 minutes (41), whereas 24-hour GH profiles with sampling every 20 minutes is a well-established procedure (42, 120). Even 24-hour GH profiles with 30-minute sampling intervals yield marked contrasts in GH secretory patterns between GH-deficient adults and healthy controls (121). Since low-normal serum IGF-1 concentrations were a criterion for including women in the trial, the association between IGF-1 and GH secretion indexes was not estimated. As all the women were menopausal and were not receiving oestrogen-replacement therapy, varying oestrogen was not a confounding factor.

The findings in paper IV are in line with previous studies that increased VAT is linked with blunted GH secretion (38, 122). In healthy, pre-menopausal women, increased VAT accumulation was found to be associated with both reduced basal and pulsatile GH secretion and with a loss of regularity of GH release (17). Furthermore, the relationship between VAT and GH was independent of age and gender. From current and previous data, it is thus possible to conclude that the relationship between VAT and basal GH secretion is stronger in women than in men.

The mechanism for the relationship between VAT and blunted GH secretion has not yet been established, but increased serum levels of FFA in abdominal obesity may reduce GH secretion (123), as is also indicated in simple regression analysis. Subjects with abdominal obesity have blunted lipolysis during fasting which may be an effect of attenuated GH response during fasting (28). Abdominal obesity per se may therefore help to augment fat accumulation, possibly through a state of relative GH insufficiency (18)(30). It has also been suggested that insulin resistance induced by intra-abdominal fat accumulation may contribute to blunted GH secretion by suppressing serum IGFBP-1, leading to increased free IGF-1 levels (124). In Paper IV, simple regression analysis revealed inverse correlations between fasting insulin and both basal and pulsatile GH secretion and positive correlations between IGFBP-1 and both basal and pulsatile GH secretion support this mechanism.

IMAT has been described by using CT scan or more recently by using magnetic resonance imaging as the accumulation of adipose tissue surrounding skeletal muscles bundles. The inverse relationship between pulsatile GH secretion and IMAT found in this study has not previously been described. However, there is evidence that basal and pulsatile

GH secretion are regulated in a distinct manner (41). In rodents, data suggest that the pulsatile component of GH secretion is more important for body growth (125-127), whereas the basal component is more important for the lipolytic effect (128, 129). This may support our finding that thigh IMAT was more strongly related to the pulsatile component, whereas VAT was more strongly related to the basal component of GH secretion. It is therefore possible to hypothesise that there are different mechanisms for the interaction between thigh IMAT and pulsatile GH secretion compared with that between VAT and basal GH secretion. The mechanism for this depåt dependent association has not been studied and is not known. Hepatic fat content was not predictor of GH secretion indicating that hepatic fat infiltration is rather dependent of intra-abdominal fat accumulation.

Only abdominal fat depots correlated positively with GHBP concentration. GHBP levels may interfere with the GH analysis and could therefore be a confounding factor in the association between basal GH secretion and VAT. GHBP has been reported to cause disturbances in the GH immunoassays by competing with the antibodies for ligands as circulating GHBP are closely related to the amount of body fat in humans and subject to large interindividual variation (130). However, GHBP had no predictive value in the multiple regression model. This suggests that GHBP probably did not exert a major influence on the results.

IMAT, but not mean muscle attenuation, demonstrated a clear association with insulin sensitivity and GH secretion. The reason for these differences is unknown, but it may be related to the CT technique that was used to quantify the lipid content within muscle. Although the method of measuring muscle attenuation has proved to have low variability (<1%), it is not capable of differentiating between intramyocellular lipids and lipids outside the myocyte. Moreover, factors such as variations in the protein content of muscle, skeletal muscle perfusion and extracellular water may alter muscle attenuation (131). The muscle attenuation assessed using CT may therefore be a less specific measure of intra- rather than inter-muscular fat content.

Previous studies (41)ʼ (17) have shown a positive association between VAT and ApEn ratio, suggesting a loss of regularity of GH secretion with increasing VAT. In women with polycystic ovary syndrome (PCOS), a loss of regularity in the pattern of GH secretion was associated with VAT accumulation (43).

No correlation between ApEn and VAT was found in Paper IV. However, the selection of women with low-normal serum IGF-1 concentrations, together with the small range of VAT in this study, could possibly explain the lack of correlation.

The metabolic impact of IMAT has not been explored until recently. A relationship exists between thigh IMAT accumulation and insulin resistance in healthy individuals (131). Thigh IMAT is also associated with the abdominal fat accumulation seen in male and elderly female patients with DM type 2 (20). Furthermore, it has been proposed that, with increasing levels of adiposity, differences in regional AT distribution, with a greater IMAT accumulation in relation to VAT, could explain differences in glucose intolerance and DM that are related to ethnicity (132). In this study, a multiple regression model showed that VAT and hepatic fat content

were independent predictors of insulin resistance (35), whereas IMAT or muscle fat content were not. This is consistent with the hypothesis that the mechanism by which muscle metabolism causes insulin resistance is different from that induced by VAT (133). However, it does not exclude the possibility that impaired lipid oxidation in skeletal muscle is linked to both muscle fat accumulation and the development of visceral adiposity (26). Further studies are needed to explore this issue to determine whether increased IMAT deposition is simply the result of more severe obesity or whether it is specifically associated with intra-abdominal fat, as is suggested by our analysis, as women with increased thigh IMAT and abdominal fat showed more marked disturbances in their metabolic profile.

General Summary

1. GH treatment in abdominally obese women reduced VAT, increased thigh muscle mass, and reduced serum total and LDL-cholesterol (Paper I).

2. Insulin sensitivity was improved within the GH treated women (Paper I).

3. GH treatment reduced the subclinical inflammation but did not affect endothelial dysfunction (Paper II).

4. Abdominally obese men were more responsive to GH treatment than women in terms of reduction in VAT (Paper III).

5. GH treatment in abdominally obese men and women were well tolerated. Although, mild side-effects related to fluid retention were common, particularly during the first months of treatment (Papers I-III).

6. The 12-h GH profiles showed that crosstalk between GH secretion and body fat is depot dependent (paper IV).

General conclusions and perspectives for the future

Treatment of general as well as abdominal obesity include changes in lifestyle and eating habits, and / or increased physical activity. Behavioural therapy is also be considered to overcome problems related to compliance (134). However, a major problem is that sustained weight loss in the long term is generally poor. Surgical interventions have been reported to be promising (135). The medical treatment of specific metabolic disturbances such as hypertension, dyslipidaemia, insulin resistance and type 2 DM, is considered a part of an integrated strategy (89).

The results of the papers included in this thesis suggest that the relative state of hyposomatotropism present in abdominal obesity affects the cardiovascular risk profile. One of the strength of these studies is their double blind, placebo-controlled study design (Papers I and II). In addition, the use of high standard methodology for assessment of insulin sensitivity, body composition and estimate of GH secretion status strengthens the present findings.

Some important limitations to take into account are, in paper III, there were differences in the length of the dose titration phase of the GH dose between men and women that could influence the outcome. In paper IV, the relative small number of participants and the homogeneity of the group to postmenopausal women from European origin is an important limitation. A larger study, including subjects of both genders and different ethnical groups would be needed to further investigate the importance of the association between IMAT and pulsatile GH secretion.

The findings of this thesis are supported by studies in adult GHD patients in whom GH replacement has shown to improve most of the metabolic abnormalities also present in individuals with metabolic syndrome (78). Therefore, these studies suggest that GH treatment might be beneficial for women and men with low GH secretion associated with severe abdominal obesity. However, it is still not clear whether there is a causal relationship between alterations in the GH-IGH-1 axis and abdominal obesity. In addition, the use of GH treatment is limited to some extent, because it must be administered by daily subcutaneous injections and for its cost. For these reasons GH treatment to subjects with the metabolic syndrome is currently not recommended.

Larger and longer studies than those in the present study, including abdominally obese individuals of both genders, are needed to determine the efficacy of GH therapy on cardiovascular morbidity, insulin resistance, quality of life and long-term safety. In Paper I, the patients with the lowest insulin sensitivity at baseline displayed the most marked response to treatment. The future studies could therefore include patients with more severe baseline abnormalities than the present studies. Furthermore, GH secretion declines with increasing age, and it could also be of interest to study elderly patients with the metabolic syndrome. It is less likely that GH intervention will be a treatment alternative in all patients with abdominal obesity, so one of the main aims of future studies will be to identify subgroups of abdominally obese subjects that could benefit from GH treatment.

Orally active GH secretagogues could be considered an appropriate alternative therapy. GH secretagogues enhances the physiological pulsatile GH secretion by acting on specific receptors with multiple sites, interacting with the neuroendocrine hypothalamus (136), and thereby stimulating secretion of GH-releasing hormone from the hypothalamus into portal blood (137). This may result in fewer side effects than subcutaneous GH injections. However, GH secretagogue treatment in the human has not shown impressive effects on body fat. The GH secretagogues are, however, a less likely treatment alternative since they may, in addition to stimulate GH, also stimulate appetite (138). However, in the future, GH secretagogues could be available with fewer appetite-stimulating effects.

In conclusion, the studies presented in this thesis have shown that GH intervention improves the cardiovascular risk profile in abdominally obese postmenopausal women, and that the mechanisms modulating interactions between fat mass and the somatotropic axis are depot dependent. In abdominal obesity, there is a gender difference with a more marked treatment response in men than in women in terms of body composition. Low GH secretion may therefore be one of the mechanisms underlying the metabolic abnormalities associated with the metabolic syndrome.

Celina Franco

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