Ghrelin regulates mitochondrial-lipid metabolism gene expression and tissue fat distribution in liver and skeletal muscle

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Barazzoni, Rocco, Alessandra Bosutti, Marco Stebel, Maria Rosa Cattin, Elena Roder, Luca Visintin, Luigi Cattin, Gianni Biolo, Michela Zanetti, and Gianfranco Guarnieri. Ghrelin regulates mitochondrial-lipid metabolism gene expression and tissue fat distribution in liver and skeletal muscle. Am J Physiol Endocrinol Metab 288: E228–E235, 2005. First published August 24, 2004; doi:10.1152/ajpendo.00115.2004.—Ghrelin is a gastric hormone reported to increase body weight and body fat (24, 26, 34, 57), also independently of changes in food intake (50). Ghrelin favors adipogenesis in vivo (49) and impairs adipocyte lipolysis in vitro (12), whereas sustained peripheral and central ghrelin administration increase respiratory quotient directly, indicating reduced whole body lipid oxidative utilization (50). Fat accumulation in obese individuals is associated with fat deposition in nonadipose tissues, including liver and skeletal muscle (3, 19, 20, 25), and these changes can contribute to the onset of insulin resistance (45). Excess fat in the obese general population is, however, characterized by hypogrelinemia (51), whereas circulating ghrelin is increased after caloric restriction and weight loss (8, 13), which can in turn be associated with tissue-specific liver fat deposition (3).

In vitro studies have indicated that ghrelin can exert direct metabolic effects in lean tissues, since ghrelin was shown to activate the insulin-signaling cascade and to independently stimulate expression of glucogenic genes in cultured hepatocytes (32). Known mediators of central ghrelin effects include neuropeptide Y (24), which was reported to regulate lean tissue lipid metabolism, favoring lipogenesis in liver but not skeletal muscle (53, 59). Taken together, the above observations suggest a role of ghrelin in changes of lean tissue lipid metabolism and distribution after changes in nutrient availability, but these potential effects have not been investigated. To elucidate the role of ghrelin in the regulation of lipid metabolism and distribution in metabolically relevant lean tissues and its potential molecular mediators, we determined the in vivo effects of peripheral ghrelin administration at a dose not affecting food intake on liver and skeletal muscle triglyceride content and mitochondrial-lipid metabolism gene expression in a rodent model in vivo. Both mixed type I-type II gastrocnemius and type I soleus muscles were studied to assess effects of different muscle fiber composition and oxidative capacities. Expression levels of genes involved in the regulation of lipogenesis [acyetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS)] and lipid oxidation [carnitine palmitoyltransferase (CPT) I and mitochondrial uncoupling proteins (UCPs)] were investigated. Peroxisome proliferator-activated receptor (PPARγ) and -α transcript levels were also measured, since PPARs are important regulators of tissue fat metabolism and content (38), and ghrelin was reported to enhance adipocyte PPARγ expression (12). Mitochondria are the site of tissue fat oxidative disposal, and activities of cytochrome c oxidase (COX) and citrate synthase (CS) were measured as representative mitochondrial enzymes because of their flux-generating role in the respiratory chain and tricarboxylic acid cycle, respectively (15). Potential involvement of AMP-activated protein kinase (AMPK) in ghrelin effects was finally studied, since AMPK is a major regulator of tissue lipid and glucose metabolism, the activating phosphorylation of which favors tissue fatty acid oxidation (56) and downregulates hepatic glucogenetic molecules (58) in direct contrast with reported ghrelin effects (32, 50).

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MATERIALS AND METHODS

Experimental protocol. Male Wistar rats (10 wk old) were purchased from Harlan Italy (San Pietro al Natisone), kept in individual cages in the Animal Facility of the University of Trieste in a controlled environment (22°C, 12:12-h light-dark cycle), and fed a standard commercial chow diet (3.4 kcal/g Harlan 2018; Harlan). Experimental procedures were approved by the Institutional Review Committee for animal studies. After arrival (2 wk), rats were randomly assigned to undergo twice daily (8:00 PM and 8:00 AM) subcutaneous injections of rat ghrelin (n = 8, 200 ng/injection; AnaSpec, San Jose, CA) or vehicle (water, n = 8). The ghrelin dose was chosen to be lower but in the same order of magnitude of the one previously reported to markedly increase body weight without affecting food intake in mice (50), and lack of food intake effects were verified in preliminary short-term experiments. Injections were started in the evening (day 7), with the last injection in the morning (day 4). Animals were killed 3.5 h after the last ghrelin injection, with food withdrawal 2 h before death. Food intake and body weights were measured before injections and before death. Food intake between the last injection and food withdrawal was comparable in the two study groups (0.81 vs. 0.89 g/h, control vs. ghrelin, P = 0.4). Animals were not killed under prolonged fasting conditions to avoid potential confounding effects of fasting-related hyperghrelinemia in the control group. After intraperitoneal pentobarbital overdose, gastrocnemius muscles, liver, and abdominal epididymal white adipose tissue were collected, frozen in liquid nitrogen, and stored at −80°C. Blood was collected through cardiac puncture.

RNA analyses (real-time PCR and Northern blot). Total RNA was isolated from 40- to 60-mg tissues by the guanidinium method (Tri Reagent; MRC, Cincinnati, OH). Transcript levels of pivotal regulators of lipid metabolism were measured by real-time PCR (7900 Sequence Detection System; Applied Biosystems). Total RNA (1 μg) was reversed transcribed (RNA Reverse Transcription KIT; Applied Biosystems) to cDNA. Primers and probes for real-time PCR amplification were selected using Primer Express Software (Applied Biosystems; Table 1). The probe for target genes was labeled at the 5’ end with a reporter dye FAM (6’-carboxyfluorescein) and at the 3’ end with a quencher dye TAMRA (6’-carboxytetramethylrhodamine). The reporter and quencher dyes are in close proximity on the probe, resulting in suppression of reporter fluorescence. The probe is designed to hybridize to a specific sequence within the PCR product. The 5’- to 3’-exonuclease activity of the Taq DNA polymerase allows separation of the reporter from the close proximity of the quencher dye, resulting in fluorescence of the reporter dye. The resulting signal is measured at each amplification cycle on the ABI Sequence Detection System (Applied Biosystems), thus allowing the measurement of sample abundance in the linear phase of amplification. Target genes were amplified using aliquots of the same cDNA sample, and final quantitation of each sample was achieved by a coamplified relative standard curve. Abundance of 28S rRNA was measured separately in the same fashion and used to normalize against potential differences in RNA isolation, RNA degradation, and efficiency of reverse transcription reactions. All values (arbitrary units) were finally divided by the average of the control group and multiplied by 100 to express them as percent of the control group. Mitochondrial UCP2 and -3 mRNA was measured by Northern blot, as previously described, and also expressed as a percentage of control (6, 7).

Western blot. For measurement of activated (phosphorylated) AMPK and inactivated (phosphorylated) and total ACC, total tissue proteins were extracted from liver and muscles and quantitated as described (6). Total protein (40 μg) was separated on 12% (phosphorylated AMPK) or 4–12% (ACC) gradient acrylamide gels and transferred to PVDF membranes (Bio-Rad Laboratories, Hercules, CA) that were blocked overnight and subsequently hybridized overnight at 4°C to rabbit antibodies for Thr172 phosphorylated AMPK, phosphorylated ACC, or total ACC (Upstate Biotechnology, Lake Placid, NY). The secondary antibody was peroxidase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA) used at a 1:20,000 dilution for 1 h at room temperature. Membranes were then exposed to films for 4–8 min (Kodak Biomax MR; Kodak, Rochester, NY), and resulting images were quantified by densitometry. Similar to mRNA measurements, each value (arbitrary units) was divided by the average of the control group and multiplied by 100 to express data as a percentage of the control vehicle-treated group.

Table 1. Forward and reverse primer and probe sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Seq Accession</th>
<th>Forward (FP) Primer Sequence</th>
<th>Reverse (RP) Primer Sequence</th>
<th>Probe Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>GenBank J03808</td>
<td>FP: GAAGCTCAGCAAAACCAACCAAA</td>
<td>RP: ATGGCCAAATGGAAGGCAATAA</td>
<td>TGGTAAACTCCATCTAAGGCTAAATACCGGC</td>
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<tr>
<td>FAS</td>
<td>GenBank X62889</td>
<td>FP: TGGAGTCCACGCATGTGAAG</td>
<td>RP: TCTACCTTGCGGCTCACTTTC</td>
<td>TGCAAGGGCTTCTTTCGGC</td>
</tr>
<tr>
<td>CPT I L</td>
<td>GenBank NM031559</td>
<td>FP: TGGTAAACTCCATCTAAGGCTAAATACCGGC</td>
<td>RP: TCTACCTTGCGGCTCACTTTC</td>
<td>TGCAAGGGCTTCTTTCGGC</td>
</tr>
<tr>
<td>CPT I M</td>
<td>GenBank AF029875</td>
<td>FP: TGGTAAACTCCATCTAAGGCTAAATACCGGC</td>
<td>RP: TCTACCTTGCGGCTCACTTTC</td>
<td>TGCAAGGGCTTCTTTCGGC</td>
</tr>
<tr>
<td>PPARγ</td>
<td>GenBank NM 03124</td>
<td>FP: TGGTAAACTCCATCTAAGGCTAAATACCGGC</td>
<td>RP: TCTACCTTGCGGCTCACTTTC</td>
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<tr>
<td>PPARα</td>
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<td>RP: TCTACCTTGCGGCTCACTTTC</td>
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<tr>
<td>G-6-Pase</td>
<td>GenBank L37333</td>
<td>FP: TGGTAAACTCCATCTAAGGCTAAATACCGGC</td>
<td>RP: TCTACCTTGCGGCTCACTTTC</td>
<td>TGCAAGGGCTTCTTTCGGC</td>
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<td>28S rRNA</td>
<td>GenBank V01270</td>
<td>FP: TGGTAAACTCCATCTAAGGCTAAATACCGGC</td>
<td>RP: TCTACCTTGCGGCTCACTTTC</td>
<td>TGCAAGGGCTTCTTTCGGC</td>
</tr>
</tbody>
</table>

Table 1. Forward (FP) and reverse (RP) primer and probe sequences for real-time PCR gene quantitation for acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), carnitine palmitoyltransferase (CPT) I liver (L) and muscle (M) isoforms, peroxisome proliferator-activated receptor (PPAR)γ and --, glucose-6-phosphatase (G-6-Pase), and 28S rRNA.
Mitochondrial enzyme activities. Activities of mitochondrial COX and CS were measured spectrophotometrically from whole tissue homogenates, as previously reported (44).

Tissue triglycerides and plasma biochemical profile. Triglyceride content was measured from 35 to 40 mg liver and each muscle after lyophilization. Dry samples were homogenized in 2:1 chloroform-methanol solution in a 20:1 volume-to-weight ratio and kept at 4°C overnight with gentle shaking. Phase separation was performed using H2SO4 (1 mmol/l), lipid phase was dried under nitrogen and dissolved in 100 μl ethanol, and triglyceride content was measured using a commercially available kit (TG; Roche Diagnostics, Indianapolis, IN).

Plasma insulin and growth hormone concentrations were measured by RIA (Linco, St. Louis, MO; see Ref. 8). Blood glucose concentration was measured by reflectometer (Roche Diagnostics).

Statistical analysis. Student’s t-test for unpaired data was used to compare variables in the two groups. Linear regression analysis was used to study the relationships between variables. P values <0.05 were considered statistically significant.

RESULTS

Food intake, body weight, and plasma metabolic profile. Ghrelin resulted in a moderate increment of body weight gain (control: 14 ± 2 g, ghrelin: 19 ± 1 g, P = 0.04) over the 4-day study period, whereas overall food intake was comparable in the two groups (control: 79 ± 2 g, ghrelin: 82 ± 3 g, P = 0.39). Plasma insulin, growth hormone, and free fatty acid concentrations were comparable in ghrelin- and vehicle-treated rats, whereas blood glucose was increased (P < 0.01) in the ghrelin-treated group (Table 2).

Ghrelin modifies lipid metabolism gene expression and increases triglyceride content in rat liver. Ghrelin administration markedly increased liver triglyceride content (control: 0.0073 ± 0.001, ghrelin: 0.0125 ± 0.017 mg/g dry wt; Fig. 1). Transcript levels of lipogenic enzymes ACC (37%) and FAS (42%) were increased, whereas those of the rate-limiting enzyme of lipid oxidation, CPT I, were reduced (30%) after ghrelin compared with vehicle injections. In addition, ghrelin treatment upregulated glucose-6-phosphatase expression (+46%; all P < 0.05 vs. control; Fig. 2A). These changes were associated with reduced tissue-phosphorylated AMPK (42%) and increased total (+77%) but not phosphorylated ACC protein levels, indicating a relative reduction of phosphorylated inactive ACC form (Fig. 2B). Tissue COX and CS activities (Fig. 2C) were unchanged, whereas UCP2 expression was increased (+268%, Fig. 2D) after ghrelin treatment. Transcript levels of hepatic PPARγ (control: 100 ± 23 and ghrelin: 123 ± 20%) were comparable in the two experimental groups.

Table 2. Initial body weight and body weight changes, food intake, plasma insulin, growth hormone, and free fatty acid and blood glucose concentrations in the two experimental groups

<table>
<thead>
<tr>
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<th>Control</th>
<th>Ghrelin</th>
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<tbody>
<tr>
<td>Initial body weight, g</td>
<td>341 ± 4</td>
<td>331 ± 6</td>
</tr>
<tr>
<td>Body weight change, g</td>
<td>14 ± 2</td>
<td>19 ± 1*</td>
</tr>
<tr>
<td>Total food intake, g</td>
<td>79 ± 2</td>
<td>82 ± 3</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>7.8 ± 1.3</td>
<td>9 ± 1.2</td>
</tr>
<tr>
<td>Growth hormone, ng/ml</td>
<td>14.3 ± 3.9</td>
<td>15.9 ± 3.8</td>
</tr>
<tr>
<td>Free fatty acids, meq/l</td>
<td>0.47 ± 0.09</td>
<td>0.35 ± 0.07</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>109 ± 2</td>
<td>144 ± 5*</td>
</tr>
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Values are means ± SE. *P < 0.05 vs. control.

Ghrelin reduces triglyceride content and increases mitochondrial oxidative capacity in rat mixed skeletal muscle. In mixed gastrocnemius muscle, no changes were observed in transcript levels of ACC and CPT I (Fig. 3A), in phosphorylated AMPK, or phosphorylated or total ACC protein (Fig. 3B). Activities of both COX (control: 125 ± 9, ghrelin: 192 ± 16 μmol·min⁻¹·g protein⁻¹) and CS (control: 118 ± 11, ghrelin: 173 ± 21 μmol·min⁻¹·g protein⁻¹) were increased after ghrelin treatment in mixed gastrocnemius muscle (P < 0.05 vs. control; Fig. 3C), and a positive correlation was observed between the two (r = 0.86, P < 0.001, data not shown). Ghrelin treatment also increased UCP2 (+86%) but not UCP3 transcript levels (Fig. 3D). Transcript levels of PPARγ (control: 100 ± 24, ghrelin: 188 ± 18%, P < 0.05) were also higher after ghrelin administration in gastrocnemius muscle and were positively related to mitochondrial COX activities in all animals (r = 0.75, P < 0.001, data not shown) as well as in each group considered separately (control: r = 0.70, P = 0.07; ghrelin: r = 0.71, P = 0.07). These changes were associated with marked reduction of tissue triglyceride content in ghrelin- compared with vehicle-treated rats (control: 0.0072 ± 0.0012, ghrelin: 0.00423 ± 0.0005 mg/g dry wt; Fig. 1). In contrast to gastrocnemius, mitochondrial oxidative enzyme activities, transcript levels of ACC, CPT I, UCPs, and PPARγ, as well as tissue triglyceride content were comparable in highly oxidative soleus muscle from ghrelin-treated and control rats (Fig. 1 and Table 3).

Ghrelin favors expression of lipogenetic genes in white adipose tissue. To further test the hypothesis that ghrelin would favor a lipogenic pattern of gene expression in white adipose tissue, transcript levels of ACC, FAS, CPT I, and PPARs were also determined in this tissue. Ghrelin resulted in increased transcript levels of both ACC (+84%) and FAS (+109%) in abdominal white adipose tissue (Fig. 4, P < 0.05), whereas PPARγ expression was increased to a nonsignificant extent (control: 100 ± 33; ghrelin: 163 ± 51%). Ghrelin did not change white adipose tissue expression of CPT I (Fig. 4). PPARα expression was not modified by ghrelin treatment in any tissue (data not shown).

DISCUSSION

The current data demonstrate a role of ghrelin in the in vivo regulation of fat distribution and metabolism in nonadipose tissues. Ghrelin administration was reported to increase adipo-
genesis and adiposity (12, 49, 50), consistent with the current findings of enhanced adipose tissue lipogenic gene expression. It is shown here that weight gain caused by sustained ghrelin administration is further associated with altered lean tissue fat distribution with triglyceride deposition favored in liver over skeletal muscles. Circulating ghrelin is inversely related to body weight and fat mass in pathophysiological conditions, including obesity and anorexia (35, 51), suggesting that ghrelin is involved in adaptation to altered body fat content. Tissue-specific liver fat deposition occurs during sustained or marked caloric restriction and weight loss (3) that can also be associated with reduction of skeletal muscle fat (20). The current observations therefore suggest a potential contribution of ghrelin to the regulation of tissue fat distribution during reduced nutrient availability and loss of body weight and body fat.

Ghrelin treatment altered hepatic transcriptional and posttranscriptional expression of major regulators of lipid metabolism favoring lipogenesis over lipid oxidation. In addition, ghrelin enhanced mitochondrial UCP2 transcript levels, in agreement with UCP2 upregulation reported in experimental fatty liver (11). Caution should be used in interpreting changes in transcript levels with respect to their potential impact on correspondent enzyme activities and functions. The coordinated pattern of changes in transcript and protein levels and concomitant marked increase in triglyceride content, however, suggest that changes in fatty acid metabolism were involved in liver triglyceride accumulation. Sustained ghrelin treatment was also associated with hyperglycemia and increased transcript levels of the key enzyme of the gluconeogenic pathway, glucose-6-phosphatase, in keeping with in vitro findings (32).

Fig. 2. Ghrelin effects on liver mitochondrial-lipid metabolism gene expression. A: transcript levels (real-time PCR) of carnitine palmitoyltransferase I (CPT I), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and glucose-6-phosphatase (G-6-Pase). B: protein levels of phosphorylated (p) AMP-activated protein kinase (AMPK) and total (t) and phosphorylated ACC. C: enzyme activities of cytochrome c oxidase (COX) and citrate synthase (CS). D: transcript levels of uncoupling protein 2 (UCP2) measured by Northern blot. B and D: bands under each bar are representative samples from each experimental group. Data are means ± SE from 8 animals/group. *P < 0.05, ghrelin vs. control by Student’s t-test for unpaired data.
These combined observations suggest that enhanced hepatic production contributed to increase circulating glucose. Changes in phosphorylated AMPK and total and phosphorylated ACC (56) further support the involvement of altered AMPK signaling in hepatic effects of ghrelin treatment and indicate a link between ghrelin and AMPK in vivo. A major role of PPARγ and mitochondrial changes in ghrelin-induced triglyceride deposition are in turn not supported by the current data. Mitochondrial abnormalities reported in fatty liver in the general population are indeed proposed to result from chronic fat-related oxidative damage (4), in keeping with lack of changes in the current short-term model.

Table 3. Transcript levels of ACC, CPT I, UCP2 and -3, PPARγ (arbitrary units, %average control value), COX and CS enzyme activities in soleus muscle in the two experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ghrelin</th>
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<tbody>
<tr>
<td>ACC</td>
<td>100 ± 16</td>
<td>98 ± 11</td>
</tr>
<tr>
<td>CPT I</td>
<td>100 ± 6</td>
<td>90 ± 8</td>
</tr>
<tr>
<td>UCP2</td>
<td>100 ± 19</td>
<td>82 ± 16</td>
</tr>
<tr>
<td>UCP3</td>
<td>100 ± 21</td>
<td>80 ± 17</td>
</tr>
<tr>
<td>PPARγ</td>
<td>100 ± 13</td>
<td>92 ± 28</td>
</tr>
<tr>
<td>COX activity</td>
<td>226 ± 11</td>
<td>236 ± 31</td>
</tr>
<tr>
<td>CS activity</td>
<td>282 ± 17</td>
<td>298 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SE. COX, cytochrome c oxidase; CS, citrate synthase. Units are μmol·min⁻¹·g protein⁻¹.

Fig. 4. Abdominal white adipose tissue transcript levels of CPT I, ACC, and FAS. Data are means ± SE from 8 animals/group. Values were expressed as %average value in the control group. *P < 0.05, ghrelin vs. control by Student’s t-test for unpaired data.
Central ghrelin mediators include hypothalamic neuropeptide Y (24), the overexpression of which was reported to favor hepatic ACC activity, lipogenesis, and glucose production independent of its orexigenic effects (59). Importantly, neuropeptide Y also enhanced adipose tissue lipogenesis but not muscle triglyceride deposition (53, 59), in strong indirect agreement with tissue-specific changes in the current study. The association between liver fat deposition and sustained reduction of nutrient availability has been established (3), and hepatic lipogenesis was reported to be enhanced during malnutrition and weight loss, presumably associated with hyper-ghrelinemia and hypoinsulinemia (28). Thus enhanced liver lipid deposition induced by ghrelin could contribute to fatty liver associated with marked or sustained reduction of nutrient intake. Because circulating insulin is commonly reduced in calorie-restricted states and high insulin can favor hepatic lipogenesis (3), it is possible that maintenance of basal plasma insulin concentrations played a permissive role in the rapid onset of ghrelin-induced changes in liver triglyceride content in the current experimental setting. Ghrelin-induced lipogenic patterns of gene expression in liver and adipose tissue are further consistent with and could mediate excess fat gain during refeeding in different clinical models of undernutrition (17, 41).

Preferential fat deposition in liver and adipose tissue (12, 49) could have also contributed to prevent muscle triglyceride deposition in the current model by preventing increments of circulating free fatty acids and muscle free fatty acid supply that commonly occur in ghrelin-independent weight gain (9). Unchanged plasma free fatty acids could have also prevented major changes in muscle CPT I transcriptional expression, since fatty acid availability is an independent activator of muscle fat oxidative disposal (40). Ghrelin-induced weight gain was conversely not associated with downregulation of transcriptional and posttranscriptional expression of measured mitochondrial-lipid metabolism genes, at variance with observations in short-term diet-induced weight gain and human models of ghrelin-independent obesity (25, 37). In particular, the increase of representative mitochondrial enzyme activities and UCP2 transcript levels in mixed muscle (representing a majority of muscle tissue in rodents and humans (5)) suggests a role of ghrelin in the regulation of muscle mitochondrial oxidative capacity. Enhanced gastrocnemius muscle mitochondrial gene expression is reported during chronic caloric restriction (47). Early increments of the ability for muscle substrate oxidative disposal also occur in the presence of acute or short-term reduction of nutrient intake (42, 52). Expression of mitochondrial genes is in turn rapidly reduced in rodent muscle after 3 days of overfeeding and weight gain possibly associated with ghrelin suppression (37). The above observations suggest that early changes of muscle mitochondrial oxidative capacity contribute to adaptive changes in substrate disposal after modifications of nutrient intake. This hypothesis is also consistent with rapid changes in muscle lipid deposition after treatment with nutrient-sensing hormones insulin (2) and leptin (43) for 3–7 days in human and rodent models. It is therefore possible to hypothesize that early ghrelin increments (8, 13) contribute to muscle metabolic adaptive changes in calorie-restricted states (47, 52). Enhanced mitochondrial oxidative capacity could also have independently contributed to initiate or maintain ghrelin-induced triglyceride depletion in mixed gastrocnemius muscle, since reduced mitochondrial enzyme activities are related to impaired substrate utilization and muscle triglyceride accumulation in obese, type 2 diabetic, and aging individuals (21, 25, 39). Notably increased muscle fat oxidative gene expression during acute nutrient deprivation is more pronounced in moderately than in highly oxidative muscle (42), consistent with the current muscle-specific ghrelin effects that could be the result of higher basal oxidative reserve and capacity for fat utilization in type 1 fibers (33). A direct link between increased PPARγ expression or activation by thiazolidinediones and increased expression of skeletal muscle mitochondrial oxidative genes are indicated by several studies (23, 27, 31). Knockout mouse models showing muscle insulin resistance and tendency to accumulate muscle fat (22, 36) highlighted the metabolic relevance of muscle PPARγ. Although changes in transcript levels do not imply corresponding effects on protein levels and activity, upregulation of PPARγ transcriptional expression parallel to oxidative enzyme activities suggests involvement of PPARγ in ghrelin-induced muscle mitochondrial effects.

Intravenous bolus ghrelin administration can acutely increase growth hormone and, to a lesser extent, cortisol and prolactin plasma concentrations (48). However, sustained stimulation of growth hormone secretagogue receptor (46) and sustained repeated ghrelin administration in rodents (24, 57) did not result in increased circulating growth hormone. These findings are in excellent agreement with the current results in supporting the concept that repeated administration blunts or abolishes the growth hormone-secreting effects of ghrelin. Because acute ghrelin-induced increments of cortisol and prolactin plasma concentration are markedly less pronounced than that of growth hormone (48), comparable circulating growth hormone levels indirectly suggest that no major changes in cortisol and prolactin occurred after ghrelin treatment. In addition, available data do not indicate that cortisol and prolactin effects would account for the pattern of changes in tissue mitochondrial-oxidative metabolism gene expression and lipid distribution. In particular, reported prolactin effects on liver and white adipose tissue lipogenic gene expression and function are opposite to those observed after ghrelin treatment in the current study (1). In addition, excess glucocorticoids are not reported to exert independent effects on hepatic lipogenesis and lipid deposition (14, 29, 54), whereas most (16, 18, 30), although not all (55), reports agree on their suppressive or null effect on skeletal muscle mitochondrial function. Thus, taken together, the above observations do not support a major role of additional hypothymal hormonal changes in observed metabolic effects.

In conclusion, ghrelin induces tissue-specific changes in mitochondrial and lipid metabolism gene expression favoring triglyceride deposition in liver over skeletal muscle. These results suggest that ghrelin could be involved in adaptive changes of lipid distribution and metabolism in the presence of caloric restriction and loss of body fat.

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