Enhancement of Muscle Mitochondrial Function by Growth Hormone

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Context: Although GH promotes growth and protein anabolism, which are ATP-dependent processes, the GH effect on mitochondrial regulation remains to be determined.

Objective: Our objective was to determine the acute effect of GH on mitochondrial oxidative capacity in skeletal muscle of healthy subjects.

Design and Setting: The study was a randomized crossover design at an academic medical center.

Participants: Nine healthy men and women completed the study.

Intervention: GH (150 μg/h) or saline was infused for 14 h on separate days, and muscle biopsies were obtained.

Main Outcome Measures: Outcome measures included mitochondrial function, gene expression, and protein metabolism.

Results: The 4-fold increase in plasma GH caused elevations in plasma IGF-I, insulin, glucose, and free fatty acids and a shift in fuel selection, with less carbohydrate (−69%) and leucine (−43%) oxidation and 29% more fat oxidation. Muscle mitochondrial ATP production rate and citrate synthase activity were increased 16–35% in response to GH. GH also resulted in higher abundance of muscle mRNAs encoding IGF-I, mitochondrial proteins from the nuclear (cytochrome *c* oxidase subunit 4) and mitochondrial (cytochrome *c* oxidase subunit 3) genomes, the nuclear-derived mitochondrial transcription factor A, and glucose transporter 4. Although GH increased whole-body protein synthesis (nonoxidative disposal of leucine), no effect on synthesis rate of muscle mitochondrial proteins was observed.

Conclusions: These results demonstrate that acute GH action promotes an increase in mitochondrial oxidative capacity and abundance of several mitochondrial genes. These events may occur through direct or indirect effects of GH on intracellular signaling pathways but do not appear to involve a change in mitochondrial protein synthesis rate. (***J Clin Endocrinol Metab*** **93: 597–604, 2008)**

Human GH promotes organ growth and is an important regulator of fuel metabolism in both health and disease. GH receptors have been identified in most tissues including muscle, adipose tissue, liver, heart, kidney, brain, and pancreas (1, 2). The recognized actions of GH include protein anabolism, promotion of lipolysis, and resistance to insulin-induced glucose metabolism in liver and peripheral tissues (1–3). Several studies demonstrated that both acute and chronic GH infusion reduces urea synthesis, promotes protein synthesis, and typically results in reduction of protein breakdown in humans (4–10). These anabolic actions are particularly evident when GH is replaced in patients with GH deficiency or when given therapeutically

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Abbreviations: AU, Arbitrary units; BHAD, β-hydroxyacyl coenzyme A dehydrogenase; COX3, cytochrome *c* oxidase subunit 3; ERR-α, estrogen-related receptor-α; GCRC, General Clinical Research Center; GLUT4, glucose transporter 4; IGFBP, IGF-binding protein; MFN2, mitofusin 2; MHCI, slow-twitch isoform of the contractile protein myosin heavy chain; mTOR, mammalian target of rapamycin; NEFA, nonesterified fatty acids; NRF1, nuclear respiratory factor 1; PDK4, pyruvate dehydrogenase kinase 4; PGC-1α, peroxisome-proliferator receptor-γ coactivator 1α; STAT5, signal transducer and activator transcription 5; TFAM, mitochondrial transcription factor-α; UCP3, uncoupling protein 3.

in catabolic conditions like fasting, surgery, burns, or illness (1, 11).

Growth, protein synthesis, and many components of fuel metabolism are ATP-requiring processes that may increase energetic demands on mitochondria, but GH effects on mitochondrial function are not fully established. There is some evidence that GH action may be important for mitochondrial regulation in skeletal muscle. A case study of a patient with acromegaly demonstrated that structural abnormalities of muscle mitochondria were resolved after surgical treatment, although no functional results were provided (12). Lange *et al.* (13) reported that when older women combined GH injections with aerobic exercise training for 12 wk, they had a greater increase in activity of muscle mitochondrial oxidative enzymes than exercising women who received a placebo injection. However, that study did not include a group taking GH without exercise, so it is not known whether GH could regulate mitochondria oxidative capacity independent of the exercise program. In contrast, when nonexercising rats were given daily GH injections for 2 wk, there was no change in muscle mitochondrial respiration rate (14). The last dose of GH in that study was given 1–2 d before the muscle measurements, however, so any acute effects of GH action may not have been detectable. To our knowledge, no studies have reported whether administration of GH regulates muscle mitochondrial function in humans. The present study was designed to test the hypothesis that GH would induce an increase in mitochondrial oxidative capacity and expression of oxidative genes and to define the effects of GH on muscle protein synthesis, including mitochondrial proteins. We investigated the acute effect of GH by infusing GH or saline for 14 h in young healthy volunteers in the postabsorptive state in a randomized crossover study design. Muscle biopsies were used to measure mitochondrial ATP production rate, mitochondrial protein synthesis rate, and the abundance of several gene transcripts that regulate to the muscle oxidative phenotype.

Subjects and Methods

Participants

Nine healthy volunteers (five men, four women) completed the study. Average characteristics (mean ± SD) of the group were age 33 ± 10 yr, body mass index 25.2 ± 3.2 kg/m^2, body fat-free mass 50.8 ± 14.6 kg, and body fat 26.4 ± 7.4%. Body composition was determined using dual-energy x-ray absorptiometry. Health status was assessed by medical history, physical exam, blood chemistries (including liver enzymes, creatinine, electrolytes, and glucose), complete blood count, urinalysis, and electrocardiogram. Inclusion criteria included age (18–45 yr) and body mass index (20–30 kg/m^2). Exclusion criteria included diabetes, chronic renal, liver, or other metabolic/endocrine disorders, or use of tobacco or medications that could affect metabolism. None of the participants were taking medications at the time of the study. Female participants were studied during the luteal phase of their menstrual cycle. The Institutional Review Board of Mayo Foundation approved the study protocol. All procedures were performed in accordance with the guidelines in The Declaration of Helsinki and were clearly explained to each study volunteer before obtaining informed oral and written consent.

Protocol and procedures

Each participant was studied once with saline infusion and once with GH infusion in a randomized crossover design. The two trials were separated by an average of 10 wk with regular lifestyle patterns maintained in the interim. For 3 d before each study, strenuous physical activity was avoided and a weight-maintaining diet (55:30:15% of energy as carbohydrate, fat, and protein, respectively) was provided by the Metabolic Kitchen at the Mayo Clinic General Clinical Research Center (GCRC). On the evening before each study, participants were admitted for an overnight stay in the GCRC. After the evening meal (1800 h), no food was consumed until completion of the study the next day.

A polyethylene catheter was placed in an antecubital arm vein for infusion of either saline or GH (150 μg/h; 2.1 ± 0.1 μg/kg·h) beginning at 2200 h and continuing for 14 h. At 0400 h the next morning, priming doses of L-[1,2-^{13}C]leucine (6.9 μmol/kg, 97 atom percent excess; MA Trace, Woburn, MA) and [^{13}C]bicarbonate (2.4 μmol/kg; 99 atom percent excess; Cambridge Isotopes, Andover, MA) were given, and the [1,2-^{13}C]leucine was continued at 6.9 μmol/kg·h until the end of the study (1200 h). Isotope solutions were prepared under sterile conditions and were determined to be bacteria and pyrogen free before their administration. At 0600 h, a second catheter was placed in the hand opposite the infusion site and used to obtain arterialized blood each hour using the heated hand vein method (15). Expired breath was sampled hourly, coinciding with blood collections. Muscle biopsies of the vastus lateralis were obtained under local anesthesia (16) at 0700 and 1200 h, each obtained from a different leg within the study day. Both biopsies of each study day were used for protein synthesis and mRNA measurements. Mitochondrial and signal protein analyses were performed only on the second biopsy. A portion of the muscle was kept on ice in saline-soaked gauze for mitochondrial studies, and the remainder was rapidly frozen in liquid nitrogen and stored at −80 C until analysis.

Resting energy expenditure was determined by indirect calorimetry (DeltaTrac; SensorMedics, Yorba Linda, CA) for 45 min beginning at approximately 0800 h. The last 20 min of this measurement were used for data analysis. Urinary nitrogen content was measured using a Beckman GM7 Analox Microstat (Beckman Instruments, Fullerton, CA).

Muscle mitochondrial function

Mitochondria were isolated by centrifugation from fresh muscle tissue, and ATP production capacity was assessed using a bioluminescent method (17, 18). Briefly, mitochondria were added to cuvettes containing luciferin/luciferase (BioTherma, Haninge, Sweden), 0.3 mM ADP, and one of six substrate combinations. Substrates used were (in mM) 10 glutamate plus 1 malate (GM), 1 pyruvate plus 0.05 palmitoyl-L-carnitine plus 10 α-ketoglutarate plus malate (PPKM), 10 α-ketoglutarate (KG), 20 succinate plus 0.1 rotenone (SR), 1 pyruvate plus 1 malate (PM), and 0.05 palmitoyl-L-carnitine plus 1 malate (PCM). ATP production was measured simultaneously for all reactions in triplicate at 25 C in a BioOrbit 1251 luminometer. Each reaction was calibrated using an internal ATP standard. A separate piece of muscle (20 mg) was used to measure the activity of citrate synthase and β-hydroxyacyl coenzyme A dehydrogenase (BHAD) using spectrophotometric assays (17).

Quantification of mRNA

Abundance of selected mRNAs in muscle was measured with a real-time quantitative PCR system (ABI Prism 7700; PE Biosystems, Foster City, CA). RNA was extracted by TRIZOL method (Life Technologies, Gaithersburg, MD), treated with DNase (Life Technologies), and reverse-transcribed using TaqMan reverse transcription reagents (PE Biosystems). Transcripts measured included IGF-I, because it is a major effector of GH action, and the mitochondrial components cytochrome *c* oxidase subunit 3 (COX3) and 4 (COX4), and uncoupling protein 3 (UCP3). Nuclear transcription factors involved in regulation of muscle oxidative genes were also measured: peroxisome-proliferator receptor-γ coactivator 1α (PGC-1α), nuclear respiratory factor 1 (NRF1), estrogen-related receptor-α (ERR-α), mitofusin 2 (MFN2), and mitochondrial transcription factor-α (TFAM). Two additional genes associated with

muscle oxidative capacity were measured: glucose transporter 4 (GLUT4) and the slow-twitch isoform of the contractile protein myosin heavy chain (MHCI). Samples were run in triplicate with coamplification of the target gene and 28S rRNA (as a housekeeping gene) and quantified by normalizing the target signal for the 28S rRNA signal. For each gene, the average transcript abundance in the saline trial was assigned a value of 1.0, and all individual values from both the saline and GH trials were linearly transformed relative to this value and expressed in arbitrary units (AU).

Sequences for the following primers and probes have been previously published: COX3 (18), COX4, PGC-1α, NRF1, TFAM, GLUT4 (19), MHCI, and 28S rRNA (20). The primers used for IGF-I (GenBank accession number X57025) were forward CCAGCGCCACACCGA and reverse CTCCCTCTACTTGCGTTCTTCAA, and the probe sequence was ATGCCCAAGACCCAGAAGGAAGTACA. Corresponding information for the other genes is as follows: UCP3 (GenBank AF011449) forward primer CTCAAGGAGAAGCTGCTGGACTA, reverse primer GCTCCAAAGGCAGAGACAAAGT, and probe ACCTGCTCACTGACAACTTCCCCTGC; ERR-α (GenBank NM004451) forward primer AGATTGTGGTCACCATCAGCTG, reverse primer TCCACACGCTCTGCAGTACTG, and probe CCAAGAGCATCCCAGGCTTCTCA; and MFN2 (GenBank NM014874) forward primer GGCTCGGAGGCACATGAA, reverse primer CGGTGCTCTTCCCATTGC, and probe CGTCCGGCCAAAAAAAGCCA.

Western blotting

Sufficient tissue was available in five of the nine subjects to measure the phosphorylation status of two key signaling molecules that regulate protein synthesis. Frozen muscle samples were prepared, separated by polyacrylamide gel electrophoresis, and transferred to polyvinylidene difluoride membranes as previously described (21). After blocking in nonfat milk, membranes were incubated overnight at 4 C with primary antibodies directed against the total or phosphorylated (Ser 2448) forms of the mammalian target of rapamycin (mTOR; Cell Signaling Technology, Danvers, MA), and the total or phosphorylated (Thr 37/46) form of translation elongation binding protein 4E-BP1 (Cell Signaling). After incubation with horseradish peroxidase-conjugated secondary antibodies and the ECL-Plus detection system (Amersham Biosciences, Piscataway, NJ), images were captured on Biomax XAR film (Kodak Scientific, New Haven, CT) and analyzed using Kodak Molecular Imaging software. Data are expressed as the ratio of phosphorylated to total protein signal for each subject. The group average in the saline trial was then assigned a value of 1.0, and all individual values from both the saline and GH trials were linearly transformed relative to this value and expressed in AU.

Hormone and metabolite assays

Glucose was measured with a Beckman Glucose Analyzer (Beckman Instruments, Porterville, CA). Nonesterified fatty acids (NEFA) were measured using an enzymatic colorimetric assay (NEFA C; Wako Chemicals USA, Richmond, VA). Plasma levels of amino acids were measured by an HPLC system (HP 1090, 1046 fluorescence detector and cooling system) with precolumn *o*-phthaldehyde derivatization (22). Hormone assays were performed by the Mayo Clinic Clinical Chemistry Laboratory. Insulin and human GH were measured with two-site immunoenzymatic assays (Access system; Beckman Instruments, Chaska, MN). Glucagon and C-peptide were measured by direct RIAs (Linco Research, St. Louis, MO). After separation from their binding proteins with a simple organic solvent, total IGF-I and IGF-II were measured with two-site immunoradiometric assays (Diagnostic Systems Laboratories, Webster, TX). IGF-binding protein (IGFBP)-1 and -3 were also measured with two-site immunoradiometric assays, whereas IGFBP-2 was measured by a double-antibody RIA (Diagnostic Systems Laboratories).

Plasma amino acid kinetics

The enrichment level of [1,2-^{13}C]leucine in plasma was determined using a gas chromatograph/mass spectrometer (HP5973; Hewlett-Packard Instruments, Avondale, CA) by multiple ion monitoring at m/z 342/344 under positive ion methane chemical ionization conditions. [1,2-^{13}C]Ketoisocaproate in plasma was determined as its quinoxalinol-trimethylsilyl derivative under electron ionization conditions using an HP5988 gas chromatograph/mass spectrometer (23). Isotopic enrichment of breath $^{13}CO_2$ was measured by isotope ratio mass spectrometry (24). Average steady-state enrichment values from 0700–1200 h (corresponding to the muscle biopsy times) were used to calculate whole-body rates of leucine flux, oxidation, and nonoxidative disposal using standard equations (24).

Muscle protein synthesis

A portion of each muscle sample was used for the isolation of mitochondrial and sarcoplasmic protein fractions by differential centrifugation (16). A separate piece of tissue was used to prepare total mixed muscle proteins and to isolate free tissue fluid amino acids (25). The muscle protein fractions were hydrolyzed overnight in 0.6 M HCl in the presence of cation exchange resin (AG-50; Bio-Rad, Hercules, CA) and purified the next day using a column of the same resin. The amino acids were dried and then derivatized as their trimethyl acetyl methyl ester. [^{13}C]Leu enrichment in muscle proteins was determined using a gas chromatograph-combustion-isotope ratio mass spectrometer (δ Plus; Finigan MAT, Bremen, Germany) as described (26). Tissue fluid amino acids were derivatized as their *t*-butyldimethylsilyl ester and analyzed using an

TABLE 1. Plasma metabolites and hormones (mean ± SEM)

	Saline	GH	% Difference[a]	*P* value
Glucose (mmol/liter)	5.10 ± 0.13	5.41 ± 0.15	6	0.004
Fatty acids (mmol/liter)	0.452 ± 0.036	0.892 ± 0.063	97	0.001
Insulin (pmol/liter)	34 ± 6	57 ± 6	68	0.001
C-peptide (nmol/liter)	0.42 ± 0.04	0.63 ± 0.03	48	0.001
Cortisol (μg/dl)	14.8 ± 1.1	14.2 ± 1.9	−4	0.689
Glucagon (pg/ml)	141 ± 8	135 ± 8	−4	0.007
GH (μg/liter)	2.3 ± 1.0	12.2±1.1	425	0.001
IGF-I total (ng/ml)	314 ± 37	367 ± 33	17	0.096
IGF-I free (ng/ml)	0.87 ± 0.10	1.74 ± 0.26	101	0.004
IGF-II (ng/ml)	717 ± 70	651 ± 49	−9	0.090
IGFBP-1 (ng/ml)	37.9 ± 6.7	20.5 ± 5.9	−46	0.030
IGFBP-2 (ng/ml)	372 ± 60	344 ± 54	−7	0.211
IGFBP-3 (ng/ml)	3621 ± 170	3444 ± 143	−5	0.019

Values shown are the average of blood samples collected at 0700 and 1200 h, corresponding to 9 and 14 h of infusion with either saline or GH.

[a] GH *vs.* saline.

HP5973 gas chromatograph/mass spectrometer under electron ionization conditions (26). The fractional synthetic rate of muscle proteins was calculated from the increment in protein-bound enrichment between biopsies, using muscle tissue fluid enrichment as the precursor pool (25).

Statistical analysis

Summary data are reported as mean ± SEM. Differences between the saline and GH trials were analyzed using paired *t* tests. P values < 0.05 were considered statistically significant.

Results

Plasma metabolites and hormones

GH infusion resulted in significant elevations in circulating GH, glucose, NEFA, insulin, C-peptide, and free IGF-I, whereas there were reductions in glucagon, IGFBP-1, and IGFBP-3 during the last 5 h of the study (Table 1). There were also trends for increased total IGF-I and reduced IGF-II. There were no differences between trials for cortisol or IGFBP-2. The total concentration of measured amino acids in plasma was not different between trials (2.47 ± 0.11 and 2.40 ± 0.12 mmol/liter for saline and GH, respectively, $P = 0.525$), nor was there a difference for individual amino acids, including leucine (157 ± 10 and 150 ± 10 μmol/liter for saline and GH, respectively, $P = 0.471$).

Indirect calorimetry

GH infusion did not significantly alter resting energy expenditure or urinary nitrogen excretion rate, although there was a reduction in the nonprotein respiratory quotient (Table 2), indicating a shift in fuel metabolism away from carbohydrate utilization and toward greater reliance on fat. Calculated carbohydrate oxidation (grams per hour) was 69% lower, whereas fat oxidation tended to be higher (29%, $P = 0.056$) in the GH trial compared with saline.

Muscle mitochondrial function

Enzymatic activity of citrate synthase in muscle homogenates was significantly increased (16%), and BHAD showed a trend to increase (13%) in the GH trial *vs.* saline (Fig. 1). Likewise, GH infusion resulted in an 8–35% higher mitochondrial ATP production rate, reaching statistical significance or a strong trend ($P \leq 0.051$) for five of the six substrates tested, as shown in Fig. 1.

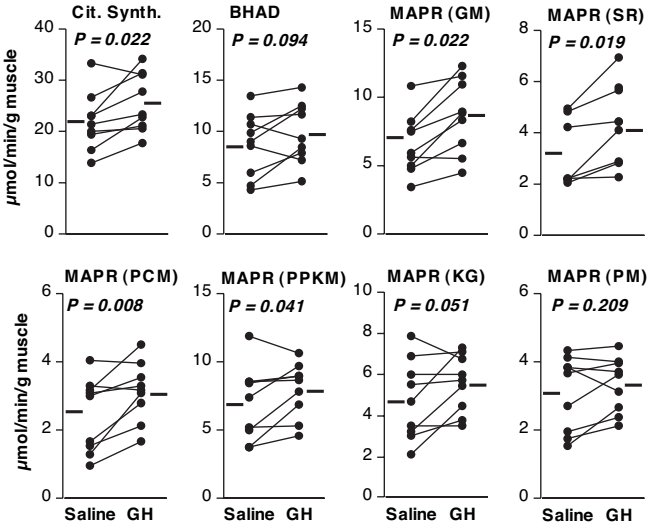

FIG. 1. Effect of GH on muscle oxidative capacity. Enzymatic activity of citrate synthase (Cit. Synth.) and BHAD and mitochondrial ATP production rate (MAPR) are shown. Paired data from individual participants are shown as *connected circles*, with treatment means denoted by the *adjacent bars*. For MAPR, mitochondria were incubated with glutamate plus malate (GM), succinate plus rotenone (SR), palmitoyl carnitine plus malate (PCM), pyruvate plus palmitoyl carnitine plus ketoglutarate plus malate (PPKM), ketoglutarate (KG), or pyruvate plus malate (PM). *P* values are for paired *t* test comparisons between treatments.

Abundance of mRNAs

Compared with saline, GH infusion resulted in a 240 and 119% increase in IGF-I mRNA abundance at 9 and 14 h, respectively (Fig. 2). None of the other mRNAs were significantly different between trials at the 9-h measurement time. By 14 h, however, both COX3, encoded by the mitochondrial DNA, and COX4, encoded by the nuclear DNA, were increased by 342 and 78%, respectively, during the GH trial. However, GH did not alter mRNA content for another mitochondrial membrane protein, UCP3. Among the nuclear transcription factors measured, only TFAM increased (34%) with GH infusion. GLUT4 mRNA abundance increased 52% with GH infusion. MHCI mRNA increased from 1.00 ± 0.23 AU in the saline trial to 1.43 ± 0.25 AU in the GH trial but did not reach statistical significance ($P = 0.25$).

Western blotting

The relative Ser 2448 phosphorylation level of mTOR was 1.00 ± 0.13 during saline and increased in each of the five sub-

TABLE 2. Indirect calorimetry (mean ± SEM)

	Saline	GH	% Difference[a]	*P* value
VO_2 (ml/min)	256 ± 18	245 ± 17	−4	0.318
VCO_2 (ml/min)	207 ± 17	184 ± 12	−11	0.063
Urinary urea nitrogen excretion (g/h)	0.38 ± 0.04	0.36 ± 0.04	−6	0.717
Nonprotein RQ	0.81 ± 0.02	0.75 ± 0.02	−8	0.035
Energy expenditure (kcal/min)	1.22 ± 0.09	1.15 ± 0.08	−6	0.208
Fat oxidation (g/h)	5.02 ± 0.47	6.49 ± 0.65	29	0.056
CHO oxidation (g/h)	6.43 ± 1.41	1.98 ± 0.78	−69	0.027

CHO, Carbohydrate; RQ, respiratory quotient (VCO_2/VO_2) after adjustment for protein loss.

[a] GH *vs.* saline.

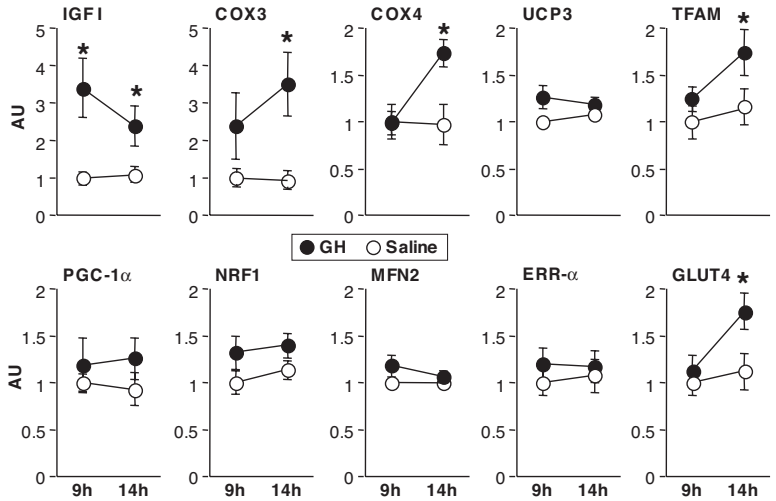

FIG. 2. Abundance of mRNA transcripts for selected genes measured at 9 and 14 h of GH or saline infusion. Values for each gene are expressed in AU after normalizing for 28S rRNA signal and adjusting the mean of the saline trial at 9 h to 1. Results are shown for IGF-I, COX3 and -4, UCP3, TFAM, PGC-1α, NRF1, MFN2, ERRα, and GLUT4. *, GH greater than saline, $P < 0.05$.

jects measured to 1.76 ± 0.16 during the GH trial ($P = 0.020$). Phosphorylation of Thr 37/46 on 4E-BP1 was 1.00 ± 0.22 during saline and increased in three of the five subjects to 1.42 ± 0.41 during the GH trial, but this was not a statistically significant difference ($P = 0.457$).

Amino acid kinetics

Whole-body amino acid kinetics are shown in Table 3. Leucine oxidation was reduced during GH infusion, and nonoxidative leucine disposal was increased. There was a trend for reduced leucine flux that did not reach statistical significance. As shown in Table 3, the infusion of GH did not result in significant changes in the fractional synthetic rate of muscle proteins.

Discussion

The current study demonstrated that a 14-h infusion of GH causing physiological elevation of GH in healthy people resulted in increased skeletal muscle mitochondrial oxidative capacity, as shown by increased mitochondrial ATP production rate, increased citrate synthase activity, and a trend for higher BHAD activity. GH infusion also resulted in higher muscle content of mRNA transcripts encoding oxidative proteins in mitochondria (COX 3 and COX4), a nuclear transcription factor that regulates mitochondrial biogenesis (TFAM), and the glucose transport protein GLUT4. These effects of GH action on muscle oxidative capacity were accompanied by a shift in whole-body fuel utilization reflecting reduced carbohydrate and leucine oxidation and a trend toward enhanced fat oxidation, although whole-body energy expenditure was not changed.

To our knowledge, this is the first report of an acute effect of GH infusion on muscle mitochondrial function and gene transcripts in healthy human subjects. A prior study suggested that GH injections for 12 wk may add to the effect of aerobic exercise to increase muscle oxidative capacity in older women (13), but the effect of GH alone has not been reported. The current study indicated that GH enhanced transcript abundance of genes involved in mitochondrial biogenesis, including TFAM, the key nuclear transcription factor regulating mitochondrial transcription and replication. These findings are similar to recent reports that showed that acute insulin infusion, like GH, stimulates mitochondrial oxidative capacity and transcript levels of several mitochondrial genes (18, 21, 27, 28), raising the possibility that the actions of these hormones may overlap.

The existing literature, however, suggests that insulin and GH probably regulate mitochondrial function though different pathways. For example, GH infusion caused an increase in circulating insulin in the present study, but the concentration remained at least 5-fold lower than used in studies reporting a stimulatory effect of insulin on muscle mitochondrial ATP production (18, 21, 27, 28). GH action is known to cause insulin resistance (1–3, 10), which could further diminish any effects of the modest increase in insulin during GH infusion. In the present study, GH resulted in increased mitochondrial function despite evidence of insulin resistance as indicated by higher insulin, glucose, and fatty acids. This observation is an important point for distinguishing the regulatory actions of GH and insulin because it was demonstrated that the stimulatory effect of insulin on mitochon-

TABLE 3. Whole body amino acid kinetics and fractional synthetic rates of muscle proteins (mean ± SEM)

	Saline	GH	% Difference[a]	*P* value
Whole-body kinetics (μmol/h·kg body weight)				
Leucine rate of appearance	103.6 ± 4.0	96.9 ± 2.6	−7	0.091
Leucine oxidation	40.9 ± 2.3	23.5 ± 1.3	−43	0.001
NOLD	62.7 ± 3.7	73.4 ± 3.0	17	0.018
Muscle protein synthesis (%/h)				
Mixed protein	0.078 ± 0.010	0.072 ± 0.007	−7	0.587
Mitochondrial protein	0.102 ± 0.014	0.108 ± 0.013	6	0.815
Sarcoplasmic protein	0.077 ± 0.007	0.067 ± 0.013	−13	0.596

NOLD, Nonoxidative leucine disposal.

[a] GH *vs.* saline.

drial ATP production is blunted in subjects with clinical insulin resistance (18, 21, 27, 28) and in healthy individuals in whom insulin resistance is acutely induced by raising plasma lipid concentration (27). In the present study, plasma fatty acid concentration was nearly doubled during GH infusion compared with saline and was accompanied by a shift in fuel metabolism toward greater reliance on fat. This increase in fatty acids did not appear to limit mitochondrial function, and the mitochondrial data indicate that GH increased the capacity to produce ATP with the fatty acid substrate palmitoyl carnitine or the tricarboxylic acid cycle intermediates α-ketoglutarate (and glutamate, which is exchanged with α-ketoglutarate) and succinate. GH action may therefore activate proteins in the β-oxidation or tricarboxylic acid cycles (*e.g.* BHAD and citrate synthase enzymes) or other components of the mitochondrial fuel delivery and oxidation machinery. In contrast, GH did not stimulate ATP production with pyruvate plus malate. This may be due to regulation of the pyruvate dehydrogenase complex, a known control point for fat and glucose oxidation (29, 30). During insulin-resistant conditions, including fatty acid elevation, pyruvate dehydrogenase activity is inhibited by an increase in pyruvate dehydrogenase kinase 4 (PDK4) (29). It has been shown that GH induces PDK4 expression in adipose cells through a signal transducer and activator of transcription 5 (STAT5) binding site in the PDK4 promoter region (31, 32). The only study, of which we are aware, to examine whether this also occurs in muscle found that a single bolus dose of GH given to healthy young men resulted in STAT5 phosphorylation (activation) 30–60 min later but did not change STAT5 binding to DNA (32). However, this single dose of GH may not be sufficient to induce the characteristic insulin resistance and altered fuel selection. It is also possible that changes in DNA binding events in muscle may require more than 60 min to occur. Nevertheless, the mechanism through which GH action regulates muscle mitochondrial substrate oxidation remains to be elucidated.

GH action resulted in increased expression of mRNAs that encode mitochondrial proteins COX3 and COX4 and the major nuclear-derived transcription factor that regulates replication and transcription of the mitochondrial genome, TFAM (33). These responses are similar to the acute effects of insulin infusion (18, 21, 34) and the effects of acute or chronic aerobic exercise training (19, 35). Exercise is a potent stimulus of GH release (36), and thus it is possible that GH action contributes to many exercise-induced adaptations, including increased mitochondrial biogenesis and fat utilization. We explored the potential role of the nuclear transcription factor PGC-1α in mediating the changes in mitochondria because PGC-1α is a key regulator of muscle mitochondrial biogenesis and several related proteins that ultimately determine muscle oxidative phenotype (37–40). PGC-1α exerts its effects by promoting the transcription of, and working in conjunction with, other transcription factors, including NRF-1, ERR-α, MFN2, and TFAM, all of which have been reported to increase in muscle in response to endurance exercise (19, 35, 37, 38, 41). PGC-1α has also been reported to exert positive regulatory control on the GLUT4 gene (39), which was increased by GH in the present study and increases in response to exercise (19, 38). There were no significant differences in PGC-1α mRNA or the other transcription factors measured, besides TFAM, at either 9 or 14 h of GH infusion. Therefore, if the PGC-1α transcriptional pathway is responsible for the downstream increases in COX3, COX4, TFAM, and GLUT4 mRNA during GH infusion, then either there were transient changes in transcript levels occurring before our first measurement (9 h) or the regulation occurred through changes in protein content or DNA binding. In response to acute exercise, it was reported that PGC-1α and ERR-α mRNA abundance is transiently increased for only a few hours (35, 37). Whether that is also true in response to GH elevation needs to be determined. Alternately, regulation of the mitochondrial pathways may occur through direct actions of GH (perhaps the STAT5 pathway), or secondarily by increasing IGF-I and fatty acids. Chronic fatty acid elevation, for example, was shown to increase the muscle protein content of citrate synthase, subunits of COX, and related mitochondrial proteins and did not appear to be regulated by PGC-1α, which was unchanged (42). Whether the fatty acid-mediated events also occur within the time frame of our overnight GH infusion remains to be shown.

The increase in GLUT4 mRNA in the GH trial appears to contradict the muscle and whole-body changes favoring increased fat utilization, including the insulin resistance for glucose metabolism shown to occur with GH treatment (2, 10). It was previously shown that GH infusion for 6 h in healthy young men did not alter muscle GLUT4 protein content (3). There was also no change in muscle GLUT4 mRNA or protein in rats treated with GH for 4 wk (43). Thus, the mechanism and functional impact of the increased GLUT4 mRNA in the present study is not yet clear.

We found that GH had no effect on muscle protein synthesis, including mitochondrial proteins. We previously showed that insulin infusion could selectively increase synthesis of mitochondrial proteins in skeletal muscle, thus potentially contributing to insulin-mediated stimulation of mitochondrial oxidative capacity (18, 44). This is the first report, to our knowledge, on the effect of GH on synthesis of subfractions of the total muscle protein such as mitochondrial or sarcoplasmic proteins. This measurement technique has been used to demonstrate changes in synthesis of muscle proteins with aging or exercise (20, 45), so it seems unlikely that an effect of GH would not be detected. A limitation, however, is that the measurements represent the average synthesis rates of multiple individual mitochondrial or sarcoplasmic proteins. It is possible that GH has a selective effect on synthesis of specific proteins, particularly those proteins with increased mRNA abundance. Interestingly, the lack of effect of GH on muscle protein synthesis rate occurred despite an increased activation (phosphorylation) of mTOR, a key energy-sensing anabolic signal molecule that contributes to the regulation of transcription and translation. However, the lack of change in the phosphorylation of 4E-BP1, a downstream effector of mTOR action, indicates that the pathway leading to increased protein synthesis rate was not fully activated. mTOR and 4E-BP1 are rapidly stimulated by insulin, amino acids, and exercise (46, 47), but their response to GH in human muscle was unknown. Because we made our measurements at rest during the postabsoptive state, it is possible that the addition of exercise (13)

and/or protein meals (46, 47) while GH is elevated could have a synergistic effect on activating mTOR, downstream signaling pathways, and muscle protein synthesis.

The lack of change in muscle protein synthesis in response to GH infusion is consistent with most previous studies in which healthy humans received GH acutely (3–10 h) or up to 3 months (6, 9, 10, 48) and protein metabolism measurements were performed in the postabsorptive state. The daily equivalency of GH doses in those studies were similar to the amount administered in the current investigation, and the resulting serum GH was similar to that observed after prolonged exercise or clinically stressful conditions (1, 36). In comparison, studies in which GH increased muscle protein synthesis in healthy volunteers used larger doses (1.7- to 4-fold higher than the present study) either alone (49) or in combination with high-dose insulin (50). In contrast to the results in muscle, GH exerted an anabolic effect on whole-body amino acid metabolism, as shown by reduced leucine oxidation, increased nonoxidative leucine disposal (an index of protein synthesis), and a trend for a lower rate of leucine appearance (a measure of protein breakdown). These findings on whole-body kinetics are consistent with previous reports and collectively demonstrate that a major effect of short-term administration of GH on protein metabolism in healthy subjects occurs in nonmuscle tissues (1, 6, 10).

Because we measured total GH action using a continuous infusion of GH, we recognize that the observed changes may differ if a more physiological (*i.e.* pulsatile) pattern of GH delivery is used. We also note that changes in muscle mitochondrial function and mRNA expression could result from either primary effects of GH or secondary changes in metabolites and hormones, such as IGF-I. IGF-I has similar effects as GH on whole-body protein turnover but may have an additional stimulatory effect on protein synthesis in muscle (51–54). We are not aware of any studies that tested whether IGF-I exerts control of muscle mitochondrial function, gene expression, or protein synthesis. GH action may also be related to increased lipolysis. There is evidence that GH-mediated suppression of protein breakdown occurs through the elevation of fatty acids (5). As already noted, elevated fatty acids, induced by a 4-wk high-fat diet in rats, can stimulate an increase in many of the muscle mitochondrial proteins that were shown to change with GH infusion in the present study (42). The relative importance of primary and secondary GH actions requires further careful investigation.

A potential area of application is assessing whether GH may have beneficial effects on muscle mitochondrial function in elderly people because circulating GH concentration, the magnitude of exercise-mediated GH release, and muscle mitochondrial ATP production capacity are reduced with age (17, 36, 55, 56). However, it remains to be determined how long the changes in mitochondrial function and gene expression persist after cessation of GH infusion and how this may affect muscle performance. The observed effects may have little or no benefit to physically active people for whom regular exercise may provide equal or greater stimulus for mitochondrial biogenesis. Still, there may be some benefits for select clinical populations, such as GH-deficient individuals.

In conclusion, the current study demonstrates that overnight infusion of GH stimulates muscle mitochondrial metabolism by increasing the mRNA levels of specific genes and raising the capacity for oxidative ATP generation in healthy subjects. This response in mitochondrial functional capacity is consistent with the shift in fuel utilization away from carbohydrate toward fat use and appears to involve the activation of specific enzymes but does not appear to involve a change in mitochondrial protein synthesis rate.

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