Effect of Short-Term Fasting and Refeeding on Transcriptional Regulation of Metabolic Genes in Human Skeletal Muscle

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During short-term fasting, substrate utilization in skeletal muscle shifts from predominantly carbohydrate to fat as a means of conserving glucose. To examine the potential influence of short-term fasting and refeeding on transcriptional regulation in skeletal muscle, muscle biopsies were obtained from nine male subjects at rest, after 20 h of fasting, and 1 h after consuming either a high-carbohydrate (CHO trial) or a low-carbohydrate (FAT trial) meal. Fasting induced an increase in transcription of the pyruvate dehydrogenase kinase 4 (PDK4) (10-fold), lipoprotein lipase (LPL) (~2-fold), uncoupling protein 3 (UCP3) (~5-fold), and carnitine palmitoyltransferase I (CPT I) (~2.5-fold) genes. Surprisingly, transcription of PDK4 and LPL increased further in response to refeeding (both trials) to more than 50-fold and 6- to 10-fold, respectively, over prefasting levels. However, responses varied among subjects with two subjects in particular displaying far greater activation of PDK4 (>100-fold) and LPL (>20-fold) than the other subjects (mean ~8-fold and ~2-fold, respectively). Transcription of UCP3 decreased to basal levels after the CHO meal but remained elevated after the FAT meal, whereas CPT I remained elevated after both refeeding meals. The present findings demonstrate that short-term fasting/refeeding in humans alters the transcription of several genes in skeletal muscle related to lipid metabolism. Marked heterogeneity in the transcriptional response to the fasting/refeeding protocol suggests that individual differences in genetic profile may play an important role in adaptive molecular responses to metabolic challenges. *Diabetes* 52:657–662, 2003

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CHO trial, high-carbohydrate regimen; CPT I, carnitine palmitoyltransferase I; FAT trial, low-carbohydrate/high-fat regimen; FFA, free fatty acid; gDNA, genomic DNA; HKII, hexokinase II; LCAD, long-chain acyl-CoA dehydrogenase; LPL, lipoprotein lipase; PDH, pyruvate dehydrogenase; PDK4; pyruvate dehydrogenase kinase 4; UCP 3, uncoupling protein 3.

Skeletal myofibers possess an extraordinary ability to undergo adaptive changes in metabolic gene expression when faced with sudden or sustained increases in metabolic demand; e.g., as encountered in response to contractile activity induced by exercise or chronic motor nerve stimulation (1,2). Fasting also represents a metabolic challenge to skeletal muscle, requiring a sustained increase in the reliance on lipid and protein metabolism as part of the body's overall effort to minimize total carbohydrate utilization. It is not clear, however, whether the metabolic stress imposed by short-term fasting in humans elicits enough of a disturbance to metabolic homeostasis in muscle to require adaptive changes in gene expression. An additional possibility is that changes in circulatory factors induced by fasting such as reduced insulin, elevated counterregulatory hormones, or elevated free fatty acids (FFAs) may alter the regulation of metabolic gene expression in skeletal muscle (3).

In rats, fasting has previously been shown to induce an increase in the transcription and/or mRNA content for several lipid metabolism genes in skeletal muscle, including lipoprotein lipase (LPL), carnitine palmitoyltransferase 1 (CPT I), and long-chain acyl-CoA dehydrogenase (LCAD) (4,5). Of particular interest with respect to fasting is the regulation exerted over the pyruvate dehydrogenase (PDH) complex in muscle. PDH catalyzes the irreversible conversion of pyruvate to acetyl-CoA, allowing for entry of glycolytic products into the citric acid cycle. In response to fasting, PDH activity is inhibited via phosphorylation of the E1 component of the enzyme, a reaction that is catalyzed in muscle by pyruvate dehydrogenase kinase 4 (PDK4) (6). Fasting causes a marked induction in PDK4 mRNA and protein in both heart and skeletal muscle of rats, a response that is completely reversed upon refeeding (7–9). Thus, induction of PDK4 is thought to be a primary means by which glucose oxidation is suppressed in skeletal muscle during starvation, presumably as part of an integrative response to conserve glucose.

The aim of the present study was therefore to determine whether short-term fasting in humans elicits an adaptive response in skeletal muscle at the level of transcription of genes involved in lipid metabolism. In addition, the acute effect of a refeeding meal on transcription in human muscle was studied. Muscle biopsies were obtained before and after 20 h of fasting as well as 1 h after refeeding a meal either high (CHO) or low (FAT) in carbohydrates.

TABLE 1
Primers and probe sequences used for real-time PCR

Gene	Forward primer	Reverse primer	TaqMan probe
PDK4	5′ TCCACTGCACCAACGCCT 3′	5′ TGGCAAGCCGTAACCAAAA 3′	5′ ATAATTCCCGGAATGCTCCTTTGGCTG 3′
UCP3	5′ TGACTCCGTCAAGCAGGTGTAC 3′	5′ CAAAATCCGGGTAGTGAGGCT 3′	5′ CCCCCAAAGGCGCGGACAAC 3′
LPL	5′ GCTAGTGAAGTGCTCCCACGA 3′	5′ GGCCTTACTTGGATTTTCTTCATTC 3′	5′ CGCTCCATTCATCTCTTCATCGACTCTCTG 3′
CPT1	5′ CTGCAGTGGGACATTCCAAA 3′	5′ CAACGCCTTGGCCACCT 3′	5′ ACTCTCGATGACCGCCTGGCACTG 3′
GLUT4	5′ CCTGCCAGAAAGAGTCTGAAGC 3′	5′ ATCCTTCAGCTCAGCCAGCA 3′	5′ CAGAAACATCGGCCCAGCCTGTCA 3′
HKII	5′ TTGTCCGTAACATTCTCATCGATT 3′	5′ TGTCTTGAGCCGCTCTGAGAT 3′	5′ ACCAAGCGTGGACTGCTCTTCCGA 3′
β-actin	5′ CCTGGCACCCAGCACAAT 3′	5′ GCCGATCCACACGGAGTACT 3′	5′ ATCAAGATCATTGCTCCTCCTGAGCGC 3′

The transcriptional response of select glucose (GLUT4, hexokinase II [HKII], PDK4) and lipid (LPL, CPT I, uncoupling protein 3 [UCP3]) metabolism genes was measured by RT-PCR–based nuclear run-on and mRNA analyses.

RESEARCH DESIGN AND METHODS

Subjects. Nine healthy male subjects ranging in age from 22 to 28 years, with an average height of 185 cm (range 175–192) and a mean weight of 81 kg (range 65–110) participated in the study. The subjects were habitually physically active and maintained their normal activity pattern between the two trials. The subjects were given both oral and written information about the experimental procedures before they gave their informed consent. The study was approved by the Copenhagen and Frederiksberg Ethics Committee (Denmark) and the Human Investigations Committee (Yale University).

Experimental design. The subjects completed two trials (separated by 2–3 weeks), each consisting of 20 h of fasting followed by intake of a standardized refeeding meal, which in one trial was a carbohydrate-rich meal (CHO trial) and in the other a low-carbohydrate/high-fat meal (FAT trial). The diets were isocaloric (total amount of 4MJ) and the composition was 76% carbohydrate, 9% fat, and 15% protein in the CHO trial and 9% carbohydrate, 76% fat, and 15% protein in the FAT trial. Muscle biopsies were obtained from the middle portion of the vastus lateralis muscle using the percutaneous needle biopsy technique with suction (10) 3 h after a light standardized meal (control), after 20 h of fasting and 1 h after finishing the refeeding meal. The fasting and refeeding biopsies were obtained through the same incision (opposite leg from the control biopsy) but at sites ~6 cm apart. The major part of the muscle biopsies (110–140 mg) was used for isolation of nuclei, and 20–25 mg were frozen in liquid nitrogen for mRNA determinations.

Nuclei isolation. Nuclei were isolated from muscle biopsies as previously described (11). In brief, fresh muscle tissue was immediately placed in a precooled aluminum block, weighed, and placed in ice-cold buffer. The tissue was thoroughly minced, gently homogenized, and the nuclei spun down. The crude nuclear pellet was gently resuspended in Triton X-100 containing buffer and filtered through prewetted gauze. Nuclei were repelleted and gently resuspended in 10 ml of rinsing buffer. The final nuclear pellet was resuspended in 200 μl of storage buffer, quick-frozen in liquid N_2, and stored at −80°C.

Nuclear run-on. Relative transcriptional activity of selected genes was determined by an RT-PCR–based nuclear run-on technique as previously described (5,11). Briefly, incomplete transcripts were allowed to proceed to completion in the presence of nonradioactive nucleotides ATP, CTP, GTP, and UTP. After lysing the nuclei, nuclear proteins and genomic DNA were enzymatically digested by proteinase K and DNase I, respectively, and nascent RNA transcripts were isolated by extraction (TriZol; Gibco-BRL) and precipitation. Nascent transcripts were then subjected to a second DNase digestion and precipitation followed by thorough rinsing and resuspension overnight (4°C) in 22 μl of DEPC-treated water containing 0.1 mmol/l EDTA, 10 mmol/l Tris (pH 8.0).

Isolation of genomic DNA. Genomic DNA (gDNA) was isolated from 20 μl of each nuclei preparation as previously described (5,11). Final gDNA pellets were resuspended overnight (4°C) in 50 μl nuclease-free water containing 0.1 mmol/l EDTA, 10 mmol/l Tris (pH 8.0). The relative gDNA content of the nuclei samples was determined by PCR amplification of the 18S gene (described in RT-PCR section) and was used to adjust the volumes of the nascent transcript samples for differences in nuclei content before the nuclear run-on reaction.

RNA isolation. Total RNA was isolated from ~20–25 mg of tissue by a modified guanidinium thiocyanate-phenol-chloroform extraction method adapted from Chomczynski and Sacchi (12) and as described previously (11). To facilitate localization of the RNA pellet, 100 μg of yeast tRNA was added to the aqueous phase before precipitation. RNA was resuspended overnight (4°C) in DEPC-treated H_2O containing 0.1 mmol/l EDTA (2 μl per mg original tissue weight).

Reverse transcription and PCR. Reverse transcription (RT) of both nascent RNA from the nuclear run-on reactions and of total RNA samples was performed using the Superscript II RNase H^- system (Gibco-BRL) as previously described (5,11). RT products of nascent nuclear run-on RNA were diluted with nuclease-free H_2O based on the relative gDNA content of each nuclei preparation (see above) with the average volume set to 150 μl. For RT products of total RNA (RT-RNA), each sample originating from 11 μl of total RNA was diluted to a total volume of 150 μl.

Transcription and mRNA content of a given gene as well as the 18S content in the gDNA samples were determined by PCR as previously described (11) or by fluorescence-based real-time PCR (TaqMan) technology (ABI PRISM 7700 Sequence Detection System; Applied Biosystems). For real-time PCR, forward and reverse primers and TaqMan probe (Table 1) were designed from human specific sequence data (*Entrez*, National Institutes of Health; and *Ensembl*, Sanger Institute) using computer software (Primer Express; Applied Biosystems). For each of the genes, a Blast Search revealed that sequence homology was obtained only for the target gene. All TaqMan probes were 5′-6-carboxyfluorescein (FAM) and 3′-6-carboxy-*N*,*N*,*N*′,*N*′-tetramethylrhodamine (TAMRA) labeled, except for β-actin, which was 5′ VIC and 3′ TAMRA (Applied Biosystems) labeled. Prior optimization was conducted for each oligo set determining optimal primer concentrations, probe concentration, and verification of the efficiency of the amplification. The expected size of the PCR products was confirmed by separating PCR products by gel electrophoresis using a 2.5% agarose gel stained with ethidium bromide and visualizing the products by UV exposure using a Kodak image station E440CF (Kodak). The β-actin mRNA was amplified using a predeveloped assay reagent (5′ VIC and 3′ TAMRA labeled). A validating test was performed ensuring that the amplification efficiency was similar for the target genes and the endogenous control (β-actin). Finally, the relative nuclei content of the gDNA samples was determined by amplifying the 18S gene using a predeveloped assay reagent (Applied Biosystems) designed to amplify genomic DNA. PCR amplification was performed (in triplicate) in a total reaction volume of 25 μl. The reaction mixture consisted of 2.5 μl diluted template, TaqMan probe, and forward and reverse primers as determined from the prior optimization, 2× TaqMan Universal MasterMix (Applied Biosystems) optimized for TaqMan reactions (containing AmpliTaq Gold DNA polymerase, AmpErase Uracil *N*-glycosylase, dNTPs with dUTP, ROX as passive reference, and buffer components) and nuclease-free water. An identical cycle profile was used for all genes: 50°C 2 min + 95°C for 10 min + [94°C for 15 s + 60°C for 1 min] × 40 cycles. Data were analyzed using a comparative critical threshold (*C*t) method where the amount of target normalized to the amount of endogenous control (β-actin) and relative to the control sample is given by $2^{-\Delta\Delta Ct}$ (Applied Biosystems). For each subject, all samples were run together allowing relative comparisons of the samples of a given subject.

Statistics. Transcriptional activity and mRNA contents were normalized to β-actin transcription and mRNA levels, respectively, and samples were expressed relative to the corresponding control (pre) sample, which was set to 1. An average value was calculated from the two identical fasting trials. Data are presented as means ± SE. Statistical analysis was performed after log transformation of the data. A paired *t* test was used to compare transcription and mRNA content before and after fasting. One-way ANOVA for repeated measures was applied to evaluate the effect of the fasting/refeeding protocol on transcription and mRNA content using Student-Newman-Keuls's post hoc test to locate differences. Differences were considered significant at $P < 0.05$.

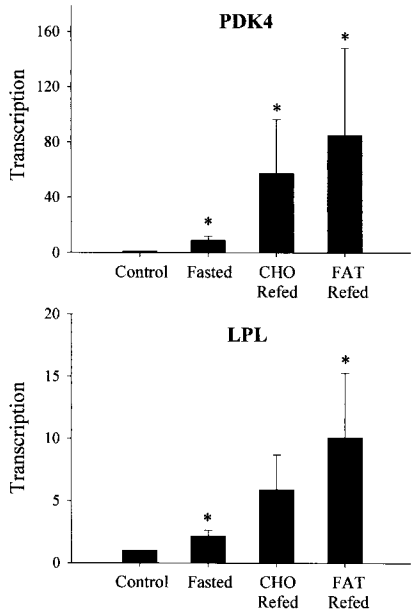

FIG. 1. Transcriptional responses to fasting and refeeding. Transcription of the PDK4 and LPL genes was determined by RT-PCR–based nuclear run-on analyses. Muscle biopsies were obtained from the vastus lateralis muscle 3 h after a light standardized meal (Control), after 20 h of fasting (Fasted), and 1 h after a refeeding meal that was either high (CHO Refed) or low (FAT Refed) in carbohydrates. The target gene response was normalized to β-actin transcription and expressed as fold change relative to the control sample. For each subject, the fasting data from the two identical trials were averaged. Values are means ± SE with $n = 9$. *Significantly ($P < 0.05$) different from control.

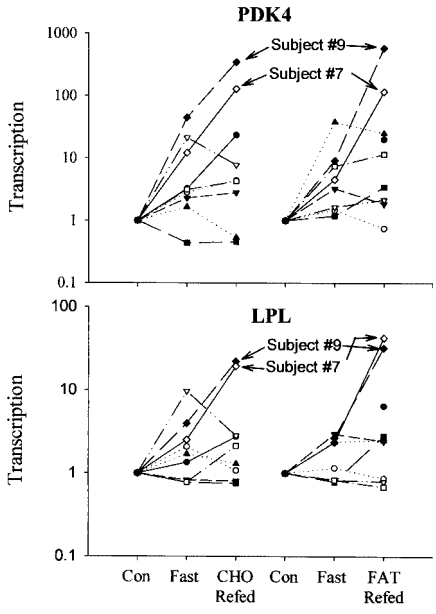

FIG. 2. Individual responses in PDK4 and LPL transcription. Transcription was determined by RT-PCR–based nuclear run-on analyses from muscle biopsies obtained from the vastus lateralis muscle 3 h after a light standardized meal (Con), after 20 h of fasting (Fast), and 1 h after a refeeding meal that was either high (CHO Refed) or low (FAT Refed) in carbohydrates. Data were normalized to β-actin transcription and expressed as fold change relative to the control sample for each subject. Note that *y*-axis is logarithmic scale.

RESULTS

Transcription. Combining the data from both trials, fasting for 20 h induced a significant ($P < 0.05$) ninefold increase in PDK4 transcription (Fig. 1). Surprisingly, within 1 h after refeeding, average transcription of PDK4 increased ($P < 0.05$) further to 60- to 80-fold over control levels, independent of the type of meal ingested (CHO or FAT) (Fig. 1). Transcription of the LPL gene followed a similar pattern, characterized by a slight increase in response to fasting followed by a marked induction within 1 h after CHO (~6-fold, $P = 0.059$) or FAT (~10-fold, $P = 0.024$) refeeding. An important feature of the data from this study, however, was that the absolute change in transcription in response to fasting and refeeding varied considerably among subjects. Inspection of the individual data revealed that two subjects were particularly sensitive to the fasting/refeeding protocol, displaying far greater activation of PDK4 (>100-fold) and LPL (>20-fold) in both trials than the remaining seven subjects (Fig. 2). PDK4 transcription in these seven subjects was also significantly ($P < 0.05$) increased in response to fasting (6.5 ± 2.6-fold) and refeeding (CHO 6.2 ± 3.0-fold, FAT 9.2 ± 3.7-fold). Average LPL transcription was significantly ($P < 0.05$) elevated (2.0 ± 0.5-fold) in response to fasting in these seven subjects, but failed to reach statistical significance ($P > 0.05$) in response to refeeding.

Fasting and refeeding also significantly affected transcription of the UCP3 and CPT I genes. Transcription of UCP3 was fivefold higher ($P < 0.05$) after fasting (Fig. 3). Interestingly, CHO refeeding rapidly (1 h) reversed this response causing transcription of UCP3 to return to basal levels, whereas UCP3 transcription remained elevated ($P < 0.05$) over control levels in response to the FAT refeeding meal. Fasting caused an ~2.5-fold increase ($P < 0.05$) in CPT I transcription that was still present 1 h after refeeding in both the CHO and FAT trial (Fig. 3). Responses in the two "sensitive" subjects were also among the highest for both UCP3 and CPT I transcription; analysis of the remaining seven subjects yielded the same

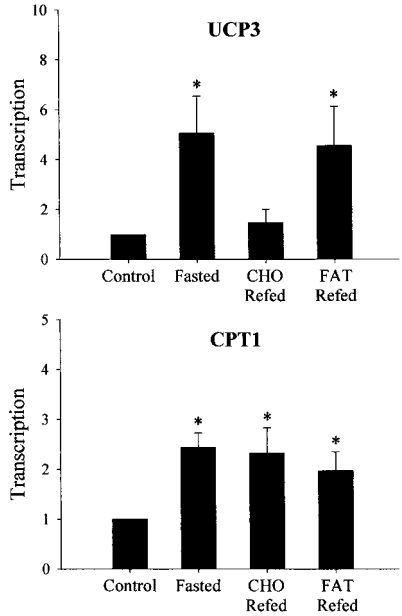

FIG. 3. Transcriptional responses to fasting and refeeding. Transcription of the CPT I and UCP3 genes was determined by RT-PCR–based nuclear run-on analyses as described in the Fig. 1 legend. Values are means ± SE with $n = 9$. *Significantly ($P < 0.05$) different from control.

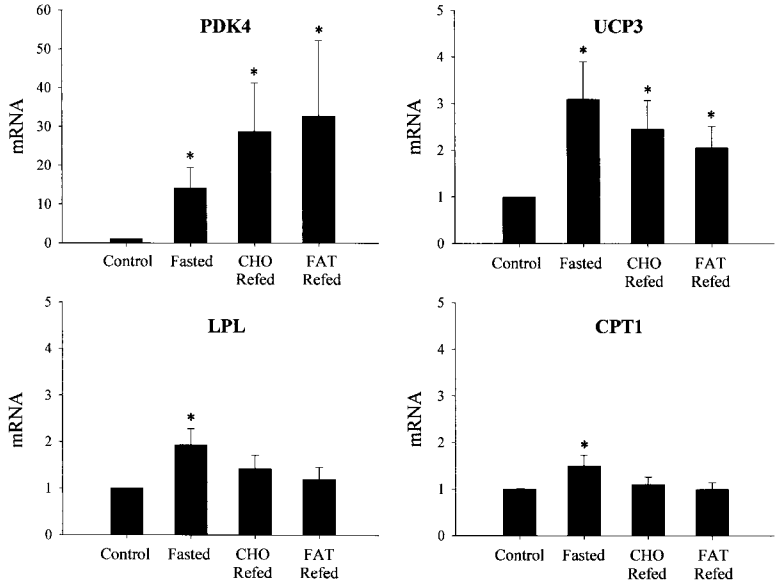

FIG. 4. Response in mRNA content to fasting and refeeding. The mRNA contents of the PDK4, LPL, CPT I, and UCP3 genes were determined by RT-PCR from experiments described in the Fig. 1 legend. Values are means ± SE with $n = 9$. *Significantly ($P < 0.05$) different from control.

pattern of response for both genes ($P < 0.05$). GLUT4 and HKII transcription could only be determined for four of the subjects, but interestingly, refeeding elicited marked increases in GLUT4 (3- to 5-fold) and HKII (~30-fold) transcription in the same two "sensitive" subjects with little change in the other two subjects (not shown).

Content of mRNA. PDK4 mRNA increased ($P < 0.05$) in response to fasting (~14-fold) and even further after refeeding (~30-fold, independent of the meal ingested) (Fig. 4), although, as with transcription, the magnitude of the response varied considerably among subjects. Small increases were observed in the mRNA content of LPL (~2-fold, $P < 0.05$) and CPT I (1.5-fold, $P < 0.05$) after 20 h of fasting, but were no longer present 1 h after refeeding in either trial (Fig. 4). UCP3 mRNA increased (~3-fold, $P < 0.05$) in response to fasting and remained signficantly elevated 1 h after refeeding independent of the meal ingested. GLUT4 and HKII mRNA (measured on four subjects) did not change in response to either fasting or refeeding (not shown).

DISCUSSION

Three new findings have come from the present study. First, short-term fasting (20 h) in humans elicits a marked activation of transcription and increase in mRNA content of the PDK4, LPL, UCP3, and CPT I genes in skeletal muscle. Second, refeeding appears to be more complex with evidence presented that transcription may be further induced (PDK4 and LPL), may remain elevated (CPT I and UCP3), or may rapidly decline (UCP3), depending on the gene and the type of meal consumed. Third, and perhaps most intriguing, the molecular response to fasting and refeeding in human skeletal muscle appears to vary considerably among individuals as evidenced by the dramatic induction of PDK4, LPL, and HKII in the same two subjects in both trials.

Consistent with an increased reliance on FFA utilization associated with starvation (13), the results from the present study demonstrate that short-term fasting in humans activates the transcription of at least a subset (PDK4, LPL, and CPT I) of genes associated with lipid metabolism in skeletal muscle. These data agree with and extend our previous findings in rats in which transcription of several lipid metabolism genes was found to increase in both red/oxidative and white/glycolytic skeletal muscle after 24–72 h of fasting (5). However, in a recent study also in humans, Tunstall et al. (14) did not detect a significant change in skeletal muscle mRNA content for several genes involved in FFA intracellular transport (fatty acid binding protein, fatty acid translocase) and oxidation (CPT I, β-hydroxyacyl-CoA dehydrogenase, LCAD) after either 15 or 40 h of fasting. Although significant only in the present study, the increase in CPT I mRNA (the only mRNA measured common to both studies) in response to fasting was very similar (1.3- to 1.5-fold) in the two studies. It is also worth noting that the effect of fasting in the present study was assessed at the level of transcription, a more sensitive indicator of pretranslational regulation than mRNA determinations. Thus, collectively these findings suggest that short-term fasting in humans initiates an adaptive response in skeletal muscle to increase the expression of at least a subset of lipid metabolism genes, presumably as a component of the body's overall strategy to minimize glucose utilization in peripheral tissues.

In response to refeeding, it was hypothesized that the carbohydrate meal would rapidly reverse the transcriptional activation of genes induced by fasting due to the increased availability of glucose, whereas a persistent elevation in transcription was anticipated after the meal low in carbohydrate. The sharp increase in transcription of PDK4 and LPL upon refeeding independent of the meal ingested was therefore a surprising finding (Fig. 1). However, inspection of the individual data revealed considerable heterogeneity in the response to the fasting/refeeding protocol with two subjects in particular showing marked increases in PDK4 and LPL transcription (Fig. 2). Although repeated muscle sampling represents a potential source of variability, we have previously found a coefficient of

variation of 10.2 ± 0.7% (mean ± SE) for β-actin/GAPDH mRNA (determined by real-time PCR) between duplicate muscle samples obtained at the same time from opposite legs (total of 16 paired measurements; unpublished data). This is similar to a coefficient of variation of 12.3 ± 9.4% (mean ± SD) reported by Watt et al. (15) for measurements of intramuscular triglyceride content in duplicate biopsy samples obtained from the same leg. Moreover, the observation that the same marked increase in PDK4 and LPL occurred in the same two subjects in response to the fasting/refeeding protocol in two separate trials argues against a technical artifact. In fact, the high level of sensitivity to fasting and refeeding in these two subjects strongly suggests that genetic profile is largely responsible for setting the cellular and molecular sensitivity and responsiveness to metabolic challenges in skeletal muscle.

The observed fasting-induced increase in the expression of PDK4, LPL, and CPT I in skeletal muscle of rats (4,5,8) and humans (present study) suggests that the level of these proteins under basal conditions is insufficient to meet the metabolic demand imposed by fasting. The PDK4 gene for example is expressed primarily in skeletal muscle and heart, where it catalyzes the phosphorylation and inactivation of the PDH enzyme (6). Although expressed at low levels at rest, PDK4 expression increases dramatically in response to not only fasting (7,8,16), but also exercise (11,17), streptozotocin-induced diabetes (8), and high fat feeding (18), inhibiting the conversion of pyruvate to acetyl-CoA and effectively preventing the oxidation of carbohydrate-derived substrates. In contrast to PDK4, the LPL and CPT I genes are expressed at moderate levels in skeletal muscle under resting conditions, consistent with their respective roles in lipid metabolism (19–21). Elevations in muscle LPL expression during fasting would be expected to enhance the capacity for hydrolyzing circulating triglycerides, thereby improving the local availability of FFAs within the tissue, while fasting-induced increases CPT I would be expected to improve the capacity for FFA transport into mitochondria. It is tempting to speculate that the metabolic changes encountered during fasting lead to an activation of the PDK4, LPL, and CPT I genes in skeletal muscle as part of a concerted effort to conserve total body carbohydrate reserves. By extension, the further enhanced activation of PDK4 and LPL during the initial 1 h of refeeding would serve to ensure that the ingested carbohydrate is preferentially utilized for fuel storage. This response seems, however, to be unique to the initial period of refeeding after fasting, as carbohydrate intake over several hours to days leads to a downregulation of PDK4 expression as compared with a low-carbohydrate/high-fat diet (18,22,23).

Results from the present study also demonstrate that fasting in humans induces a marked increase in transcription and mRNA concentration of the UCP3 gene in skeletal muscle (Figs. 3 and 4). These findings extend previous work in both rats and humans (5,14,24–26), providing direct evidence that UCP3 expression in human skeletal muscle is acutely regulated by fasting primarily at the level of transcription of the UCP3 gene. Interestingly, the fasting-induced elevation in muscle UCP3 transcription was completely reversed within 1 h after refeeding the CHO meal but remained significantly elevated after the FAT meal. Regulation of UCP3 appears to be particularly sensitive to elevations in circulating FFA levels as inhibition of lipolysis during fasting by nicotinic acid administration in rats abolishes the food restriction–induced increase in UCP3 expression in both oxidative skeletal muscle and cardiac muscle (27,28). Direct infusion of intralipids also leads to an increase in muscle UCP3 mRNA (26,29), implying a direct role for FFA-mediated regulation of UCP3 in muscle, possibly through interaction and functional activation of the peroxisomal proliferator–activated-receptor-α (PPARα) (30). The close relationship between changes in FFA levels in response to a variety of nutritional interventions and the regulation of UCP3 expression in skeletal muscle has led to the suggestion that UCP3 plays a role in FFA metabolism (31,32). However, more recent biochemical studies on isolated mitochondria have shown that UCP3-mediated uncoupling is activated by superoxide (33), thereby supporting earlier findings from UCP3 knockout mice that UCP3 may be induced in muscle as a protective mechanism to limit the formation of reactive oxygen species (34). Considering that FFA oxidation in skeletal muscle is driven primarily by plasma FFA concentration (35), it is proposed that UCP3 may be induced under conditions in which FFA oxidation/respiratory chain activity is accelerated in muscle without a corresponding increase in demand for ATP synthesis (e.g., as during fasting, recovery from exercise, diabetes, etc.), thereby providing an alternative mechanism for dissipating the H^+ gradient and limiting the formation of reactive oxygen species.

In summary, the results from the present study demonstrate that short-term fasting in humans increases the transcription of at least a subset of genes encoding for proteins involved in lipid metabolism in skeletal muscle. These fasting-induced changes in gene expression are likely contributing to the overall shift in lipid metabolism in skeletal muscle and are, thus, an important component of the body's overall strategy to conserve carbohydrates during fasting. It is important to emphasize that these alterations in gene expression occurred in response to a relatively minor metabolic stress, illustrating the sensitivity of skeletal muscle tissue to changes in intermediary metabolism. That skeletal muscle comprises a high proportion of the total body mass and accounts for a substantial portion of energy utilization (36) suggests that even relatively small changes in the expression of lipid metabolism genes in muscle may be an important component of the overall counterregulatory response to the metabolic stress of fasting. Finally, the marked heterogeneity evident in the gene-activation response to fasting/refeeding among subjects in the present study suggests that individual differences in genetic profile may set the cellular and molecular sensitivity and responsiveness of skeletal muscle. Delineating how genetic diversity affects the adaptive response of muscle to metabolic stress may be an important step in understanding the etiology of metabolically based diseases such as obesity and type 2 diabetes.

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