GH receptor signaling in skeletal muscle and adipose tissue in human subjects following exposure to an intravenous GH bolus


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GH receptor signaling in skeletal muscle and adipose tissue in human subjects following exposure to an intravenous GH bolus. Am J Physiol Endocrinol Metab 291: E899–E905, 2006. First published June 6, 2006; doi:10.1152/ajpendo.00024.2006.—Growth hormone (GH) regulates muscle and fat metabolism, which impacts on body composition and insulin sensitivity, but the underlying GH signaling pathways have not been studied in vivo in humans. We investigated GH signaling in biopsies from muscle and abdominal fat obtained 30 (n = 3) or 60 (n = 3) min after an intravenous bolus of GH (0.5 mg) vs. saline in conjunction with serum sampling in six healthy males after an overnight fast. Expression of the following signal proteins were assayed by Western blotting: STAT5/p-STAT5, MAPK, and Akt/PI 3-kinase (3). GH-induced STAT5 DNA binding and the expression of IGF-I and SOCS mRNA was measured by real-time RT-PCR. GH induced a 52% increase in IGF-I mRNA expression of IGF-I and SOCS mRNA was detected in fat, whereas expression activity was affected by GH. GH-induced STAT5 DNA binding and expression of IGF-I mRNA were detected in fat, whereas expression of SOCS-1 and -3 tended to increase after GH in muscle and fat, respectively. We conclude that 1) STAT5 is acutely activated in human muscle and fat after a GH bolus, but additional downstream GH signaling was significant only in fat; 2) the direct GH effects in muscle need further characterization; and 3) this human in vivo model may be used to study the mechanisms subserving the actions of GH on substrate metabolism and insulin sensitivity in muscle and fat.

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IN HEALTHY HUMAN SUBJECTS, growth hormone (GH) is predominantly secreted in peaks shortly after the onset of sleep and a few hours postprandially (11). On the basis of classical forearm studies, Zierler and Rabinowitz (23) hypothesized that GH acts in concert with insulin during repetitive feast-famine cycles. In the early postprandial phase, insulin levels are high and GH secretion is suppressed, which favors disposal of glucose and fat. In the postabsorptive phase, GH is secreted and insulin levels are low, which promotes lipolysis and fat oxidation. It was furthermore suggested that protein synthesis was stimulated in the intermediate phase by synergistic effects of insulin and GH. The recognition of IGF-I as a second messenger of GH mediating important anabolic effects was at that time not yet matured, but it is interesting that both portal insulin and GH promote hepatic IGF-I production. Møller et al. (19) demonstrated that administration of a physiological GH pulse was associated with distinct time course effects on substrate metabolism with a rapid decrease in skeletal muscle glucose uptake followed by stimulation of lipolysis and a net muscle uptake of lipid intermediates.

These data suggest acute and direct GH effects in human muscle and fat, but the signaling mechanisms of GH in human subjects have so far not been studied in vivo. Activation of the Janus kinase (JAK) and tyrosine phosphorylation of signal transducer and activator of transcription (STAT) pathways have been detected in cultured human fibroblasts incubated with GH (28), and absence of STAT5b expression due to a gene mutation is a recognized cause of severe GH insensitivity in children (13).

We hypothesized that a GH pulse in human subjects translates into detectable in vivo GH receptor signaling in muscle and adipose tissue. To test this, we measured phosphorylation of Tyr946 and DNA binding activity of STAT5 as well as the expression of target genes in muscle and fat biopsies obtained from healthy human subjects shortly after exposure to an intravenous GH bolus.

SUBJECTS AND METHODS

Subjects and study design. Six healthy male subjects (mean ± SE age 26 ± 4 yr, BMI 23.7 ± 0.8 kg/m²) were studied on two separate occasions after an overnight fast in the supine position. At 0830 (t = −30 min), an intravenous cannula was inserted into an antecubital vein for administration of either GH or saline, and another cannula was inserted in a heated dorsal hand vein on the contralateral arm for frequent blood sampling. At 0900 (t = 0 min), an intravenous bolus of either 0.5 mg GH (Norditropin, Novo Nordisk) or saline was administered. Muscle and fat biopsies were taken 30 (n = 3) or 60 (n = 3) minutes after GH/saline. Frequent blood sampling was conducted from −30 to 180 min (0830–1500). The study was performed in a randomized, single-blinded, crossover manner with ≥14 days elapsing between each study. The study was approved by the Ethics Committee System of Aarhus University Hospital and conducted in accordance with Good Clinical Practice standards.

Biopsies. A muscle biopsy was taken from the vastus lateralis muscle with a Bergstöm biopsy needle under local anesthesia (1% Lidocaine), a small incision having been made through the skin and muscle sheath 15–20 cm above the knee corresponding to the vastus lateralis. A total amount of ∼200 mg muscle was aspirated, and biopsies were cleaned for blood (within 15 s) and snap-frozen in liquid nitrogen. Muscle biopsies were stored at −80°C until analyzed.

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Subcutaneous fat biopsies from the periumbilical region were obtained by liposuction and snap-frozen in liquid nitrogen.

Western Blotting for STAT5 and MAP kinases. Before analysis, muscles and fat tissues were homogenized on ice with a polytron in lysis buffer (20 mM Tris, pH 7.0, 1% Triton X-100, 0.27 M sucrose, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 5 mM tetrassodium pyrophosphate, 10 mM glycerolphosphate, 1 mM benzamidine, 4 μg/ml leupeptin, 1 mM DTT, 10 mM Na3VO4). After 10 min of solubilization at 4°C, extracts were centrifuged for 20 min at 10,000 g and the supernatants collected for further analysis. Protein concentration the lysates was determined by the Bradford assay (Eppendorf BioPhotometer). Primary antibodies were as follows: anti-STAT5b (Tyr694), anti-SAPK/JNK, anti-phospho-(Thr183, Tyr185) SAPK/JNK, anti-p38, anti-phospho-(Thr180, Tyr182) p38, anti-p44/p42 ERK, anti-phospho-(Thr202, Tyr204) p44/p42 ERK (Cell Signaling Technology, Beverly, MA), and anti-phospho-(Tyr694) STAT5 (Santa Cruz Biotechnology). An anti-rabbit IgG horseradish peroxidase (HRP) was used as secondary antibody. Western blotting was performed as described by the manufacturer (Invitrogen). In short, lysates (10 μg protein) were subjected to NuPAGE followed by electroblotting onto nitrocellulose membranes. Membranes were blocked with 5% skim milk powder before primary antibody was added overnight at 4°C. After incubation with secondary antibody for 60 min, the protein of interest was detected by a chemiluminescence detection system (LumiGlow, Cell Signaling) and visualized using an imaging system (Las3000, Fuji Film) according to the manufacturer’s instructions. Band intensities were quantitated using Multigauge software (Fuji Film).

PI 3-kinase assay and Western blotting for Akt/PKB. Frozen muscles biopsies (~40 mg) were homogenized in ice-cold solubilization buffer [50 mM HEPES, 137 mM NaCl, 10 mM Na2PO4, 10 mM NaF, 1 mM MgCl2, 1 mM CaCl2, 1% NP-40, 10% glycerol, 2 mM Na3VO4, 2 μg/ml aprotinin, 5 μg/ml leupeptin, 0.5 μg/ml pepstatin, 10 μg/ml antipain, 1.5 mg/ml benzamidine, and 100 μmol/l 4-(2-aminoethyl)benzenesulfonyl fluoride, hydrochloride (AEBSF), pH 7.4] and rotated end over end for 1 h at 4°C. Insoluble materials were removed by centrifugation at 16,000 g for 60 min at 4°C, and protein content in the supernatant was determined using a bicinchoninic acid protein assay reagent (Pierce Chemical, Rockford, IL). Western blotting for assessment of Akt/PKB protein expression and phosphorylation was performed with the Bio-Rad Mini Protein II system with a primary Akt/PKB and pAkt/PKB (Ser473) antibody from New England BioLabs (Beverly, MA) that recognizes the three isoforms of Akt/PKB. HRP-conjugated anti-rabbit IgG antibody (Pierce Chemical, Rockford, IL) was used as secondary antibody. Akt/PKB proteins were visualized by BioWest enhanced chemiluminescent (UVP, Upland, CA) and quantified by UVP BioImaging System (UVP).

PI 3-kinase activity was assessed as previously described (30), with minor modifications. Briefly, aliquots of protein were immunoprecipitated overnight with anti-IRS-1 antibody (Upstate Biotechnology, Lake Placid, NY) coupled with protein A-agarose (Sigma, St. Louis, MO). The immune complexes were washed thoroughly, and IRS-1-associated PI 3-kinase activity was assessed directly on the protein-agarose complex in a buffer containing 10 mM Tris·HCl, 1 mM EDTA, 1 mM MgCl2, 75 μM ATP, 50 mM NaCl, and 6 μCi of [γ-32P]ATP (NEN, Boston, MA), pH 7.5. Reaction products were

Table 1. Primer sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer 1</th>
<th>Primer 2</th>
<th>Product Length, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOCS-1</td>
<td>ACACGCACTTCCGCCGACATTCC</td>
<td>GAGAAGCACATTCTCGAGTAAG</td>
<td>209</td>
</tr>
<tr>
<td>SOCS-2</td>
<td>GTGCCGACGCCATCAAGTGCA</td>
<td>TCCCTAGGACTAGTGGCAGAC</td>
<td>152</td>
</tr>
<tr>
<td>SOCS-3</td>
<td>TGGGCTCAAGACCTTTTCCAA</td>
<td>CGGACTGACAGAAGCTGTTT</td>
<td>110</td>
</tr>
<tr>
<td>IGF-I</td>
<td>TTGCTAGTGGCTCTTCCAGTTT</td>
<td>GAAGAGGAGGTTGAGCTTG</td>
<td>191</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GTGAAGGCTGAGAGGCTGCAAC</td>
<td>CAGATGCGGAAATGCAATTG</td>
<td>154</td>
</tr>
<tr>
<td>β-Actin</td>
<td>ACGGGTGCCAGCAGACTGGGCG</td>
<td>CTAGAGAGATTGTTGGTGCAGG</td>
<td>658</td>
</tr>
</tbody>
</table>

SOCS, suppressor of cytokine signaling.
resolved by thin-layer chromatography and were quantified using a PhosphorImager (Packard BioScience, Meriden, CT).

Electrophoretic mobility shift assay. To measure STAT5 DNA binding activity an electrophoretic mobility shift assay (EMSA) using the SPI-GLE1 (5′-agtcATGGTTCTGAAATACT-3′) promoter element was used. After annealing, the double-stranded oligonucleotide was 32P-radiolabeled in a fill-in reaction using DNA polymerase (Klenow fragment). Cellular lysates from the muscle and fat biopsies (10 μg protein) were incubated for 30 min at 30°C with a 20-fmol probe in a 20-μl reaction containing 20 mM HEPES, pH 7.9, 50 mM NaCl, 1 mM MgCl2, 1 mM EDTA, 1 mM DTT, 10% glycerol, and 0.1 mg/ml poly(dIdC)·poly(dIdC). Unlabeled double-stranded oligonucleotides were included to test specificity. A double-stranded oligonucleotide containing a binding site for the transcription factor NF-κB [5′-agtcaGCTTCAGAGGGACTTTCCGAGAGG-3′ (lower case indicates nucleotides added for cloning; upper case indicates the promoter sequence)] was used as a nonspecific competitor. Antibodies against STAT5 or STAT1 (1 μl; Santa Cruz Biotechnology) were included in the reaction for supershift analysis. Free and bound probes were separated on a 5% polyacrylamide gel containing 2% glycerol and 0.25× TBE (25 mM Tris·HCl, 25 mM boric acid, 0.25 mM EDTA, pH 7.9). The gel was dried and exposed to PhosphorImager analyzer.

Real-time RT-PCR. Total RNA was isolated from muscle and fat biopsies using the TRIzol reagent (Invitrogen, Carlsbad, CA). cDNA synthesis was performed with the TaqMan Gold RT-PCR kit (PerkinElmer, Boston, MA). Real-time PCR analysis was performed to analyze the levels of suppressor of cytokine signaling (SOCS)-1, SOCS-2, SOCS-3, and IGF-I mRNA. Primers used for the amplification were as seen in Table 1.

The mRNA levels of GAPDH and β-actin were measured as an internal control gene. The PCR reaction was performed as described in the manual for the ABI prism 7700 Sequence Detector (AB Applied Systems). Each cDNA sample was subjected to two individual PCR analyses using either the SOCS-3 or IGF-I primer pair. For the real-time PCR analysis, every PCR reaction was performed using a mastermix supplemented with SYBR Green (Applied Biosystems, Foster City, CA). Analysis of SOCS-1 and SOCS-2 gene expression was performed using PCR mastermix containing the specific primers Taq DNA polymerase (HotStar Taq, Qiagen) and SYBR-Green real-time PCR, as described in the iCycler manual from Bio-Rad. The target gene and the internal control gene were amplified in separate tubes. 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 calculated essentially as described in User Bulletin no. 2, 1997, from PerkinElmer (PerkinElmer Cetus, Norwalk, CT). Briefly, the target gene (X0)-to-control gene (R0) ratio in each sample before amplification was calculated as X0/R0 = k × [I/ΔΔCt]. For SOCS-1 and SOCS-2 analysis, β-actin was used as a control gene. All samples were amplified in duplicate. A similar set-up was used for negative controls, except that the reverse transcriptase was omitted and no PCR products were detected under these conditions.

Analytes in serum. All samples were analyzed in duplicate. Plasma glucose was measured immediately with a glucose analyzer (Beckman Instruments, Palo Alto, CA). Serum insulin concentrations were measured in duplicate with a two-site immunospecific insulin enzyme-linked immunosorbent assay (1). Serum free fatty acid (FFA) concentrations were determined by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany). Serum GH was measured with radioimmunoassays (DELFIA; Wallac Oy, Turku, Finland).

Statistics. Values are means ± SE. Time series of analytes in serum were analyzed by ANOVA. Paired data were analyzed by Student’s t-test or rank sum test where appropriate. For statistical analysis, data from all six subjects were pooled.

RESULTS

GH exposure resulted in a mean (±SE) peak level of 94 ± 5 μg/l, which occurred after 10 min in all subjects (Fig. 1). In the saline experiment, small, spontaneous GH bursts were recorded in three subjects (subject 1: 5.6 μg/l at 0 min; subject 4: 9.6 μg/l at 10 min; subject 5: 6.7 μg/l at 0 min). Serum FFA levels increased with time following both saline and GH exposure.
injection ($P < 0.001$), but the increment was larger following GH administration compared with saline ($P = 0.03$; Fig. 1). In the GH experiment, peak FFA levels were reached $155 \pm 5$ min after GH administration. No significant changes with time or between groups in the circulating levels of insulin (data not shown) or glucose were recorded (Fig. 1).

As STAT5 has been shown to be the major transcription factor responsible for the effects of GH on gene transcription, we analyzed the activation of STAT5 by measuring the induction of tyrosine phosphorylation and DNA binding activity in response to GH stimulation. Figure 2, which represents single biopsies from muscle and fat from all six subjects, demonstrates that GH administration was associated with tyrosine phosphorylation of STAT5 in muscle tissue in all subjects. After saline, evidence of less intense STAT5 phosphorylation was detectable in subjects 4 and 5, but the difference between GH and saline was highly significant ($P = 0.001$). Of note, the level of tyrosine-phosphorylated STAT5 appeared to be similar $30$ and $60$ min after GH exposure (Fig. 2). In adipose tissue, STAT5 tyrosine phosphorylation was observed in all subjects injected with GH and for five of the subjects was higher than the level of phosphorylation seen after saline injection (Fig. 2). However, the GH-dependent increase failed to reach statistical significance ($P = 0.07$), because in subject 1 the level of phospho-STAT5 was higher after saline treatment than after GH treatment. GH-induced DNA binding activity of STAT5 was detectable in fat but not in muscle (Fig. 3).

Fig. 3. GH induced DNA-binding activity of STAT5 from muscle and fat biopsies. Top: electrophoretic mobility shift assay using the STAT5-binding element from the Spi 2.1 promoter as a probe and extracts (10 μg protein) made from muscle or fat biopsies. Biopsies were obtained from subjects 1–6 treated with GH (+) and saline (−). In lane 13, a control extract from insulin-producing Ins-1 cells was included as a positive control. Bottom: fat tissue extract from subject 2 treated with GH was used to verify specificity. This extract was incubated with 10- or 100-fold molar excess unlabeled double-stranded oligonucleotide from the Spi.2.1 promoter (specific) or from an NF-kB binding sequence (nonspecific). Supershift analysis was performed using antibodies against STAT1 or STAT5.

Fig. 4. IRS-1-associated PI 3-kinase activity after saline (□) and GH stimulation (■). NS, not significant. Values are means ± SE expressed as % saline-injected subjects.

A

\[
\begin{array}{c}
\text{Saline} \\
\text{GH} \\
\end{array}
\]

B

\[
\begin{array}{c}
\text{Saline} \\
\text{GH} \\
\end{array}
\]
tion between GH and saline could be demonstrated. In accordance with this, IRS-1-associated PI 3 kinase activity was not significantly affected by GH (Fig. 4).

Regarding induction of SOCS mRNA expression in response to GH, there was a trend toward increased SOCS-1 in muscle and increased SOCS-3 in fat (Fig. 5). GH-dependent stimulation of IGF-I mRNA expression was significantly elevated in muscle tissue, whereas the level of expression of IGF-I mRNA in muscle was severalfold lower without a significant GH effect (Fig. 6).

DISCUSSION

The biological effects of GH in human subjects on endpoints such as longitudinal growth and substrate metabolism have been demonstrated in numerous publications, but the underlying mechanisms in terms of signaling pathways in target tissues have so far been experimentally investigated only in cell cultures and animal models. In the present study, we tested the hypothesis that administration of an intravenous GH bolus to human subjects translates into detectable GH receptor signaling in skeletal muscle and fat tissue. We observed acute GH-induced tyrosine phosphorylation of STAT5 in both tissues in all subjects, in addition to evidence of target gene expression. Our data emphasize the central role of the JAK/STAT pathway as a mediator of GH effects also in humans, which is supported by in vitro data (28) as well as by case reports of children with severe GH insensitivity and growth failure due to mutations in the STAT5b gene (13).

Biopsies were obtained 30–60 min after the GH/saline bolus, because STAT5 tyrosine phosphorylation (29) as well as induction of IGF-I and SOCS mRNA expression have been reported both in vitro (26) and in skeletal muscle of rats in vivo (4) within this window of time after GH exposure. A larger time series of biopsies in each individual would have been appropriate from a scientific point of view, but such an approach poses ethical as well as technical problems. It is also noteworthy that spontaneous GH bursts prior to biopsies were revealed in three of six individuals during the saline study, which could have obscured some of the results. We decided not to suppress endogenous GH secretion with somatostatin, because this could have influenced GH signaling either directly (21) or by its concomitant suppression of insulin (15). We did not have access to a specific GH receptor antagonist for this study, but it is obvious that administration of Pegvisomant, which is a licensed GH receptor antagonist for treatment of acromegaly (17), as a control experiment would be of major relevance in future studies.

GH has been shown to stimulate IGF-I mRNA expression in rat muscle cells in vivo (12) and in cultured muscle cells via the JAK/STAT signaling proteins (9, 26). We could not, however, demonstrate an effect of GH on either STAT5-induced DNA binding activity or IGF-I mRNA expression in human muscle. We were also unable to detect a statistically significant effect of GH on SOCS-1, -2, and -3 mRNA expression, which has previously been shown to occur in cultured murine muscle cells less than 30 min after GH exposure (24, 26). All the evidence taken together, we found no statistically significant downstream effect of the GH-induced activation of STAT5 in muscle within the window of time provided by our study design. The lack of detectable STAT5 DNA binding in muscle is surprising, considering the induction of STAT5 tyrosine phosphorylation. It should, however, be noted that the level of...
total and tyrosine-phosphorylated STAT5 observed in muscle was lower than that seen in adipose tissue. The exposure time of the Western blots for both the tyrosine-phosphorylated and total STAT5 from muscle tissue shown in Fig. 2 is three times longer than that shown for adipose tissue, indicating a lower level of STAT5 phosphorylation in muscle tissue. The mechanism underlying this difference is unknown but could be due to lower levels of GH receptor or JAK2 expression in muscle compared with adipose tissue. On the other hand, SOCS-1 mRNA expression tended to reach statistical significance in muscle after GH, even though the degree of target gene expression was lower compared with fat (Fig. 5). Direct effects of GH on substrate metabolism in human skeletal muscle in vivo have been reported (10, 23), and data from animal models clearly show that circulating IGF-I does not fully account for the growth-promoting effects of GH (31). Future studies involving muscle biopsies obtained at multiple time points after GH, and including a control situation with Somavert administration, should be undertaken to further our understanding of the mechanisms subserving the anabolic effects of GH in human muscle. In adipose tissue, acute GH exposure was associated with evidence of downstream effects in terms of DNA binding activity, IGF-I mRNA expression, and a trend toward increased SOCS-3 expression. GH-induced lipolysis has been detected in human GH receptor-transfected adipocytes in association with JAK/STAT activation (2). In support of a critical role of STAT5 proteins, GH-induced lipolysis is absent in adipose tissue extracts from STAT5a/b knockout mice (8). In the present study, a significant increase in circulating FFA levels, compatible with lipolysis from adipose tissue, was observed after the GH bolus. Little is known about the biochemical mechanisms subserving the lipolytic effects of GH in humans, but there is evidence to suggest that it involves activation of hormone-sensitive lipase (22, 32). Interestingly, a lag phase of several hours exists between GH exposure and lipolysis both in vitro (2) and in vivo (19), which supports the involvement of gene expression. Although a temporal relationship does not imply causality, our in vivo observations agree with in vitro data that STAT5 activation is involved in the acute actions of GH on fat metabolism, and our model provides a viable tool for future studies on this important and complex effect of GH.

GH acutely antagonizes insulin-stimulated glucose uptake in skeletal muscle, as shown originally by Zierler and Rabinowitz (23) and in a number of studies using the euglycemic hyperinsulinemic glucose clamp technique (3, 16, 18, 22). Under normal physiological conditions, glucose transport across the cell membrane is considered the rate-limiting step for insulin-stimulated glucose uptake (6). The mechanisms whereby GH impairs glucose transport in humans are unclear, but at the intracellular level it is accompanied by suppression of glucose phosphorylation and glycogen synthase activity (3, 5). Because infusion of FFA induces insulin resistance (7), a causal link between the lipolytic effects of GH and its inhibitory effect on insulin-stimulated glucose uptake in muscle is plausible. In support of this, we (22) previously observed that experimental suppression of lipolysis in conjunction with GH administration in GH-deficient adults significantly abrogates the antagonistic effects of GH on muscle glucose uptake. On the other hand, FFA infusion in human subjects induces insulin resistance via suppression of PI 3-kinase activity (7), whereas GH-induced insulin resistance during a glucose clamp is not accompanied by suppression of either PI 3-kinase or Akt/PKB (14), the latter of which is considered an important downstream signaling protein involved in the translocation and activation of GLUT4. In the present study, which was performed after an overnight fast with low levels of insulin activity, we were also unable to detect any effects of GH on PI 3-kinase activity or PKB phosphorylation. Increased expression of SOCS proteins has been suggested to mediate cytokine-induced insulin resistance by interfering with insulin signaling (20), although data in human models are ambiguous (25). As previously mentioned, there was a trend toward GH-induced SOCS expression in muscle and fat in our study. To learn more about the significance of SOCS proteins for the insulin-antagonistic effects of GH, it would be helpful to study in vivo SOCS expression in human muscle tissue during insulin stimulation in the presence and absence of GH exposure.

Prolonged fasting and other catabolic conditions are associated with suppression of serum IGF-I levels together with amplification of GH secretion and lipolysis. The signaling events underlying this shift in GH action have not been studied in humans. Hepatic GH resistance in terms of suppressed GH-induced STAT5 tyrosine phosphorylation and blunted IGF-I mRNA expression is observed in rats with chronic uremia, which is associated with increased SOCS-3 mRNA levels (27), and GH-induced IGF-I mRNA expression in skeletal muscle is blocked in rats with sepsis together with upregulation of SOCS protein (12). To study GH signaling in human muscle and fat during fasting would therefore also be of interest for the future.

In summary, this in vivo study shows that GH acutely tyrosine-phosphorylates STAT5 in skeletal muscle and adipose tissue in human subjects, which is accompanied by STAT5 DNA-binding activity and increased mRNA expression of IGF-I in fat, but not in muscle. Further studies will show whether downstream effects of GH in muscle can be detected if the experimental conditions are modified, but we believe that our model is viable and can be used to study in more detail the complex peripheral effects of GH in humans.

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