Clinical Study

P F Vestergaard and others Impact of age and gender on GH signaling

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GH signaling in skeletal muscle and adipose tissue in healthy human subjects: impact of gender and age

Poul F Vestergaard, Mikkel H Vendelbo, Steen B Pedersen, Anders Juul1, Steffen Ringgard2, Niels Møller, Niels Jessen and Jens O L Jørgensen

The Medical Research Laboratories, Department of Endocrinology and Internal Medicine, Faculty of Health Sciences, Institute of Clinical Medicine, Aarhus University Hospital, Aarhus University, Nørrebrogade 44, DK-8000 Aarhus C, Denmark, 1Department of Growth and Reproduction, University Hospital of Copenhagen, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark and 2Department of Clinical Medicine, MR Research Centre Aarhus University Hospital, Skejby, Brendstrupgaardsvej 100, DK-8200 Aarhus N, Denmark

Correspondence should be addressed to P F Vestergaard
Email poulv@ki.au.dk

Abstract

Objective: The mechanisms underlying the impact of age and gender on the GH–IGF1 axis remain unclear. We tested the hypothesis that age and gender have impacts on GH signaling in human subjects in vivo.

Design: A total of 20 healthy non-obese adults (‘young group’ < 30 years (5F/5M) and ‘old group’ > 60 years (5F/5M)) were studied after: i) an i.v. GH bolus (0.5 mg) and ii) saline.

Methods: Muscle and fat biopsies were obtained after 30 and 120 min. Total and phosphorylated STAT5B proteins, gene expression of IGF1, SOCS1, SOCS2, SOCS3 and CISH, body composition, VO2max, and muscle strength were measured.

Results: In the GH-unstimulated state, women displayed significantly elevated levels of CISH mRNA in muscle (P≤0.002) and fat (P≤0.05) and reduced levels of IGF1 mRNA in fat. Phosphorylated STAT5B (pSTAT5b) was maximally increased in all subjects 30 min after GH exposure and more pronounced in women when compared with men (P≤0.01). IGF1, SOCS1, SOCS2, SOCS3, and CISH mRNA expression increased significantly in muscle after 120 min in all subjects with no impact of age and gender. GH-induced pSTAT5b correlated inversely with lean body mass (LBM; r = −0.56, P=0.01) and positively with the CISH mRNA response (r=0.533, P≤0.05).

Conclusion: i) GH signaling in muscle and fat after a single GH bolus in healthy human subjects is age independent, ii) we hypothesize that constitutive overexpression of CISH may contribute to the relative GH resistance in women, and iii) experimental studies on the impact of sex steroid administration and physical training on GH signaling in human subjects in vivo are required.

Introduction

The activity of the growth hormone (GH)–insulin-like growth factor 1 (IGF1) axis in human subjects is determined by several factors including age and gender, but the cause–effect relationship remains uncertain (1, 2, 3).

Circulating GH and IGF1 levels start to decline shortly after the completion of puberty and this continues during adulthood and senescence. The underlying mechanisms do not seem to involve reduced pituitary responsiveness to GH secretagogues or increased sensitivity to the negative feedback by IGF1 stimulation (4). Both spontaneous and stimulated GH secretion in midlife adults are determined by abdominal adiposity rather than chronological age (5, 6), and weight loss partly restores impaired GH secretion in obesity (7). It is also well known that sex...
steroids regulate GH secretion at the pituitary level and also modulate peripheral IGF1 generation (2). In adult males, testosterone stimulates GH secretion (2) and some but not all studies suggest that testosterone enhances GH-induced IGF1 stimulation (8, 9). Adult females when compared with males exhibit higher GH levels despite similar serum IGF1 levels (1, 2, 6, 10), which seems to be at least partly explained by a suppressive effect of estrogen on hepatic IGF1 production (11).

The physiological and clinical implications of these age- and gender-dependent differences in GH secretion and serum IGF1 levels are uncertain and controversial. It was originally suggested that the senescent decline in GH secretion is causally linked to the concomitant changes in body composition and physical function and that these changes could be reversed by restoration or ‘rejuvenation’ of GH levels (12). This simple paradigm has subsequently been reuted as numerous GH intervention trials showed only marginal beneficial effects but many side effects (13, 14). In this regard, it is also noteworthy that enhanced IGF1 activity in several species is associated with reduced longevity (15). These ambiguities emphasize the need to improve our understanding of how GH actions are modulated by age and gender. In that respect, experimental studies in GH-deficient adult patients demonstrate that elderly patients are highly responsive to exogenous GH in terms of both serum IGF1 generation and side effects such as fluid retention and insulin resistance (16). Moreover, it has been confirmed that female patients exhibit relative GH resistance at the levels of serum IGF1 generation, loss of body fat, stimulation of bone turnover, and lowering the circulating cholesterol levels (16, 17). However, little is known regarding the molecular mechanisms underlying these differences in GH action at peripheral target tissue levels. We have previously documented that exposure to a physiological GH bolus translates into detectable activation of signaling mechanisms in human skeletal muscle and adipose tissue in vivo (18, 19, 20, 21). These studies, however, have only included young male subjects.

In the present protocol, we therefore included females as well as males, aged either below 30 years or above 60 years, each of whom received an i.v. GH bolus followed by muscle and fat biopsies for assessment of GH signal transduction including phosphorylated STAT5B and the expression of target genes including IGF1, suppressors of cytokine signaling 1–3 (SOCS1, SOCS2, and SOCS3), and cytokine-inducible SH2 protein (CISH), which act as feedback inhibitors of GH signaling. This allowed us to test the hypothesis that age and gender have impacts on GH signaling in human subjects.

Subjects and methods

Subjects

A total of 20 normal-weight subjects (mean ± S.E.M., BMI: 25 ± 2) comprising ten ‘young’ subjects (5F/5M) with a mean (range) age of 25 (20–28) years and ten ‘old’ subjects (5F/5M) with a mean age of 65 (60–72) years participated in this study. None were smokers or receiving prescribed drugs, and none of the females received estrogen supplementation. The study protocol was approved by the Regional Ethics committee of Denmark (m2010-0121) and conducted in agreement with the declaration of Helsinki II. All participants received oral and written information before written informed consent was obtained. Before inclusion, each subject underwent physical examination including plasma measurements of cholesterol, ALAT, HbA1c, glucose, TSH, creatinine, Hb, and electrolytes.

Body composition and physical fitness

Body composition was assessed by conventional anthropometry and dual-emission X-ray absorptiometry. MR spectroscopy was performed using a Signa Excite 1.5 tesla twin-speed scanner (GE Medical Systems) to quantify fat in muscle (IMCL) and liver (IHL) as described previously (22, 23). The spectra were quantified using the LC model software package (LCModel6.2; Stephen Provencher, Oakville, ON, Canada) by means of dedicated muscle and liver spectroscopy fitting models. The data processing provided an estimate of the ratio of lipid to water in the tissue within the voxel.

Maximal oxygen uptake (VO2max) was measured by breath by breath during an incremental bicycle test (Jaeger ER800 bicycle, Erich Jaeger, Hoechberg, Germany). The VO2max test was performed with a pedaling rate of 70 r.p.m.; for the first 3 min the subject worked at 40 W, then the workload was increased by 3 W every 10 s until exhaustion. The test subjects were verbally encouraged during the test. The maximal oxygen uptake was afterwards defined as a mean of a 30 s period during the highest oxygen uptake (Oxycon Pro, Erich Jaeger). Finally, maximal isometric knee extension was measured using the Metitut isometric muscle strength device (Metitut, Jyväskylä, Finland). All measurements of body composition and physical performance were performed within 1 month before GH exposure.
Experimental design

In a single blinded and randomized crossover design, each subject was studied twice receiving either a single i.v. bolus of 0.5 mg GH (genotropin miniquick, Pfizer) or saline. At least 14 days elapsed between each study; all five ‘young’ females were examined 14 days after the first menstrual day and again 1 month later.

Each subject attended the clinical research unit at 0800 h after an overnight fast. The subjects abstained from strenuous physical exercise 2 days before each experimental day. At 0830 h (t=0), the first blood sample was drawn followed by an i.v. bolus of GH or saline. Muscle and fat biopsies were taken at 30 and 120 min after the bolus. Blood samples were drawn at t=0, 5, 10, 20, 30, 40, 50, 60, 90, and 120 min.

Serum hormones and metabolites

Serum samples were frozen and stored at –20°C. Serum insulin and GH levels were measured using time-resolved fluoroimmunoassay (TR-IFMA, AutoDELFI; PerkinElmer, Turku, Finland), and non-esterified free fatty acids (NEFAs) were analyzed using a commercial kit (Wako Chemicals, Neuss, Germany). Total serum IGF1 was determined by time-resolved immunofluorometric assay with modifications as published recently (24), at t=0, 30, 60, and 120 min after GH and saline bolus.

Muscle and adipose tissue biopsies

Under sterile conditions, using local anesthesia (Lidokain: Amgros, Copenhagen, Denmark), skeletal muscle and adipose tissue biopsies were taken. The muscle specimen was obtained from the vastus lateralis of the quadriceps femoris muscle 12–15 cm proximal to the superior border of the patella, using a Bergström biopsy needle. The muscle tissue was immediately dissected free from fat and connective tissue and snap-frozen in liquid nitrogen. The adipose tissue was obtained by liposuction from the periumbilical subcutaneous tissue. The adipose tissue was washed and subsequently snap-frozen in liquid nitrogen.

Western blotting and quantitative RT-PCR

Muscle biopsies were freeze dried and the remaining fat was removed. Freeze-dried muscle tissue (5–6 mg) was homogenized in an ice-cold solubilization buffer containing 20 mM Tris, 50 mM NaCl, 50 mM NaF, 5 mM Na4P2O7, 250 mM sucrose, 1% (v/v) Triton X-100, 2 mM dithiothreitol, 0.1 mM benzamidine, 0.5 mM phenylmethylsulphonyl fluoride, 50 µg/ml soybean trypsin inhibitor, and 4 µg/ml leupeptin, pH 7.4, in a Precellys24 homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) at 6500 r.p.m. for 2×30 s. Samples were rotated for 15 min at 4°C and centrifuged at 13 000 r.p.m. for 20 min at 4°C. The supernatant was collected and the protein concentration was determined using the Bradford assay (Protein Assay, #500-0006, Bio-Rad Laboratories, Inc.).

Adipose tissue was homogenized in a solubilization buffer containing 20 mM HEPES, 10 mM NaF, 1 mM Na3VO4, 1 mM EDTA, 5% SDS, 50 µg/ml soybean trypsin inhibitor, 4 µg/ml leupeptin, 0.1 mM benzamidine, 2 µg/ml antipain, and 1 µg/ml pepstatin in a Precellys24 homogenizer (Bertin Technologies) for 2×30 s at 5000 r.p.m. After homogenization, the samples were thermomixed at 1000 r.p.m. for 1 h at 37°C. The infranatant was collected, frozen in liquid nitrogen, and stored at –80°C until analyses.

Western blot analyses were performed by SDS–PAGE on Stain-Free 4–15% gels using the CriterionXT-system (Bio-Rad). Proteins were transferred onto PVDF membranes, blocked for 2 h in 2.5% skim milk. Antibodies against phosphorylated STAT5B and total STAT5B from Cell Signaling Technology were used, Cat. nos: #9359 and #9358 respectively (Cell Signaling Technology, Beverly, MA, USA). STAT5B was visualized by ECL chemiluminescence using a ChemiDoc XRS system (Bio-Rad) and quantified using Image Lab (ver. 4.0.1, Bio-Rad). Quantification of STAT5B phosphorylation is expressed as a ratio of total STAT5B expression measured on the same membranes.

Total RNA was isolated from muscle and adipose tissue samples using Trizol (Gibco BRL, Life Technologies); RNA was quantified by measuring absorbance at a wavelength of 260 and 280 nm with a ratio ≥1.8 using a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Integrity of the RNA was checked by visual inspection of the two rRNAs, 18S and 28S, on an agarose gel. cDNA was synthesized using the Verso cDNA Kit AB 1453 (Thermo Fisher Scientific, Inc.) using random hexamers. Real-time PCR for target genes were carried out with β2-microglobulin levels as an internal control, and this expression did not change during intervention. The sequences of the used primers are as follows: IGF1: GACAGGGGCTTATATTCG and CTCCAGCCTCTTGATCG; SOCS1: ACGCAGCTCCGCATTTG and CGAGGCCTACCGTGAAG; SOCS2: GGTCAGGGGATCGG and TCCCTGAGTCA-GTGCGAATC; SOCS3: GGGCACCCTGACTCTATGA and
GCCCCTTGCGCCCTTT; and \textit{CISH}: GCCCTGAGCCCTGG-TAGTCC and GACACATCCACAGGCGTGG.

The PCRs were performed in duplicate using the KAPA SYBR FAST qPCR Kit (Kapa Biosystems, Inc., Woburn, MA, USA) in a LightCycler 480 (Roche Applied Science) using the following protocol: one step at 95 °C for 3 min, then 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 10 s. The increase in fluorescence was measured in real time during the extension step. The threshold cycle (CT) was calculated, and the relative gene expression was estimated essentially using the default ‘Advanced Relative Quantification’ mode of the software version LCS 480 1.5.0.39 (Roche Applied Science).

**GHR polymorphism**

Genomic DNA was extracted from blood lymphocytes. The frequency of \textit{GHR} transcript variants with retention (fl-\textit{GHR}) or exclusion (d3-\textit{GHR}) of exon 3 was tested by the multiplex PCR assay described by Pantel et al. (25). This was performed with primers G1, G2, and G3 (GenBank accession no. AF155912) as follows: initial step of denaturation for 3 min at 95 °C, followed by 25 cycles consisting of 30 s at 95 °C, 1 min at 64 °C, and 1 min at 72 °C, followed by an extension period at 72 °C for 5 min. Amplification of DNA fragments was analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide. A 935-bp band represented the full-length (fl-\textit{GHR}), and a 532-bp fragment represented the d3 allele (d3-\textit{GHR}). We have previously published the methodology (26).

**Statistical analysis**

The Shapiro–Wilk test was used to test for normal distribution. If data were not normally distributed, log transformation was applied and data were retested. Normally distributed data are expressed as mean ± S.E.M. Two-way repeated measurements (2 ANOVA) were applied to analyze the effect of GH. If there was a significant effect of GH, placebo data were removed. Thereafter, 2 ANOVA was applied to test for different outcomes in group, age, and sex respectively. Non-parametric statistics was used to test for differences in age, and basal levels of \textit{SOCS1}, \textit{SOCS2}, \textit{SOCS3}, \textit{CISH}, and \textit{IGF1} mRNA in muscle and adipose tissue (Mann–Whitney rank sum test), and data are expressed as median (range). Correlation was assessed with the Pearson correlation analysis. \( P < 0.05 \) was considered significant. Statistics and figures were produced using SigmaPlot version 11.0 (Systat Software, Germany).

**Results**

**Body composition and physical performance**

As expected, males weighed more than females, whereas fat mass was increased in females when compared with males (Table 1). In addition, muscle strength reached higher values in males when compared with females and in the young subjects when compared with the older subjects. The amount of IMCL and IHCL fat did not differ significantly between groups.

**Serum levels of GH, IGF1, and NEFAs**

Following the GH bolus, \( C_{\text{max}} \) (mean ± S.E.M.: 93.2 ± 3.6 \( \mu \)g/l) was recorded after 5 min (Fig. 1a). The serum GH profile following the GH bolus, as defined by \( C_{\text{max}} \), \( T_{\text{max}} \), \( T_1/2 \), and AUC, did not differ according to either age or gender (data not shown). In the GH-unstimulated state

| Table 1 | Data on body composition and physical fitness in the 20 participants. |
|---------|-----------------------------|-----------------------------|-----------------------------|
|         | Young \((n=10)\)             | Old \((n=10)\)               | \( P < 0.05 \)               |
|         | Male \(n=5\)                | Female \(n=5\)              | Gender | Age |
| Age (years) | 25 (20; 27) | 25 (23; 28) | 66 (63; 69) | 65 (60; 72) | * | * |
| Weight (kg) | 86 ± 13  | 72 ± 5  | 67 ± 8  | 62 ± 8  | 25 ± 3  | 24 ± 3  |
| BMI (kg/cm²) | 25 ± 2  | 24 ± 1  | 18.1 ± 4 | 21.3 ± 11 | 136.6 ± 19.4 | 122.6 ± 19.8 | * | * |
| Fat (kg) | 14.8 ± 5 | 23.0 ± 5 | 18.1 ± 4 | 21.3 ± 11 | 136.6 ± 19.4 | 122.6 ± 19.8 | * | * |
| Serum IGF1 (\(\mu\)g/l) | 197 ± 19.7 | 219 ± 24.7 | 136.6 ± 19.4 | 122.6 ± 19.8 | 562 ± 40 | 396 ± 128 | * | * |
| Knee force (N) | 851 ± 149 | 440 ± 25 | 2839 ± 292 | 2855 ± 663 | 2839 ± 292 | 2855 ± 663 | * | * |
| VO₂max (mIO₂/min per kg) | 5712 ± 574 | 3123 ± 460 | 0.006 ± 0.003 | 0.004 ± 0.001 | 0.002 ± 0.002 | 0.005 ± 0.005 | * | * |
| IMCL (AU) | 1.8 ± 1.6 | 1.3 ± 0.6 | 3.2 ± 1.2 | 1.6 ± 0.3 |  |  |
| IHCL (AU) |  |  |  |  |  |  |

IMCL, intra-myo-cellular lipid; IHCL, intra-hepatic cellular lipid. For further details, see text. Significant differences (\(*P < 0.05\)) between the ‘young’ group \((n=10)\) and the ‘old’ group \((n=10)\) and between males \((n=10)\) and females \((n=10)\).
(saline injection), spontaneous GH peaks of ≈ 10 µg/l were observed in three subjects after 20–30 min. Serum IGF1 levels (µg/l) at baseline were significantly higher in the ‘young’ group when compared with the ‘old’ group (208±15.3) vs 129.6±13.3 (P<0.001) without any impact of gender (P=0.878), and no significant changes in serum IGF1 were recorded in response to either GH or saline. Serum NEFA levels increased significantly with time only after the GH bolus, and the peak level occurring at t=120 min was significantly elevated when compared with the GH-unstimulated state (P<0.001).

STAT5B phosphorylation

Phosphorylated STAT5B (pSTAT5B) (thy694) in muscle was significantly increased in all subjects at 30 min after GH injection followed by a reduction in activity after 120 min. In the GH-unstimulated state, no consistent changes in pSTAT5b activity were recorded (Fig. 2). The response to GH was more pronounced in females when compared with males (P<0.001) without a significant impact of age (P=0.132) (Fig. 3a). A significant GH-induced pSTAT5B response (P=0.003) was also recorded in adipose tissue with a maximal activity after 30 min without any significant impact of either age (P=1.0) or gender (P=0.65) (Fig. 3b).

IGF1, SOCS, and CISH mRNA levels

In the GH-unstimulated state, CISH mRNA expression in muscle and fat was more pronounced in females when compared with males, corresponding to 359% higher expression in muscle (P=0.002) and a 130% higher expression in fat (P=0.05) (Fig. 4), whereas IGF1 mRNA expression in adipose tissue was more pronounced in males compared with females (P=0.013), corresponding to a 83% higher level in males (Fig. 4). The GH-unstimulated expression of SOCS1, SOCS2, and SOCS3 did not differ between the groups (data not shown). In muscle tissue, a significant GH-stimulated response in IGF1 mRNA was observed after 120 min in all subjects (P=0.003) (Fig. 5). No significant gender effect was recorded (P=0.437). Furthermore, muscle SOCS1, SOCS2, SOCS3, and CISH mRNA expression increased significantly after 120 min (P<0.001) (Fig. 5), with no impact of age (P=0.950) or gender (P=0.136). Adipose tissue CISH mRNA was significantly increased 120 min after injection of GH (P<0.001) without any impact of age (P=0.157) or gender (P=0.114) (Fig. 5). We did not record a significant GH-induced increase in mRNA expression of IGF1 (P=0.962), SOCS1 (P=0.386), SOCS2 (P=0.091), nor SOCS3 (P=0.637) in adipose tissue.

Correlations

The GH-induced increase in muscle pSTAT5B activity in all participants at t=30 min was inversely correlated with LBM (r=−0.558, P=0.010) and positively correlated with the concomitant increase in muscle CISH mRNA at t=120 min (r=0.533, P=0.028). No correlations were observed between the GH-induced elevations in muscle pSTAT5b and serum NEFA levels (r=−0.155, P=0.513), strength (r=−0.326, P=0.186), or VO2max (r=−0.261, P=0.296) respectively. A significant inverse correlation between IHL and serum IGF1 was observed for the study population as a whole (r=−0.552, P=0.027).

In the GH-unstimulated state, IGF1 mRNA expression in muscle biopsies 30 and 120 min after exposure to saline (a) and GH (b) in females (open triangles) and males (filled triangles).
The pSTAT5b response to GH in adipose tissue was not correlated with body composition, serum NEFA levels, or CISH mRNA (data not shown).

**GHR polymorphism**

A total of 18 subjects were analyzed for GHR polymorphism in exon 3, of whom 12 were homozygous (fl/fl), four were heterozygous (d3/fl), and two were homozygous (d3/d3). In the analysis, the two d3-GHR groups were pooled, hereafter t-test was applied to test for differences in outcome between the GHR WT and the d3-GHR isoform. No differences were found between the GHR WT and the d3-GHR isoform in any of serum IGF1 (P = 0.613), muscle pSTAT5b (P = 0.962), adipose tissue pSTAT5b (P = 0.962), NEFA (P = 0.707), IMCL (P = 0.813), or IHCL (P = 0.813).

**Discussion**

This study was undertaken to test the hypothesis that GH signaling in human muscle and fat in vivo is determined by age and gender. To this end, muscle and adipose tissue biopsies were obtained in 20 healthy human subjects of both sexes before and after i.v. exposure to either GH or saline. In the GH-unstimulated state, the expression of CISH mRNA in muscle and fat was significantly elevated in females when compared with males, whereas IGF1 mRNA expression in adipose tissue was more pronounced in males. GH exposure significantly induced pSTAT5 in both tissues in all subjects, but the response was more pronounced in females without age dependency. This was accompanied by a significant increase in the transcription of canonical GH-dependent genes after 2 h without a distinct impact of age or gender.

Based on our data, we hypothesize that the sexually dimorphic pattern of GH activity in human subjects may be causally linked to constitutive overexpression of CISH in females. This is in accordance with the data obtained in primary hepatocytes from male and female rats (27). In the latter study, pSTAT5 following episodic GH exposure was more pronounced in male hepatocytes. In contrast to this, we observed increased pSTAT5b in women following a single exogenous GH bolus. It has, however, also been recorded in vitro that the inhibitory effect of CISH on STAT5b activity is more complete with lower amounts of STAT5b and following more prolonged GH exposure (28). It is therefore conceivable but as yet unproven that the elevated unstimulated levels of CISH mRNA recorded in our female subjects are induced by continuous elevations.
in endogenous GH levels and that its inhibitory effect on STAT5b activation therefore predominates during chronic GH exposure.

We did not measure endogenous GH secretion in our subjects, but a recent comprehensive study in 100 healthy adults of both sexes over a wide range of age reveals that female subjects exhibit higher mean and nadir levels of GH in addition to greater irregularity (29). We furthermore recorded reduced IGF1 mRNA expression in adipose tissue from females when compared with males in the GH-unstimulated state, which is compatible with relative GH resistance.

It is well established that estrogen status is an important determinant of GH secretion although it does not fully account for the sexual dimorphism (2, 11, 29). One mechanism of estrogen in this regard is direct suppression of hepatic IGF1 production, where the subsequent decline in serum IGF1 levels is assumed to stimulate GH secretion secondary to reduced feedback inhibition (11). At the molecular level, it has been reported that estrogen inhibits GH activation of the JAK/STAT pathway in vitro via stimulation of SOCS2 expression (30). In our study, we did not record differences in the levels of SOCS2 expression between males and females either before or after GH exposure. We did not see any impact of age on GH signaling either at the level of STAT5b phosphorylation or the expression of IGF1, SOCS1, SOCS2, SOCS3, and CISH mRNA. We are not aware of published data on the impact of age on GH signaling in any species, but it is well recognized that aging rats as well as human subjects are highly responsive to GH stimulation induced by administration of both exogenous GH and GH secretagogues (3, 31). This could indicate that the senescent decline in GH secretion and serum IGF1 levels is primarily driven by concomitant changes in, e.g. physical activity, dietary habits, and body composition. In our study, the GH-induced activation of STAT5b in muscle was inversely correlated with lean body mass. A similar inverse correlation has been reported between LBM and

**Figure 4**
Mean ± S.E.M. of CISH and IGF1 mRNA expression (percent of maximal response in young males) in muscle and adipose tissues after saline exposure (= baseline). CISH mRNA expression in muscle was significantly elevated in females compared with males ($P=0.002$). IGF1 mRNA expression in adipose tissue was significantly elevated in males compared with females ($P=0.013$).

**Figure 5**
Mean ± S.E.M. increases (percent of maximal response in young males) in mRNA expression of IGF1, SOCS1, SOCS2, SOCS3, and CISH in muscle and adipose tissue 120 min after GH exposure relative to the GH-unstimulated state. The GH-induced increase in mRNA expression was significant for CISH in adipose tissue and for all genes in the muscle (*$P<0.01$, §$P<0.001$).
mean 24-h GH concentrations in a group of males and females aged between 27 and 59 years (6). In the previous study, however, visceral adiposity was the major and negative determinant of several attributes of GH secretion in both sexes. In this study, we only measured total fat mass by means of DEXA scan, which did not correlate with GH signaling. We did, however, observe a significant inverse correlation between intrahepatic fat and serum IGF1. This is in line with studies on rodents and human subjects, where intrahepatic fat is determined by reduced GH secretion and action (32, 33, 34). Moreover, treatment of acromegalic patients with a GH antagonist increases intrahepatic fat content (22).

The common exon 3-deleted GH receptor polymorphism (d3-GHR) is attributed to enhanced GHR activity both in vitro and in vivo (35, 36), and we therefore found it relevant to evaluate its impact on GH signaling in vivo. We could not detect a significant difference in GH responsiveness at the level of STAT5b activation when comparing carriers and non-carriers of the supposedly more active d3-GHR variant. Whether this reflects a low sample size remains yet to be elucidated.

Certain limitations of this study merit attention. First, our sample size was relatively small, which increases the risk of type 2 errors. Secondly, we focused on the impact of a single exogenous GH bolus rather than different patterns of exogenous GH exposure or endogenous GH levels, which would have been informative in the context of the sexual dimorphism of endogenous GH secretion. Thirdly, we only studied skeletal muscle and adipose tissue; in particular, it is likely that the liver – also in human subjects – exhibits more pronounced gender differences in GH responsiveness. Nevertheless, we consider our model and the data it generates to be important for advancing our understanding of how GH works in human subjects.

In summary, this study detects distinct gender differences in intracellular GH signaling in adult human subjects in vivo. In the unstimulated state, women exhibit elevated expression levels of CISH mRNA in concomitance with reduced expression levels of IGF1 mRNA. Activation of STAT5b is detectable in human muscle and adipose tissue in vivo 30 min after i.v. exposure to an exogenous GH bolus and is more pronounced in women. The mechanistic link between these two observations remains yet to be characterized. In contrast to gender, age does not seem to be a significant determinant of stimulated GH signaling in our model system, adding support to the notion that the senescent decline in GH activity is an extrinsic property.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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