GH signaling in human adipose and muscle tissue during ‘feast and famine’: amplification of exercise stimulation following fasting compared to glucose administration

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Abstract

Objective: Fasting and exercise stimulates, whereas glucose suppresses GH secretion, but it is uncertain how these conditions impact GH signaling in peripheral tissues. To test the original ‘feast and famine hypothesis’ by Rabinowitz and Zierler, according to which the metabolic effects of GH are predominant during fasting, we specifically hypothesized that fasting and exercise act in synergy to increase STAT-5b target gene expression.

Design and methods: Eight healthy men were studied on two occasions in relation to a 1 h exercise bout: i) with a concomitant i.v. glucose infusion (‘feast’) and ii) after a 36 h fast (‘famine’). Muscle and fat biopsy specimens were obtained before, immediately after, and 30 min after exercise.

Results: GH increased during exercise on both examination days and this effect was amplified by fasting, and free fatty acid (FFA) levels increased after fasting. STAT-5b phosphorylation increased similarly following exercise on both occasions. In adipose tissue, suppressors of cytokine signaling 1 (SOCS1) and SOCS2 were increased after exercise on the fasting day and both fasting and exercise increased cytokine inducible SH2-containing protein (CISH). In muscle, SOCS2 and CISH mRNA were persistently increased after fasting. Muscle SOCS1, SOCS3, and CISH mRNA expression increased, whereas SOCS2 decreased after exercise on both examination days.

Conclusions: This study demonstrates that fasting and exercise act in tandem to amplify STAT-5b target gene expression (SOCS and CISH) in adipose and muscle tissue in accordance with the ‘feast and famine hypothesis’; the adipose tissue signaling responses, which hitherto have not been scrutinized, may play a particular role in promoting FFA mobilization.

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Introduction

Growth hormone (GH) and its principal intracellular signal mediator, STAT-5b, represent a well-preserved endocrine control system, which throughout evolution has served to maintain growth and lean body mass and to regulate intermediary metabolism (1). In adipocytes and skeletal muscle, STAT-5b serves as a transcription factor for target genes including a family of

cytokine-inducible suppressors of cytokine signaling (SOCS) (1, 2, 3), cytokine inducible SH2-containing protein (CISH) (2, 4), and insulin-like growth factor 1 (IGF1). GH has important actions to regulate anabolic signals and preserve lean body mass protein (1); some of these anabolic effects are mediated by stimulation of lipolysis (5, 6).

In a classic paper Rabinowitz & Zierler (7) introduced the concept of a 'feast and famine cycle', according to which insulin is the major anabolic hormone storing fuels during periods of food surplus ('feast') and which GH initiated activation of fat depots and high levels of free fatty acids (FFAs) safeguard the individual during stress and famine by restricting protein loss.

Since then a number of studies have lent support to the role of GH in the feast and famine concept. Studies in humans have shown that during fasting GH substantially (> 30%) reduces protein breakdown and this effect depends on mobilization of FFA from lipid stores (5, 6). In the basal state (after an overnight fast), it has been difficult to document consistently the anabolic effects of GH (1).

In addition it has been reported that GH increases phosphorylation of STAT-5b in human muscle and fat (8) and activates SOCS1–3, CISH, and IGF1 mRNA expression in skeletal muscle (4, 9, 10). Stress conditions such as exercise and fasting with increased GH secretion have also been shown to stimulate human skeletal muscle STAT-5b phosphorylation and IGF1 mRNA expression (11, 12), although it is unclear whether this involves stimulation of SOCS/CISH transcription. In adipose tissue it remains uncertain how fasting and exercise affect human STAT-5b signal transduction.

To further explore the viability of the feast and famine concept, we conducted the present study to test whether spontaneous and exercise-stimulated GH secretion and subsequent intracellular activation of STAT-5b phosphorylation and target gene expression in muscle and fat are amplified during famine conditions as opposed to feast. We used a 36 h fast to reproduce 'famine' and a continuous infusion of glucose to mimic 'feast'.

Methods

Ethical approval

All participants gave written, informed consent in accordance with the Declaration of Helsinki II. The local ethics scientific committee approved the study.

Subjects

Eight healthy men (age 27 ± 5 years, body weight 77.9 ± 6 kg, BMI 24.7 ± 2 kg/m², and $\text{VO}_{2\text{-peak}}$ 4034 ± 588 ml/min) participated.

Protocol

On both experimental days, the subjects completed a 1 h cycling exercise at 50% $\text{VO}_{2\text{-peak}}$ or until fatigued, after a

60 min baseline period. Prior to one of the experimental days the subjects fasted for 36 h. On the other experimental day, the subjects received a continuous glucose infusion after a 12 h overnight fast: 0.2 g/kg per h for 1 h (baseline period), 1 g/kg per h during the 1 h of exercise, and 0.2 g/kg per h for the remaining 2 h (post exercise). Baseline skeletal muscle biopsies and blood samples were obtained 1 h before exercise ($t = -60$), immediately after exercise ($t = 60$), and 30 min into the recovery period ($t = 90$). Biopsies were sampled alternately from the right and left M. vastus lateralis using a Bergström needle and immediately frozen in liquid nitrogen. Adipose tissue biopsies were sampled from the abdominal subcutaneous adipose tissue 1 h before exercise initiation ($t = -60$) and 30 min into the recovery period ($t = 90$) using a lipid suction technique and then immediately washed free of blood and frozen in liquid nitrogen (Fig. 1).

Blood analysis

Plasma glucose was immediately measured (Beckman Instruments, Brea, CA, USA). Serum samples were stored at -20°C , and GH and insulin were analyzed using immunoassays (AutoDELFIA; PerkinElmer, Turku, Finland), as was C-peptide (DakoCytomation, Cambridgeshire, UK). FFA was analyzed by a commercial kit (Wako Chemicals, Neuss, Germany).

Western blotting

The frozen skeletal muscle and adipose tissue samples were crushed into powder in a frozen steel mortar. The tissue powder was homogenized in an ice-cold lysis buffer (adipose tissue: 20 mM HEPES, 10 mM NaF, 1 mM Na_3VO_4 , 1 mM EDTA, 5% SDS, 50 µg/ml soybean trypsin inhibitor, 4 µg/ml leupeptin, 0.1 mM benzamidine, 2 µg/ml antipain, 1 µg/ml pepstatin; skeletal muscle: 20 mM Tris-HCl, 50 mM NaCl, 250 mM sucrose, 50 mM NaF, 5 mM $\text{Na}_4\text{P}_2\text{O}_7$, 1% Triton X-100, 5 µg/ml leupeptin, 1.5 µg/ml benzamidine, 500 µM phenylmethylsulphonyl fluoride, 50 µg/ml soybean trypsin inhibitor, and 2 mM dithiothreitol) using a Precellys homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France). Insoluble materials were removed by centrifugation at 14 000 g for 20 min at 4°C . The lipid-free infranatant was isolated three times in adipose tissue before being used for western blotting. In the skeletal muscle, protein concentration of the supernatant was determined using a Bradford assay (Bio-Rad). Samples were adjusted to equal concentrations with Milli-Q Water and denatured by mixing with

Figure 1

Schematic presentation of the study design. On two experimental days, the subjects completed a 1 h cycling exercise bout at 50% VO_{2-peak}. One bout (glucose infusion day) was performed with continuous glucose infusion (0.2 g/kg per h for 1 h (baseline period), 1 g/kg per h during the 1 h of exercise, and 0.2 g/kg per h for the remaining 2 h (post exercise)) after a 12 h overnight fast, and the other bout (fasting day) was performed

after 36 h of fasting. The order of the two experimental days was randomized and separated by ~1 month. Skeletal muscle biopsies and blood were sampled 60 min before exercise initiation, immediately after exercise termination, and 30 min into the recovery period. Furthermore, adipose tissue biopsies were performed 60 min before exercise and 30 min into the recovery period.

Laemmli's buffer and heating at 95 °C for 5 min. Equal amounts of protein were separated by SDS-PAGE using the Bio-Rad Criterion system, and proteins were electroblotted onto PVDF membranes (Bio-Rad) as described (13, 14). Control for equal loading was assessed with Stain-Free technology. Membranes were blocked for 2 h in a 2% BSA solution (Sigma-Aldrich) and incubated overnight with primary antibodies against phosphorylated STAT-5b Tyr⁶⁹⁹ and STAT-5b (Cell Signaling Technology, Beverly, MA, USA).

Real-time PCR

Frozen skeletal muscle and adipose tissue powder (~30 mg) were homogenized using TRIzol reagent added DNase and Protein kinase K (Gibco BRL, Life Technologies), and total RNA was extracted following the manufacturer's protocol. RNA was quantified by measuring absorbance at 260 and 280 nm using a NanoDrop 8000 (NanoDrop Products, Bancroft, DE, USA), and inclusion criteria were a ratio ≥ 1.8 . Integrity of the RNA was checked by visual inspection of the two ribosomal RNAs, 18S and 28S, on an agarose gel. cDNA was synthesized with the TaqMan Gold RT-PCR Kit (Perkin-Elmer, Boston, MA, USA). Real-time PCR for SOCS1-3, CISH, and IGF1 were assessed with mRNA levels of β -actin as an internal control. The following primers were used: SOCS1: 5'-ACACGCACTTCCGCACATTC-3' and 5'-CGAGGCCATCTTCACGCTAAG-3'; SOCS2: 5'-GGTCGAGGCGATCAGTG-3' and 5'-TCCTTGAAGTC-AGTGC GAATC-3'; SOCS3: 5'-GCCCTTTGCGCCCTTT-3'

and 5'-CGGCCACCTGGACTCCTATGA-3'; CISH: 5'-GCCCTGAGCCCTGGTAGTCC-3' and 5'-GACACATTCACAGACGGGTGG-3'; IGF1: 5'-GACAGGGGCTTTTATTCAAC-3' and 5'-CTCCAGCCTCCTTAGATCAC-3'; and β -actin: 5'-ACGGGGTCACCCACACTGTGC-3' and 5'-CTAGAAGCATTTGCGGTGGACGATG-3'.

Statistical analysis

Results are expressed as means $\pm$ S.E.M. Normal distribution was assessed by inspection of QQ plots, and the Levene median test was used to test for equal variance. The effects of experimental day and time/exercise interaction were assessed by one-way- and/or two-way repeated-measurements ANOVA, with Student-Newman-Keul's *post hoc* testing.

Results

Circulating hormones and metabolites

GH concentrations were unchanged after 36 h of fasting. Subjects performed 1 h ergometer cycling at 50% of VO_{2-peak}, with average workloads of 139 ± 6.4 W (glucose) and 135 ± 6.5 Watt (W) (fasting) (Table 1). All participants completed 1 h of exercise on both experimental days, apart from one subject who fatigued after 45 min of exercise on the fasting day. Exercise increased circulating GH levels on both days, significantly more so after fasting. Fasting increased FFA levels, which rose further after exercise; during glucose infusion, FFA concentrations remained suppressed and levels of glucose, insulin, and C-peptide increased.

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Table 1 Effect of 36 hours fasting and one hour ergometer cycling at 50% VO_2 -peak on growth hormone (GH), glucose, insulin, C-peptide, and free fatty acid (FFA) concentrations in serum. Throughout the table pre-exercise is indicated with $t=-60$, immediately after exercise is indicated with $t=60$, and 30 min after exercise is indicated with $t=90$ on both examination days. Furthermore, fasting experimental day is illustrated with Fasting day and glucose infusion day is illustrated with Glucose day.

	Glucose day			Fasting day		
	$t=-60$	$t=60$	$t=90$	$t=-60$	$t=60$	$t=90$
GH (ng/ml)	1.13 ± 0.4	$3.52 \pm 1.0^*$	1.10 ± 0.3	1.33 ± 0.6	$6.05 \pm 1.3^{*,\dagger}$	2.29 ± 0.6
Glucose (mM)	5.26 ± 0.1	$7.78 \pm 0.8^*$	5.35 ± 0.4	$4.37 \pm 0.2^\dagger$	$4.64 \pm 0.4^\dagger$	$4.30 \pm 0.3^\dagger$
Insulin (pM)	29.74 ± 3.7	$137.35 \pm 22.0^*$	$64.11 \pm 19.4^*$	20.87 ± 3.8	$34.74 \pm 9.5^\dagger$	$20.66 \pm 4.9^\dagger$
C-peptide (pM)	543.75 ± 33	$1475.63 \pm 16^*$	$1073.63 \pm 17^*$	341.63 ± 61	$405.50 \pm 69^\dagger$	$364.38 \pm 66^\dagger$
FFA (mM)	0.36 ± 0.07	0.18 ± 0.04	0.12 ± 0.02	$0.90 \pm 0.11^\dagger$	$1.70 \pm 0.15^{*,\dagger}$	$1.10 \pm 0.14^\dagger$

* $P < 0.05$ vs $t = -60$ within experimental day and $^\dagger P < 0.05$ vs same time point on glucose infusion experimental day.

GH signaling in adipose tissue and skeletal muscle

Phosphorylation of STAT-5b Tyr⁶⁹⁹ was unaltered in adipose and skeletal muscle after 36 h of fasting compared to the overnight fast (Figs 2A and 3A). Exercise dramatically increased STAT-5b Tyr⁶⁹⁹ phosphorylation in fat and muscle without significant differences between examination days. A trend ($P=0.074$) toward increased phosphorylation was observed immediately after exercise ($t=60$) in skeletal muscle on the fasting examination day compared to the glucose infusion day.

STAT-5b target genes in adipose tissue

In adipose tissue, a significant main effect of the experimental day was observed on mRNA expression of SOCS1–2 and CISH, while only a trend ($P=0.061$) was observed in regard to SOCS3 (Fig. 2B, C, D, E and F). *Post hoc* testing revealed that SOCS1–2 and CISH mRNA expressions were significantly elevated 30 min after exercise ($t=90$) on the fasting day compared to glucose infusion. Exercise induced detectable ($P < 0.05$) increments in SOCS1–2 and CISH mRNA after 36 h of fasting, whereas only CISH expression increased after exercise combined with glucose infusion. IGF1 mRNA expressions remained unaltered.

STAT-5b target genes in skeletal muscle

In skeletal muscle, a significant main effect of the experimental day was observed on mRNA expression of SOCS2 and CISH, and *post hoc* testing revealed that expression levels on each biopsy time point were significantly increased on the fasting experimental day compared to the glucose infusion day (Fig. 3B, C, D, E and F). A main effect of time/exercise was observed on SOCS1–3

and CISH mRNA expression. SOCS1, SOCS3, and CISH mRNA expressions were increased immediately after exercise ($t=60$) on both experimental days, SOCS3 expression being significantly higher on the glucose infusion day at $t=60$. On both examination days, SOCS3 and CISH mRNA expression was significantly increased 30 min after exercise termination ($t=90$). *Post hoc* testing revealed that SOCS2 mRNA expressions were equally suppressed on both time points ($t=60$ and 90) after exercise on the glucose infusion day, whereas this effect did not reach statistical significance on the fasting examination day. Again, IGF1 mRNA expressions remained unaltered.

Discussion

This study was designed to test whether exercise-stimulated GH signaling in fat and muscle is amplified after fasting compared to a glucose infusion. Our data show that exercise strongly stimulates STAT-5b phosphorylation and that SOCS1–2 and CISH expression in fat, and SOCS2 and CISH expression in muscle increase after fasting and remain increased in response to exercise. These findings support the ‘fast and famine’ concept by locating a stronger intracellular GH signal in two major metabolic target tissues as a mechanism, whereby GH exerts distinct metabolic effects during conditions of stress, such as fasting and exercise.

In the present study we opted for i.v. administration of nutrients to imitate fed conditions, because this allows controlled steady state conditions, which are not dependent on unpredictable gastrointestinal absorption. We chose glucose alone as an energetically simple compound, which is typically used as a supplement during prolonged exercise and excludes amino acids and lipids, which may

Figure 2

Effects of 36 h of fasting and 1 h of ergometer cycling at 50% $\text{VO}_{2\text{-peak}}$ on growth hormone signaling in adipose tissue were assessed with western blotting and real-time PCR. White bars indicate pre-exercise ($t = -60$) and black bars indicate 30 min after exercise ($t = 90$) on both examination days. +, Fasting experimental day; -, glucose infusion day; *, main effect of experimental day = $P < 0.05$; time = $P < 0.05$, main effect of time/exercise; § $P < 0.05$ vs $t = -60$ within the experimental day; §* $P < 0.05$ vs same time point on glucose infusion day. (A) STAT-5b Tyr⁶⁹⁹ phosphorylation was unaltered after 36 h of fasting but increased after exercise ($t = 90$) on both examination days. (B and C) mRNA expression of SOCS1 and SOCS2 was significantly increased after 36 h of fasting. However, *post hoc* testing revealed that SOCS1 and SOCS2 mRNA expression were only increased after exercise on the fasting experimental day compared to the glucose infusion day. (D) There was a trend ($P = 0.061$) toward increased SOCS3 mRNA expression levels after 36 h of fasting. (E) mRNA expression of CISH was significantly increased after 36 h of fasting, and *post hoc* testing revealed that it was increased on both time points ($t = -60$ and 90) on the fasting experimental day compared to the glucose infusion day. Furthermore, CISH mRNA expression increased after exercise ($t = 90$) in adipose tissue on both examination days compared to pre-exercise levels within the experimental day. (F) No changes were observed on IGF1 mRNA expression after fasting or exercise.

affect GH secretion independently (1). We did not detect increased baseline levels of GH after 36 h of fasting, which could relate to the relatively short period of fasting and the 'elusive' pulsatile nature of GH secretion. Previous studies with more frequent blood sampling have shown increased GH concentrations after 72 h of fasting (12), and GH levels during exercise were increased after fasting in the present study. It is therefore likely that the increased intracellular GH signal following exercise on the fasting examination day to a large extent is a direct consequence of increased circulating levels of GH. On the other hand, high levels of FFA as observed during fasting have been shown to dampen

muscle STAT-5b phosphorylation by up to 40%, which may counterbalance stimulation of the intracellular signal (15). The present study design does not allow discrimination between the contribution of increased GH concentrations and altered intracellular signaling cascade sensitivity and responsiveness to GH. The impact of fasting on the dose-response effects of GH on signal transduction and metabolism are not well characterized. One study has shown increased lipolytic responsiveness in the presence of decreased STAT-5b phosphorylation after a GH bolus during fasting in healthy volunteers (16), compatible with increased distal signal transduction in adipocytes. In this

Figure 3

Effect of 36 h of fasting and 1 h of ergometer cycling at 50% $\text{VO}_{2\text{-peak}}$ on growth hormone signaling in skeletal muscle tissue were assessed with western blotting and real-time PCR. White bars indicate pre-exercise ($t = -60$), dashed bars indicate immediately after exercise ($t = 60$), and black bars indicate 30 min after exercise ($t = 90$) on both examination days. +, Fasting experimental day; –, glucose infusion day; *, main effect of experimental day $P < 0.05$; \$ $P < 0.05$ vs $t = -60$ within the experimental day; † $P < 0.05$ vs $t = 60$ within experimental day; ‡ $P < 0.05$ vs same time point on glucose infusion day; and * $P = 0.074$ vs same time point on glucose infusion day. (A) STAT-5b Tyr⁶⁹⁹ phosphorylation was unaltered after 36 h of fasting but increased on both time points after exercise ($t = 60$ and 90) on both examination days. Furthermore, there was a trend toward higher STAT-5b phosphorylation immediately after exercise ($t = 60$) on the fasting experimental day compared to the glucose infusion day. (B) SOCS1 mRNA expression was unaltered after 36 h of fasting, but there was a main effect of time/exercise $P < 0.05$, and *post hoc* testing revealed that expression was significantly increased 30 min after exercise ($t = 90$) on the fasting experimental day. (C) mRNA expression of SOCS2 was significantly increased after 36 h of fasting. *Post hoc* testing revealed that SOCS2 mRNA expression was increased on all time points after fasting compared to the glucose infusion day. Furthermore, there was a main effect of time/exercise $P < 0.05$, and *post hoc* testing revealed that SOCS2 mRNA expression was significantly decreased immediately after ($t = 60$) and 30 min after exercise ($t = 90$) on the glucose infusion day. (D) SOCS3 mRNA expression was unaltered after 36 h of fasting, but there was a main effect of time/exercise $P < 0.05$, and *post hoc* testing revealed that expression was significantly increased immediately after exercise ($t = 60$) and 30 min after exercise ($t = 90$) on the glucose infusion day and 30 min after exercise ($t = 90$) on the fasting experimental day. (E) There was a main effect of fasting on CISH mRNA expression, and *post hoc* testing revealed that expression was increased on all time points after fasting compared to the glucose infusion day. Furthermore, there was a main effect of time/exercise $P < 0.05$, and *post hoc* testing revealed that CISH mRNA expression was significantly increased 30 min after exercise ($t = 90$) on both examination days. (F) No significant changes were observed on IGF1 mRNA expression after fasting or exercise.

context it should be underlined that many other factors, including insulin, cortisol, epinephrine, FFA, ketone bodies, and the autonomous nervous system, may modulate the lipolytic response to GH.

Several human *in vivo* studies have shown that during fasting the protein conserving actions of GH progressively increase (5), that these effects involve inhibition of muscle protein breakdown (5), and that protection of protein

depends critically on increased levels of FFA (6), thereby highlighting the central role of GH-stimulated lipolysis in adipose tissue. In addition, a study in GH-deficient subjects with or without GH administration reported that the most significant metabolic action of GH during exercise is the stimulation of lipolysis (17). The precise cascade of events whereby GH stimulates adipose tissue lipolysis is incompletely understood, and there is a lack of human *in vivo* studies examining these events. Administration of GH to normal subjects increases STAT-5b phosphorylation and SOCS3 mRNA in adipose tissue (10) and a recent study in JAK2 knockout mice reported impaired lipolysis (18).

The biological function of the SOCS/CISH proteins is mainly to feedback inhibit the JAK/STAT signaling pathway (1). The increments in individual SOCS/CISH proteins may thus be seen as a part of a feedback loop controlling GH effects and the ensuing stimulation of lipolysis. Interestingly, SOCS3 overexpression in mouse adipose tissue has been shown to cause local insulin resistance (19) and deletion of skeletal muscle SOCS3 prevents insulin resistance in obese mice (20).

In this study we did not observe significant effects of 36 h of fasting on STAT-5b phosphorylation or IGF1 mRNA levels. We have previously observed that more prolonged fasting tends to increase both STAT-5b phosphorylation and IGF1 mRNA levels in muscle (12), implying that intracellular GH signaling is further augmented during longer periods of fasting.

Fasting and exercise are catabolic states during which the energy balance of the body is negative and, as such, a threat to survival. In 1963 Rabinowitz & Zierler (7) introduced their 'feast and famine' cycle and proposed that GH acted as a key conservator of protein during catabolic stress. The present data add novel features to this concept by showing that exercise-induced stimulation of GH signaling activity *in vivo* is amplified by fasting at the level of SOCS and CISH target gene expression in skeletal muscle and adipose tissue in human subjects.

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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