Growth Hormone Signaling in Muscle and Adipose Tissue of Obese Human Subjects: Associations With Measures of Body Composition and Interaction With Resveratrol Treatment

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Context: Growth hormone (GH) secretion is reduced in obesity, despite normal serum insulin-like growth factor I (IGF-1) levels, but the association between obesity and the GH signaling is unknown. Furthermore, SIRT1, an nicotinamide adenine dinucleotide–dependent protein deacetylase, reduces hepatic IGF-1 production in mice via blunting of GH-induced STAT5 signaling.

Objective: To study GH signaling in muscle and fat in obese subjects and the interaction with concomitant administration of the putative SIRT1 activator resveratrol, and to assess the effects of inhibiting or knocking down SIRT1 on GH regulated genes in vitro.

Design and Participants: Twenty-four obese males were examined in a randomized, double blinded, parallel-group study with resveratrol or placebo treatment for 5 weeks followed by a GH bolus. Muscle and fat biopsies were collected before and after GH. Body composition was assessed by DEXA and MRI.

Main Outcome Measure: (1) Effect of body composition and age on GH-stimulated STAT5b phosphorylation and IGF-1, SOCS2, and CISH mRNA in muscle and fat. (2) The impact of resveratrol treatment on GH activity. (3) Impact of inhibiting or knocking down SIRT1 on effects of GH in vitro.

Results: Significant GH-induced STAT5b phosphorylation in muscle and fat in obese subjects was recorded together with increased CISH and SOCS2 mRNA. GH-induced STAT5b phosphorylation in muscle correlated positively with age [$r = 0.53$, $p<0.01$], but not with body composition. Resveratrol administration had no impact on body composition, serum IGF-1, or GH signaling in vivo, and SIRT1 knock down or inhibition did not affect GH signaling in vitro.

Conclusion: (1) GH induced STAT5b phosphorylation is detectable in muscle and fat in adult males with simple obesity, but is not determined by body composition. (2) Resveratrol supplementation does not impact circulating IGF-1 levels or GH signaling in human muscle and fat. (3) Our data speak against a major impact of SIRT1on GH action in human subjects. **(*J Clin Endocrinol Metab* 99: E2565–E2573, 2014)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received May 2, 2014. Accepted July 15, 2014.
First Published Online July 22, 2014

Abbreviations: BMI, body mass index; FFA, free fatty acid; GH, growth hormone; IGF-1, insulin-like growth factor 1; SDS, standard deviation scores; SIRT1, sirtuin 1

Both spontaneous and stimulated growth hormone (GH) secretion are blunted in obese patients (1, 2), which is mainly attributed to a diminution of secretory burst mass rather than burst frequency, basal production, or half-life (1, 3). With regard to the underlying mechanisms, three observations merit attention. First, an inverse correlation between adiposity, in particular visceral fat mass, and GH secretion exists also among nonobese subjects (4), suggesting that the association between blunted GH secretion and obesity is of a continuous rather than categorical nature. Second, the association is reversible since weight loss in obesity restores GH responsiveness (5, 6), which indicates that blunted GH secretion is a consequence rather than a cause of obesity. Third, serum insulin-like growth factor I (IGF-1) levels in obese patients are generally comparable to those of normal weight subjects (2). Whether the latter is a consequence of a compensatory increase in the sensitivity to GH-induced hepatic IGF-1 production or regulation of serum IGF-1 levels via non GH-dependent mechanisms is not known. It has, however, in several controlled trials been documented that obese patients are responsive to exogenous GH in terms of serum IGF-1 generation, predictable changes in body composition, and also GH-related side effects, such as fluid retention and insulin resistance (7).

Nothing is known about the impact of obesity on GH signal transduction in human peripheral target tissues. We have documented that exogenous, as well as endogenous elevations in serum GH levels induce robust and detectable activation of the canonical Janus Kinase and Signal Transducer and Activator of Transcription (JAK-STAT) GH signaling pathway in human peripheral targets tissues in vivo (8–11). Furthermore, we have shown that circulating free fatty acid (FFA) suppresses GH-induced STAT5b activation in human skeletal muscle in vivo, which is of particular interest since FFA levels are elevated in obesity (12). However, these studies were performed in lean healthy subjects.

It was recently reported that STAT5b acetylation is associated with enhanced hepatic GH responsiveness in mice, whereas deacetylation by sirtuin 1 (SIRT1), an nicotinamide adenine dinucleotide–dependent protein deacetylase, inhibits phosphorylation of STAT5b (13). This is interesting since SIRT1 is activated during caloric restriction (14), and caloric restriction in human subjects is associated with reduced hepatic IGF-1 production and a compensatory increase in GH secretion (15, 16). Furthermore, resveratrol has been shown to be an activator of SIRT1 in cells and animals (17). In the present trial, we characterized in vivo GH signaling in skeletal muscle and adipose tissue in healthy obese males preceded by 5 weeks administration of either placebo or resveratrol in a double-blinded, parallel design. This was related to detailed indices of body composition and measures of substrate metabolism and insulin sensitivity. We also conducted a series of in vitro experiments to further examine the relationship between SIRT1 and the effects of GH.

Materials and Methods

Ethical approval

All the studies were conducted in accordance with the Helsinki Declaration and the study protocols were approved by the Ethical Committee of Central Denmark Region. Informed consent was obtained from all subjects before participation. The protocol was registered at clinicaltrials.gov NCT01150955.

Subjects and study design

The present data derived from an investigator-initiated randomized, double blinded, placebo-controlled, parallel-group clinical study, from which data related to body composition and substrate metabolism have previously been published (18). Briefly, 24 obese but otherwise healthy males, age 38 years (range 18–68 years) and with a body mass index (BMI) > 30 kg/m^2 (34.2 ± 0.7, mean$\pm$SEM) were treated with 500 mg of transresveratrol (Fluxome Inc.) or a placebo (Robinson Pharma) three times per day for 5 weeks. Randomization, blinding, packaging, and labeling were performed by the Hospital Pharmacy at Aarhus University Hospital. The randomization code was unblinded when all predefined data were on file. The participants were instructed in maintaining their normal lifestyle in terms of diet and physical exercise throughout the trial. Body composition, substrate metabolism, and circulating hormones and metabolites were assessed before and after 4 weeks of treatment. After 5 weeks of continued treatment, each subject received an IV GH bolus (0.4 mg, Norditropin). Body composition was assessed by dual-energy x-ray absorptiometry (DEXA) scan, magnetic resonance (MR) spectroscopy (ectopic fat in liver and muscle), and imaging. Substrate metabolism and insulin sensitivity were measured as previously described (18), which included muscle and fat biopsies and frequent blood sampling over 6 h to measure serum levels of GH, IGF-1, and FFA. Muscle and adipose tissue biopsies for analysis of GH signaling were collected at week 4 (basal) and 60 minutes after the GH injection at week 5 (GH). All biopsies were collected at 10 am after an overnight fast and with no exercise on the day of the examination.

Collection and processing of muscle and adipose tissue biopsies

For a detailed description of the collection of tissue samples, see Ref. 18. Briefly, muscle biopsies were collected from vastus lateralis using a Bergström needle, dissected free from fat and connective tissue, and snap frozen in liquid nitrogen.

Muscle biopsies were homogenized using a Precellys 24 homogenizer (Bertin Technologies) in a cold buffer containing 20 mM Tris, 50 mM NaCl, 50 mM NaF, 5 mM Na4P2O7, 5 mM NAM, 5 μM TSA, protease inhibitor cocktail (Halt, Thermo Scientific), 250 mM sucrose, 1% (v/v) Triton X-100, 2 mM DTT, pH 7.4. Samples were rotated for 20 minutes at 4°C, and centrifuged at 14.000 g for 20 minutes at 4°C. Total protein con-

centration was determined using Bradford protein assay (Bio-Rad).

Adipose tissue biopsies were homogenized using a Precellys 24 homogenizer (Bertin) in a buffer containing 20 mM HEPES, 10 mM NaF, 1 mM Na3VO4, 5 mM NAM, 5 μM TSA, protease inhibitor cocktail (Halt), 1 mM EDTA, 5% SDS, pH 7.4. Homogenates were incubated in a Thermomixer (Eppendorf) at 37°C and 1000 rpm for 1 hour followed by centrifugation at 14.000 g for 20 minutes. The lipid-free infranatant was aspirated and used for western blot analysis.

Western blotting

Western blots were performed by SDS-PAGE on StainFree 4–15% gels using the CriterionXT-system (Bio-Rad). Proteins were transferred onto PVDF membranes, blocked for 2 h in 0.3% iBlock, incubated with primary antibodies overnight, incubated for 1 h with HRP-conjugated secondary AB. Proteins of interest were visualized by ECL chemiluminescence using a ChemiDoc XRS system (Bio-Rad). Protein signals were quantified using Image Lab (version 4.0.1, Bio-Rad). The Bio-Rad StainFree technique was used as a loading control as validated previously (19). STAT5 (#9358) and pSTAT5 (#9359) antibodies were from Cell Signaling Technology (Danvers) and SIRT1 (ab32424) was from Abcam.

Real-time qRT-PCR

Tissue samples were homogenized in a TriZol reagent (Life Technologies), and total RNA was extracted by following the manufacturer's protocol. RNA was quantified by measuring absorbance at 260 and 280 nm using a NanoDrop 8000 (NanoDrop Products), and the inclusion criteria was a ratio > 1.8. The integrity of the RNA was determined using Bioanalyzer 2100 (Agilent) and an RIN score > 7 was obtained for all samples. cDNA was constructed using random hexamer primers (Verso cDNA kit; Abgene). The following primer pairs were used against human transcripts: CISH: TTCGGGAATCTGGCTGGTATTGG and GCATCTTCTGCAGGTGTTGTCG; SOCS2: TCCCTTCCTAAGGCTGACCAAGAC and GCGACTTCA GCCTTTACATGCG; IGF-1: GACAGGGGCTTTTATTTCAAC and CTCCAGCCTCCTTAGATCAC; STAT5b: TTGGTCCGAGAAGCCAACAATGG and TGTTTCTGGGACATGGCATCAGC; SIRT1: GCGATTGGGTACCGAGATAAC and GGCCTTGGAGTCCAGTCACTA; β_2 microglobulin: GAGGCTATCCAGCGTACTCC and AATGTCGGATGGATGA AACCC.

TaqMan gene expression probes for mice CISH, SOCS2, and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (endogenous control) were obtained from Applied Biosystems and used per vendor's protocol.

PCR-reactions were performed in duplicate using TaqMan or KAPA SYBR® FAST qPCR Kit (Kapa Biosystems Inc.) in a LightCycler 480 (Roche Applied Science) in real time. Relative gene expression was estimated using the default "Advanced Relative Quantification" mode of the software version LCS 480 1.5.0.39 (Roche). All samples were amplified in duplicate. A similar setup was used for negative controls, except that the reverse transcriptase was omitted and no PCR products were detected.

Circulating hormones and metabolites

Plasma glucose was measured in duplicates immediately after collection on a YSI 2300 Stat Plus (YSI Inc.). Serum GH and total IGF-1 were measured using time resolved fluoroimmunoassay (TR-IFMA, AutoDELFIA; PerkinElmer, Turku, Finland), and FFA was analyzed with a commercial kit (Wako Chemicals). IGF-1 was measured at $t = 0$ and GH and FFA were measured at seven time points: 0, 160, 170, 180, 340, 350, and 360 minuntes; the last three measurements were obtained during a hyperinsulinemic, euglycemic clamp as described in detail in Ref. 18.

Cell culture

Murine 3t3-L1 fibroblast cells (ATCC) were cultured in DMEM (5 g/L glucose), 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin until ~90% confluence. The medium was changed to serum free DMEM ± 25 μM EX527 2 h prior to treatment. Cells were incubated with GH for 30 minutes in experiments assessing pSTAT5b and 120 minutes in experiments assessing mRNA levels. Cells were harvested and collected in cold PBS and centrifuged at 3.000 g for 30 seconds. Cells were lysed in NETN buffer containing 20 mM Tris-HCl, 100 mM NaCl, 5 mM NaF, 50 mM 2-glycerophosphate, 1 mM Na3VO4, 1 mM EDTA, 0.5% NP-40, pH 8.0. Homogenates were incubated at 4°C for 20 minutes under constant agitation, centrifuged at 10,000 g for 10 minutes at 4°C, and the supernatant was used for western blot analysis. Cell culture experiments were conducted in singlets, qRT-PCR was run in triplets, and experiments were repeated for validation at least once.

Nontargeting siRNA (#D001210-03-20, Dharmacon) was used as a control and SMARTpool siRNA was used to knock down SIRT1. Transfections were performed with 50 nM of siRNA using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions, and cells were treated and harvested 72 hours after transfection.

Statistics

Normality was assessed by qq-plots and the Shapiro-Wilk test. Two-way repeated measures ANOVA was used to examine the effects of resveratrol, GH, and possible interactions. A significant ANOVA was followed by the Student-Newman-Keuls method for pairwise multiple comparisons. Pearson's product moment correlation with two-tailed probability values was subsequently used to measure the strength of association between the variables. Multivariate regression analysis was performed with a maximum of two dependent variables due to the population size. Data are presented as the mean ± SE and statistical significance was assumed for $P < .05$ except for correlation analyses, where a protected p value ≤ 0.01 was used.

Results

Twenty-four obese men participated in the study. Their characteristics including age, body composition and clinical biochemistry are summarized in Table 1 (in addition to supplemental data). As previously published, 4 weeks of resveratrol treatment did not impact the insulin sensitivity, substrate metabolism, or body composition in terms of

Table 1. Baseline Characteristics

Characteristics	Placebo	Resveratrol	*P* Value
Age (y)	32 ± 3	44 ± 4	0.01
BMI (kg/m^2)	36.0 ± 1.3	32.6 ± 0.7	0.07
Fat (%)	32.1 ± 1.5	30.2 ± 0.8	0.28
Visceral fat (cm^3)	353 ± 75	348 ± 87	0.96
Intrahepatic lipids (a.u.)	3.91 ± 0.88	2.74 ± 0.67	0.29
Intramyocellular lipids (a.u.)	2.93 ± 0.41	3.21 ± 0.42	0.92
FFA (mmol/L)	0.42 ± 0.03	0.47 ± 0.04	0.39
GH (ng/mL)	0.17 ± 0.06	0.27 ± 0.11	0.68
IGF-1 (ng/mL)	157 ± 11	130 ± 8	0.054
IGF-1 SDS	0.10 ± 0.23	0.25 ± 0.16	0.58

BMI, lean body mass, fat body mass, fat percentage, visceral fat, intrahepatic lipids, intramyocellular lipids (18), or circulating levels of FFA, GH, or IGF-1, and there was no difference in baseline levels of GH or FFA between the two groups (Table 1). By chance, the participants randomized to resveratrol treatment were older as compared to those in the placebo group ($P = .01$), which translated into borderline significantly lower serum IGF-1 level in the resveratrol group ($P = .054$). However, age-corrected levels of serum IGF-1 expressed as standard deviation scores (SDS) based on normative data did not differ between the two groups ($P = .58$).

STAT5b phosphorylation in the GH-unstimulated state in both muscle and fat was weak or absent and increased 10–30-fold 60 minutes after injection of GH bolus (Figure 1) concomitantly with increased Cytokine-inducible SH2-containing protein (CISH) and suppressor of cytokine signaling 2 (SOCS2) mRNA levels in muscle and fat (Figure 2). Unexpectedly, IGF-1 mRNA expression was decreased 60 minutes after injection of GH in fat and muscle when compared to the mRNA levels in tissue collected one week earlier on the control day (Figure 2). Resveratrol treatment had no impact on GH-induced STAT5b phosphorylation or the transcription level of CISH, SOCS2, or IGF-1 in muscle or fat (Figures 1 and 2). For this reason, correlation analyses regarding GH signaling and body composition represent pooled data from both groups.

GH-induced STAT5b phosphorylation correlated positively with the transcription of canonical GH target genes in muscle [CISH: $r = 0.65$, $P < .001$; SOCS2: $r = 0.72$, $P < .001$; IGF-1: $r = 0.59$, $P < .001$], but not in fat. Likewise, a positive correlation was recorded between stimulated pSTAT5b and age in muscle [$r = 0.53$, $P = .009$]. No correlation was detected between GH-induced pSTAT5 phosphorylation and basal levels of SOCS/CISH (data not shown) in muscle or fat. Serum levels of IGF-1 correlated positively with IGF-1 and CISH mRNA expression in adipose tissue [IGF-1: $r = 0.57$, $P = .005$; CISH: $r = 0.71$, $P < .001$]. As expected, age correlated inversely with IGF-1 [$r = -0.54$, $P = .007$]. Moreover, age was also inversely correlated to basal adipose tissue expression of IGF-1 and SOCS2 mRNA [IGF-1: $r = -0.52$, $P = .010$; SOCS2: $r = -0.54$, $P = .008$]. Finally, basal IGF-1 mRNA expression in fat correlated inversely with fasting levels of serum FFA [$r = -0.60$, $P = .003$].

Resveratrol treatment had no impact on either serum IGF-1 or GH, protein levels of SIRT1 and STAT5b, or SIRT1 and STAT5b mRNA levels in muscle and adipose tissue (Figure 3). In 3t3-L1 cells, GH incubation potently induced STAT5b phosphorylation concomitantly with increased transcription of CISH and SOCS2 mRNA. Inhibiting SIRT1 with EX527 or knocking down SIRT1 with siRNA had no impact on GH induced transcription of CISH and SOCS2 mRNA or STAT5b phosphorylation (Figure 4).

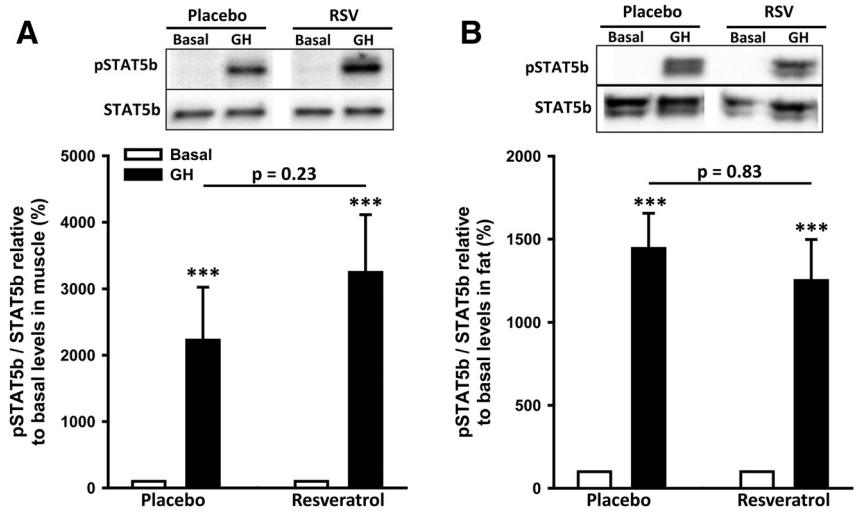

Figure 1. STAT5b phosphorylation in skeletal muscle and fat. STAT5b phosphorylation relative to total STAT5b in (A) human skeletal muscle and (B) adipose tissue presented as a percentage increase 1 hour after GH stimulation compared to the basal condition. Prior to this, the subjects had received either resveratrol ($n = 12$) or placebo ($n = 12$) for 4–5 weeks. Inserts at top are representations of the western blots from which the chart has been made. Data presented as mean values ± SE. ***, $P < .001$ compared to basal within placebo or resveratrol group.

Discussion

The aim of this study was to gain more insight into the molecular mechanisms underlying the aberrant GH-IGF-1 axis in obese human subjects. We therefore investigated GH signaling in muscle and adipose tissue following exposure to an IV GH bolus in obese, but otherwise healthy subjects who were well characterized

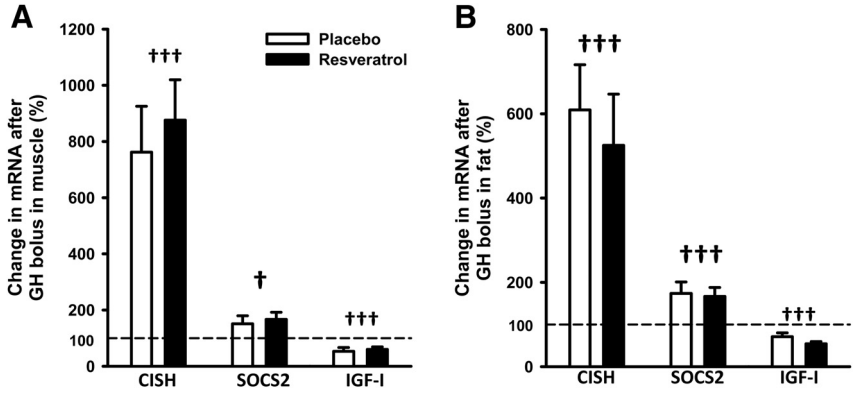

Figure 2. mRNA levels of GH responsive gene. Change in mRNA expression levels in (A) human skeletal muscle or (B) human adipose tissue of CISH, SOCS2, and IGF-1, 1 h after injection of GH bolus compared to the basal state. Prior to this, the subjects had been treated with placebo (n = 12) or resveratrol (n = 12) for 4–5 weeks. Data presented as the mean percentage increase after GH injection ± SE. †, $P < .05$ and †††, $P < .001$, 1 hour after GH stimulation compared to basal condition for both the placebo and resveratrol group. No differences between the placebo and resveratrol groups were found.

with regard to body composition, substrate metabolism, and insulin sensitivity. In addition to this, we assessed the impact of 5 weeks treatment with the putative SIRT1 activator resveratrol on the same parameters. The main findings were that distinct activation of GH signaling in terms of STAT5b phosphorylation and gene expression of SOCS/CISH genes were recorded in both tissues. Overall, the degree and distribution of adiposity did not correlate with measures of GH signaling, whereas age was a significant and positive determinant of stimulated STAT5b phosphorylation in muscle. Moreover, resveratrol did not significantly impact stimulated GH signaling either in vivo or in vitro.

Taken together, our data suggest that responsiveness to acute GH exposure is maintained in obese human subjects and speak against a specific impact of obesity on GH action at the level of GH receptor signal transduction. A decrease in the expression of the full-length GH receptor relative to its truncated isoform (trGHR) in subcutaneous adipose tissue has been observed in obese individuals and hypothesized to underlie resistance to GH-induced fat loss in obesity (20). Our finding of preserved STAT5b activation in obesity does not support this hypothesis, and it has been reported in several trials that obese subjects respond well to short-term GH administration in terms of fat mass reduction (21, 22). On the other hand, more prolonged exogenous GH exposure may induce GH resistance via the aforementioned mechanism, since a significant increase in trGHR expression has been recorded in adults with GH-deficiency after 4 months GH replacement (23). Moreover, our current data do not challenge the fact that GH secretion and action is perturbed in human obesity, and findings from several mice models with global or tissue-specific modifications of GH action emphasize that GH is an important regulator of adipose tissue mass via direct as well as indirect mechanisms (22, 24, 25). Our data show for the first time that muscle and adipose tissue from obese human male subjects are highly responsive to acute exogenous GH exposure at the level of GH signaling in vivo, which suggest that

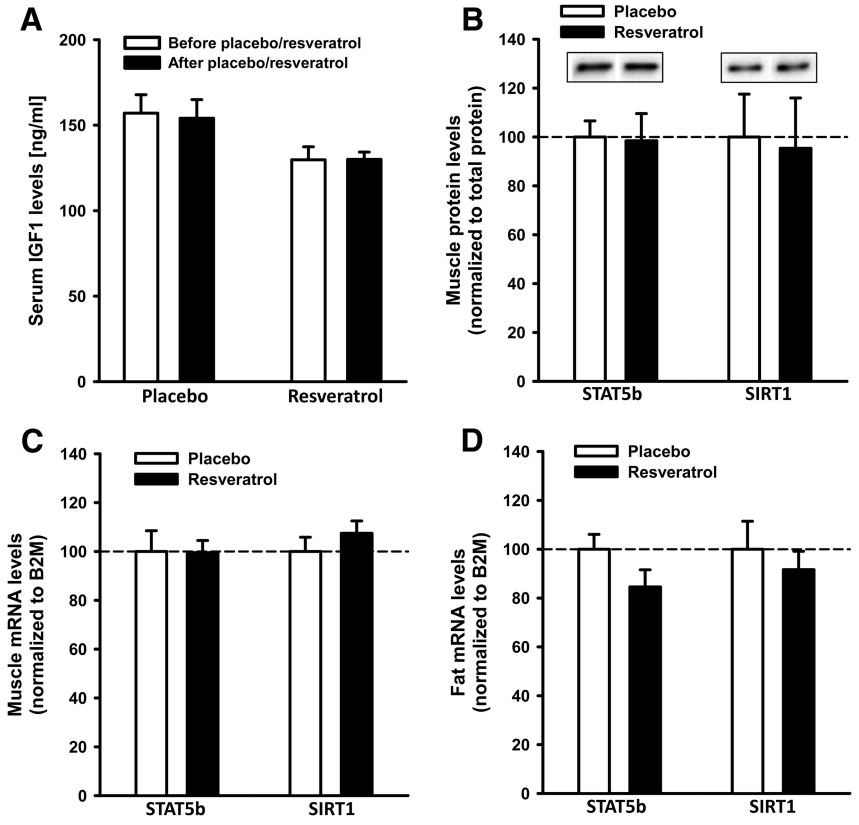

Figure 3. Effect of resveratrol treatment on IGF-1, STAT5b, and SIRT1. A, Circulating total IGF-1 before (white bar) and after (black bar) four weeks of placebo or resveratrol treatment. Change in SIRT1 and STAT5b (B) protein levels in skeletal muscle, (C) mRNA levels in muscle, or (D) mRNA in adipose tissue after placebo or resveratrol treatment. Data presented as mean percentage increase after indicated treatment ± SE. No differences between the placebo and resveratrol groups were found.

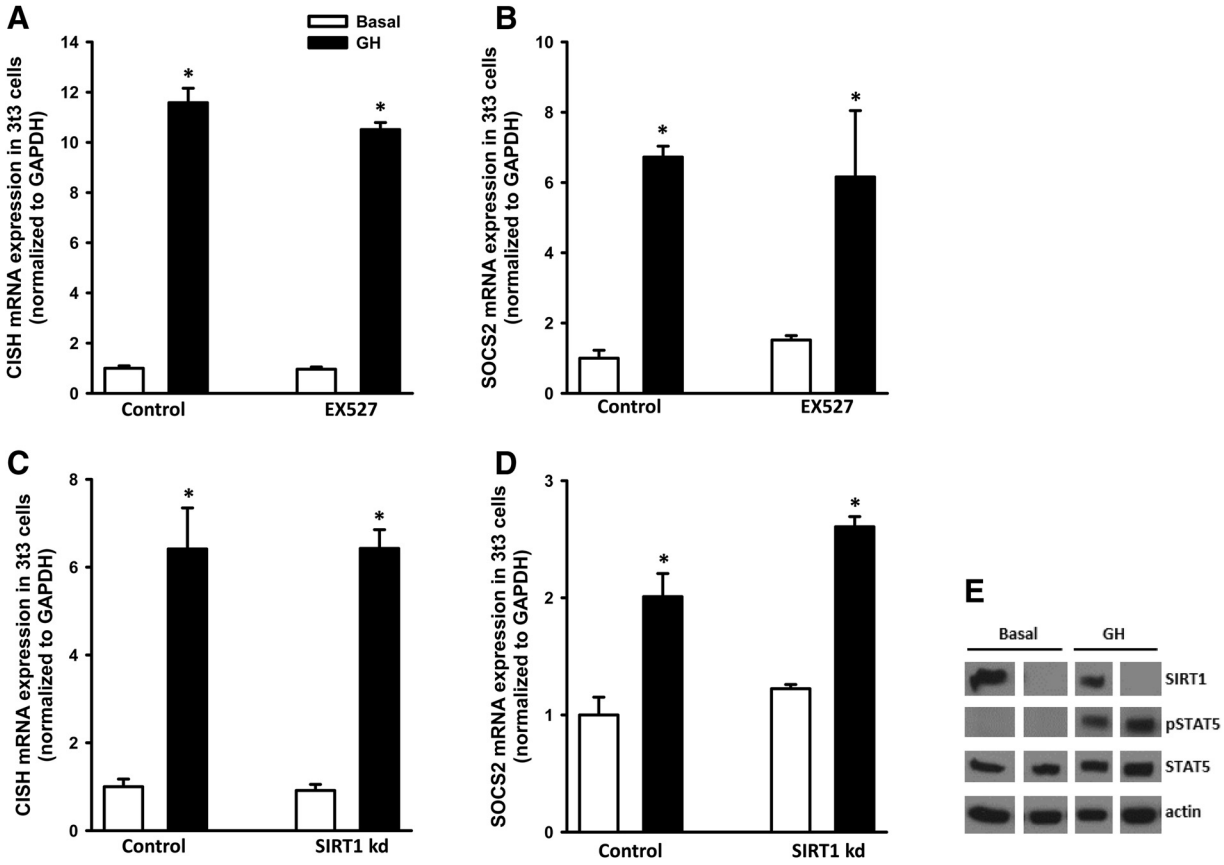

Figure 4. SIRT1 and effects of GH in 3t3 cells. Effect of SIRT1 inhibition using EX527 on (A) CISH and (B) SOCS2 mRNA levels after 30 minutes GH incubation (black bar) or without GH (white bar). Effect of knocking down SIRT1 using siRNA on (C) CISH and (D) SOCS2 mRNA levels after 30 minutes GH incubation. (E) Effect of SIRT1 knockdown on STAT5 and pSTAT5 protein levels after 120 minutes GH incubation. Cell culture experiments were conducted in singlets, qRT-PCR was run in triplets, and experiments were repeated for validation at least once. Data presented as mean values ± SE within the qRT-PCR experiment. *, $P < .05$ for GH compared to basal. No change in basal transcription levels were found between control and EX527/SIRT1 kd.

the GH signaling system is intact and not down-regulated. We included several measures of total and regional adiposity including BMI, total body fat and ectopic fat in liver and muscle all of which failed to correlate with activated GH signaling in muscle and fat. We did record several intercorrelations between GH-induced activity of pSTAT5b and expression of canonical GH target genes in muscle. This was less pronounced in fat tissue apart from intercorrelations between stimulated expression of SOCS2 and CISH. Whether the apparent dissociation between pSTAT5b activation and gene expression in fat tissue is of physiological or clinical significance remains speculative. Unexpectedly, IGF-1 mRNA expression in both muscle and fat was lower 1 h after GH exposure as compared to the level of expression in the biopsies obtained on the control day. We have previously recorded a significant increase in IGF-1 mRNA expression in adipose tissue 30–60 minutes after exposure to an IV GH bolus (8) and in muscle and fat after several hours of constant GH infusion (9, 26), but failed to detect a change in a study involving biopsies obtained 2 h after an IV GH bolus (27). In the present study, the biopsies representing the GH-unstimulated state were not obtained on the same day as the biopsy obtained after GH exposure, which may have impacted the results. Alternatively, the time window of 1 hour post GH exposure is not well suited for detection of GH-induced IGF-1 mRNA expression. At any rate we do not find it likely that GH consistently suppresses IGF-1 mRNA expression in obese subjects.

It was interesting that age was the only and positive determinant of stimulated GH signaling, given the evidence that obesity rather than chronological age per se is the major and negative determinant of spontaneous and stimulated endogenous GH secretion in nonobese human adults (4, 28, 29). Our design did not include a comprehensive assessment of endogenous GH secretion, which would require frequent measurements (every 10–20 min) over a 24 h period. We did, however, observe a significant inverse correlation with endogenous GH levels measured over 6 h and fat percentage, which is in accordance with

the literature (1, 2). Whether age per se in the context of obesity plays a more direct role in GH action in skeletal muscle therefore merits future investigations.

We have previously observed that lipid infusion suppresses pSTAT5b activity in human skeletal muscle, which could provide a mechanistic link between obesity and aberrant GH function (12). In this study we did not see a correlation between endogenous serum FFA levels and pSTAT5b activity, although serum FFA levels and pSTAT5b were not measured on the same day.

The liver is a major GH target but for good reasons we did not obtain liver biopsies in our study. It is well known that serum IGF-1 primarily derives from hepatic production and secretion (30), such that this parameter can serve as a surrogate measure of hepatic responsiveness to endogenous GH exposure. We recorded normal serum IGF-1 levels for age and gender expressed as SDS based on a large reference sample. This is in accordance with most existing data (31, 32), and therefore supports that obesity in humans is accompanied by a compensatory mechanism to maintain normal hepatic secretion of IGF-1, despite low circulating GH levels. Whether this is mediated by increased sensitivity to GH itself or other mechanisms, such as insulin-induced hepatic IGF-1 production (33), remains uncertain. In this context, it is noteworthy that serum IGF-1 correlated positively with adipose tissue IGF-1 mRNA, and that age correlated inversely with *both* serum IGF-1 and adipose tissue IGF-1 mRNA. This could suggest that IGF-1 produced in adipose tissue contribute to circulating IGF-1 levels, but since correlations do not imply causality, experimental studies are needed. It has been reported that GH reduces intrahepatic fat accumulation (34), but in the present study we did not record any association between ectopic fat as assessed by MR spectroscopy and measures of GH activity. Whether the discrepant results are attributed to obesity remains elusive.

Our study design regarding GH signaling has some limitations. Inclusion of a control group of nonobese subjects would have allowed for a direct comparison. Instead we assessed the impact of obesity by correlating GH signaling to indices of body composition. It would also have strengthened the study if the study had included serial biopsies before and after GH exposure. Finally, it would have been informative if endogenous GH secretion had been assessed more meticulously.

The present study failed to detect any effects of resveratrol treatment on levels of GH or GH signaling. We have previously documented the uptake, metabolism, and correct enantiomeric conformation of resveratrol in these subjects (18). Furthermore, we saw no association between SIRT1 and GH signaling in a cell line modeling peripheral tissue. It would be of high relevance to also assess acetylation status of STAT5b in these cells, but in our hands inhibiting or knocking down SIRT1 yielded several-fold decreased IP efficiency, making this analysis impossible. Our in vitro and human in vivo findings contrast with a recent study in a rodent model where resveratrol via deacetylation of STAT5b suppressed hepatic IGF-1 production (13). Moreover, resveratrol in our study also failed to impact body composition, substrate metabolism and insulin sensitivity as previously published (18). The latter observation is in accordance with other studies which also failed to detect any effects in a human model (35, 36), but contrasts with one study where moderate effects compatible with putative SIRT1 activation were recorded (37). From a theoretical point of view, the concept that SIRT1 activity is causally linked to GH secretion and action is compelling. During fasting and calorie restriction, serum IGF-1 levels decline, whereas GH secretion is enhanced in conjunction with amplification of the lipolytic effects of GH (38, 39). This nutrient-dependent dissociation of GH effects, characterized by suppressed anabolic signals (via IGF-1) and stimulated lipolysis (IGF-1-independent), may constitute one important mechanism underlying the favorable effects of calorie restriction on metabolic health in adult subjects (39). In the same context, SIRT1 activation has been shown to mediate many of the effects of calorie restriction in several species (40), even though the mechanism through which resveratrol activates SIRT1 is still debated (41–44). The observation that resveratrol in a rodent model suppresses hepatic IGF-1 production (13) thus provided a mechanistic link between the favorable effects of GH and SIRT1 during calorie restriction with potential clinical implications, also in the context of obesity. Another recent study reported that resveratrol suppresses GH secretion in a SIRT1 dependent manner in the rat pituitary (45). We did not find resveratrol treatment to lower circulating GH levels, but the fact that we did not perform a deconvolution-based assessment of pituitary GH secretion should be taken into consideration. At present it remains to be convincingly demonstrated that SIRT1 activation exerts robust effects on GH secretion and action in human subjects.

In conclusion, the present study demonstrates that GH signaling in response to acute exogenous GH exposure is robust in muscle and fat of obese subjects, and does not seem to depend on the degree of adiposity. Moreover, resveratrol supplementation in the same subjects did not impact either GH signaling or serum IGF-1 levels. It remains to be studied if activation by SIRT1 via other mechanisms may impact GH secretion and action in human subjects.

Acknowledgments

We give special thanks to Helle Zibrandtsen, Annette Mengel, Elsebeth Hornemann, and Hanne Fjelsted Petersen at Aarhus University Hospital, Aarhus, Denmark, for excellent technical assistance.

B.F.C. performed WB and in vitro experiments, analyzed all data, performed statistical analyses, and wrote the manuscript. J.O.J. designed the study, assisted in data analysis and writing of the manuscript and generally guided throughout the process. J.O.J., N.J., S.B.P., and in particular M.M.P. were involved and responsible for the clinical aspect of the study. S.B.P. conducted the qRT-PCR experiments. In vitro experiments were conducted in the laboratory of E.N.C. and with knowledgeable guidance from E.N.C. and C.E. All authors reviewed the article before submission. B.F.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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This work was supported by grants from the Danish Agency for Science Technology and Innovation (Grant No. 09-072323), The Novo Nordisk Foundation, Karen Elise Jensens Foundation, The Toyota Foundation, Elvira and Rasmus Riisfort Foundation, Ejnar Danielsens Foundation, and the AP Møller Maersk Foundation. The study is also part of the research program at LIRMOI Research Center (www.LIRMOI.com), which is supported by the Danish Council for Strategic Research (Grant No. 10-093499).

Clinical trial reg. no: NCT01150955, clinicaltrials.gov.

Disclosure Summary: J.O.L.J. has received unrestricted research grant and lecture fees from Pfizer Inc. No other potential conflicts of interest relevant to this article were reported.

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