Effect of 48-h Food Deprivation on the Expressions of Myosin Heavy-Chain Isoforms and Fiber Type-Related Factors in Rats

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Summary  The primary aim of this study was to examine the effects of 48-h food deprivation on rat skeletal muscle fiber type, according to myosin heavy-chain (MyHC) isoform composition and some metabolism-related factors in both slow-type dominant and fast-type dominant muscle tissues. Male Wistar rats (7 wk old) were treated with 48-h food deprivation or ad libitum feeding as control. After the treatment, the soleus muscle (slow-type dominant) and the extensor digitorum longus (EDL, fast-type dominant) were excised. We found that 48-h food deprivation did not affect MyHC composition in either the soleus or EDL, compared with fed rats by electrophoretic separation of MyHC isoforms. However, 48-h food deprivation significantly increased the mRNA expression of fast-type MyHC2B in the EDL muscle. Moreover, food deprivation increased fatty acid metabolism, as shown by elevated levels of related serum energy substrates and mRNA expression of mitochondrial uncoupling protein (UCP) 3 and lipoprotein lipase (LPL) in both the soleus and EDL. UCP3 and LPL are generally expressed at higher levels in slow-type fibers. Furthermore, we found that food deprivation significantly decreased the protein amounts of PGC1α and phosphorylated FOXO1, which are known as skeletal muscle fiber type regulators. In conclusion, 48-h food deprivation increased mRNA expression of fast-type MyHC isoform and oxidative metabolism-related factors in EDL, whereas MyHC composition at the protein level did not change in either the soleus or EDL.

Key Words  skeletal muscle, fiber type, food deprivation, UCP3, LPL.

The skeletal muscle is the largest organ in the human body, comprising about 40% of the body weight. In the skeletal muscle of most animals, there are 2 major classifications of fiber type: type 1 fibers (slow-twitch oxidative, red muscle) and type 2 fibers (fast-twitch glycolytic, white muscle). Muscle fiber type composition affects exercise performance, fatigue resistance, and metabolic capacity in humans (1). Skeletal muscle fiber types are generally classified according to myosin heavy-chain (MyHC) isoforms. In adult rodent skeletal muscles, 4 MyHC isoforms have been identified: MyHC1, 2A, 2X, and 2B (2). MyHC1 is expressed in slow-twitch oxidative type 1 muscle fibers, whereas MyHC2A, 2X, and 2B are preferentially expressed in fast-twitch type 2A, 2X, and 2B muscle fibers. Type 2A and 2X fibers have intermediate characteristics between type 1 and type 2B. Although the type 2X fibers are sometimes defined as fast-twitch glycolytic fibers, type 2B fibers have an even stronger fast-twitch, glycolytic phenotype than type 2X fibers (3–5). It is well known that cross-reinnervation, electrical stimulation, or exercise evokes fiber type transition (6, 7), even though the molecular mechanisms mediating this transformation have not been clearly elucidated. It is obvious, however, that skeletal muscle cells possess the capacity to respond and adapt to the metabolic changes imposed by physical or neural stimulation.

Food deprivation increases fatty acid oxidation and has some similarities to prolonged exercise. Food deprivation increases the expression of some genes to a greater extent in slow-type fibers than in fast-type fibers. The food deprivation increases the mRNA expressions of lipoprotein lipase (LPL) and fatty acid translocase (FAT/CD36), which are fatty acid utilization proteins (8). It was shown that upon short-term food deprivation, skeletal muscle fibers increase their capacity to take up fatty acids, likely via increased membrane content of FAT/CD36 facilitating fatty acid uptake in rats (9). Moreover, food deprivation activated the transcription of various genes with critical roles in mediating fatty acid metabolism in the skeletal muscle, such as LPL, carnitine palmitoyltransferase 1 (CPT1), and long-chain acyl-CoA dehydrogenase (LCAD) (10). These responses are also observed after prolonged exercise (8, 11). Thus, the energy metabolism condition seems similar between food deprivation and prolonged exercise, which lead to an increase in fatty acid oxidation and lipolysis. Therefore, we thought that it would be possible that food deprivation could induce fast-to-slow fiber type transition like prolonged exercise. If we point out the difference between food deprivation and prolonged exercise,
it is the amount of energy expenditure, i.e. food deprivation consumes less energy, but exercise consumes more energy than the normal condition.

Food deprivation is also known to induce muscle atrophy. Fast-twitch muscle is more sensitive than slow-twitch muscle to starvation, and fasting induces more severe muscle atrophy in fast-twitch muscle (12–15). It is possible that the slow-fiber type proportion would increase when fast-type MyHC decreases preferentially.

Thus far, the effect of food deprivation on skeletal muscle fiber type has not been fully elucidated. The primary aim of this study was to examine the effects of 48-h food deprivation on rat skeletal muscle fiber type according to MyHC isoform composition in both slow-type dominant and fast-type dominant muscle tissues. In addition, we measured the mRNA expression of MyHC isoforms, oxidative metabolism-related factors, and transcription factors regulating muscle fiber type.

MATERIALS AND METHODS

Animals and treatment. Male Wistar rats (7 wk old) weighing 210–230 g were purchased from a commercial supplier (Kyudo, Tosu, Japan). They were individually housed in stainless-steel wire-mesh cages in an animal room at 22±2°C at 50±10% humidity under an artificial lighting system of 12-h light and 12-h darkness (lights on from 0800 to 2000) and were fed a commercial diet (Type CRF-1 of Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum for 1 wk to acclimatize them to the environment. Following the acclimatization period, rats were divided into 2 groups (control and food-deprived groups, n=6 each) so that control and food-deprived groups had the same initial body weight. Each group had the same initial body weight (50.5±0.5 g). Control rats had free access to chow and water, whereas food-deprived groups had the same initial body weight. Food-deprived groups, 6 each, were given the concentrations described above.

Preparation of perirenal tissue samples. Perirenal tissue samples were collected 48 h after food deprivation. Perirenal tissue samples were divided into 2 parts: a portion for RNA extraction and another portion for protein extraction. The sample homogenates were boiled for 3 min and then electrophoresis was performed at a constant voltage. The RNA concentration was determined using a spectrophotometer. The RNA was isolated from powdered soleus and EDL muscles (approx. 50 mg) by using TRIzol Reagent (Invitrogen, Grand Island, NY), according to the manufacturer’s protocol. Briefly, muscle tissue was homogenized in 1 mL of TRIzol Reagent using a Polytron homogenizer. Cooled chloroform was added and separated by centrifugation. The aqueous phase containing RNA was transferred to a clean tube, and the RNA was precipitated by adding isopropanol and incubating at room temperature for 10 min. After another round of centrifugation, the RNA pellet was washed in 75% ethanol and finally dissolved in 200 μL of diethylpyrocarbonate-treated water. The RNA concentration was determined using a spectrophotometer and 2 μg of total RNA was reverse transcribed in a total volume of 20 μL by a reverse-transcriptase SuperScript III (Invitrogen) using Oligo d(T)16 primer (Applied Biosystems, Carlsbad, CA). The protein and reverse transcribed cDNA samples were kept at −80°C until analysis.

Analysis of MyHC isoform composition. Soleus, EDL, and gastrocnemius protein samples were subjected to high-resolution SDS-polyacrylamide gel electrophoresis for the assessment of MyHC isoform content as described in detail by Mizunoya et al. (16). Briefly, samples (50 ng) were loaded on a gel composed of 8% acrylamide (acrylamide/Bis ratio, 99 : 1) with 35% (v/v) glycerol, and the gel was run under a constant voltage of 140 V for 2 h at 4°C. The gel was then stained with Silver Stain Kanto III (Kanto Chemical Co., Inc., Tokyo, Japan) and dried. The bands were captured on an image scanner and the relative content of MyHC isoforms was quantified by densitometry using ImageJ 1.34s software (Rasband W; National Institutes of Health, Bethesda, MD). MyHC isoforms were identified according to their different migration rates (MyHC1 > 2B > 2X > 2A).

Real-time quantitative RT-PCR. The primer sets and the Universal Probe Library TaqMan probes (Roche, Tuxson, AZ) used are listed in Table 1. The Universal Probe Library is based on 165 short hydrolysis probes, labeled...
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at the 5′ end with fluorescein (FAM) and at the 3′ end with a dark quencher dye. Real-time quantitative PCR was run on the Roche LightCycler 1.5 using the TaqMan probe detection format standardized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) expression. All primers and appropriate probes are intron-spanning and were designed using ProbeFinder (version 2.35 for rat, Roche). Threshold cycles (Ct) were determined as the PCR cycle at which an increase in fluorescence above the baseline signal was first detected. Annealing temperature was set to 60˚C in all cases. Genes were analyzed using a standard curve constructed by serial dilution of aliquots of cDNA pooled from one randomly chosen sample. To clarify the difference in the amount of the 4 MyHC isoforms in the same muscle, the MyHC isoforms were analyzed using the 

\[ DC_t \] method (17) to compare the expression relative to HPRT expression. For other genes, values were standardized to HPRT expression level and expressed as the fold change in gene expression relative to the fed control group.

### Western blot analysis

For Western blot analysis, the proteins from soleus or EDL (40 μg protein/lane) were loaded onto 12% polyacrylamide gels for electrophoresis and transferred to PVDF membranes for 4 h at 38 V (constant voltage) by a tank transfer method. The membranes were then incubated with primary antibodies overnight at 4˚C with gentle agitation. The following antibodies were used: mouse monoclonal anti-myoglobin (1 : 8,000; Sigma, St. Louis, MO); rabbit polyclonal anti-UCP3 (1 : 40,000; Abcam, Cambridge, MA); anti-PPARδ (1 : 10,000; 516557, Calbiochem, Rockland, MA); rabbit monoclonal anti-phosphoFOXO1 (Ser256) (1 : 2,000; #9461, Cell Signaling Technology Inc., Danvers, MA); anti-FOXO1 (1 : 2,000; 1874-1, Epitomics, Burlingame, CA); horseradish peroxidase (HRP)-conjugated anti-β-actin antibody (1 : 5,000; #5125, Cell Signaling Technology Inc.). These primary antibodies were diluted in Can Get Signal solution 1 (Toyobo, Osaka, Japan). After washing in TBS-T 3 times for 10 min, the membranes were incubated for 1 h at room temperature with HRP-conjugated anti-β-actin antibody, developed immediately after washing. The blots were developed using enhanced chemiluminescence detection (ECL Select Western Blotting Detection Reagents, GE Healthcare Japan, Tokyo, Japan) and Hyperfilm ECL (GE Healthcare Japan). The bands were captured on an image scanner.
and quantified by densitometry using ImageJ software described above. The β-actin was used as a loading control. The multiple antigen detection was attained by cutting the membranes horizontally and hydrogen peroxide quenching method (18).

Statistical analysis. Data are expressed as the mean±SE. The Student’s t-test was used to compare the fed and 48-h FD groups. Statistics were calculated with Excel X for Macintosh (Microsoft). The level of significance was set at p<0.05.

RESULTS

Body and tissue weights

Compared with the fed rats, the 48-h FD rats showed significant decrease in body weight (Table 2). The decrease in the mass of various organ tissues was varied; in particular, perirenal fat and liver weights showed the greatest decrease in the 48-h FD rats (greater than 50% reduction compared to the fed rats). The decrease in muscle mass was also different among muscle tissues. The extent of decrease of soleus was milder than that of the other muscle tissues such as the gastrocnemius and EDL, which are mainly composed of fast-twitch fibers. The extent of decrease of EDL, which are mainly composed of fast-twitch fibers.

Table 2. Final body weight and organ weights in fed rats or 48-h FD rats (g).

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>48-h FD</th>
<th>48-h FD/Fed (%)</th>
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</thead>
<tbody>
<tr>
<td>Final body weight</td>
<td>289.5±7.7</td>
<td>224.4±12.5**</td>
<td>77.5</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.210±0.005</td>
<td>0.203±0.006</td>
<td>96.7</td>
</tr>
<tr>
<td>Plantaris</td>
<td>0.525±0.016</td>
<td>0.506±0.013</td>
<td>96.5</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>2.727±0.053</td>
<td>2.431±0.077**</td>
<td>89.1</td>
</tr>
<tr>
<td>EDL</td>
<td>0.247±0.007</td>
<td>0.228±0.004</td>
<td>92.6</td>
</tr>
<tr>
<td>TA</td>
<td>0.973±0.032</td>
<td>0.897±0.016*</td>
<td>92.2</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>1.725±0.030</td>
<td>1.635±0.047</td>
<td>94.8</td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>2.464±0.227</td>
<td>1.632±0.131**</td>
<td>66.2</td>
</tr>
<tr>
<td>Perirenal fat</td>
<td>2.762±0.297</td>
<td>1.037±0.143**</td>
<td>37.6</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>0.429±0.026</td>
<td>0.233±0.025**</td>
<td>54.2</td>
</tr>
<tr>
<td>Liver</td>
<td>14.648±0.587</td>
<td>7.270±0.290**</td>
<td>49.6</td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.405±0.048</td>
<td>2.027±0.061**</td>
<td>84.3</td>
</tr>
<tr>
<td>Heart</td>
<td>0.959±0.042</td>
<td>0.862±0.019</td>
<td>89.9</td>
</tr>
</tbody>
</table>

Values are means±SE for 6 rats. *p<0.05; **p<0.01 compared with fed group.
Muscle weights are sum of right and left muscles except for quadriceps (only right side).
EDL, extensor digitorum longus; TA, tibialis anterior; 48-h FD, 48-h food deprivation.

Table 3. Concentration of serum energy substrates in fed rats or 48-h FD rats.

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>48-h FD</th>
<th>48-h FD/Fed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>274.8±28.9</td>
<td>237.2±27.1</td>
<td></td>
</tr>
<tr>
<td>FFA, mEq/L</td>
<td>0.177±0.025</td>
<td>0.377±0.034**</td>
<td></td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>120.6±14.0</td>
<td>35.6±7.5**</td>
<td></td>
</tr>
<tr>
<td>Acetoacetic acid, μM</td>
<td>384.8±13.7</td>
<td>880.1±90.5**</td>
<td></td>
</tr>
<tr>
<td>β-Hydroxybutyric acid, μM</td>
<td>279.1±48.8</td>
<td>2.107.2±269.0**</td>
<td></td>
</tr>
</tbody>
</table>

Values are means±SE for 6 rats. *p<0.05; **p<0.01 compared with Fed group.
FFA, free fatty acids; 48-h FD, 48-h food deprivation.

Serum energy metabolites

Next, we measured the concentration of several energy substrates in the serum of rats, and these values are shown in Table 3. A significant increase in serum FFA and a significant decrease in triglycerides were observed in the 48-h FD rats compared to the fed rats, suggesting a potent degradation of body fat. The acetoacetic acid and β-hydroxybutyric acid concentrations, an index of fatty acid degradation in the liver, were significantly higher in the 48-h FD rats than in the fed rats. There were no significant differences in serum glucose concentrations between fed and 48-h FD rats.

MyHC isoform composition

Composition of MyHC isoforms is a common index for determining overall skeletal muscle fiber type. Figure 1 illustrates the electrophoresis pattern of MyHC isoform proteins in the EDL, soleus and gastrocnemius muscles of the rats in the fed and 48-h FD groups. The bands were quantified using densitometry to indicate MyHC isoform protein level. The composition of MyHC isoforms clearly differed among the muscles. The EDL was composed of mainly MyHC2B, 2X, and 2A, whereas the soleus was composed of exclusively MyHC1. The gastrocnemius composition was similar to EDL; however, it contained MyHC1 at a visible level. The mean percentage MyHC composition of EDL, soleus and gastrocnemius was not affected by 48-h food deprivation.

The mRNA expression level of the MyHC isoforms largely corresponded to the MyHC isoform composition of the EDL or soleus muscles (Fig. 2); mRNA of MyHC2B, 2X, and 2A were much higher than that of MyHC1 in the EDL, whereas the soleus showed exclusively MyHC1 mRNA expression. The 48-h food deprivation significantly increased MyHC2B mRNA expression in EDL.

Transcript levels of fiber type-related genes

To further reveal the effects of 48-h food deprivation on muscle fiber type, we used quantitative RT-PCR to analyze the expression of genes relating to energy
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metabolism (myoglobin, UCP3, LPL); mitochondria (porin); and muscle fiber type regulation [peroxisome proliferator-activated receptor (PPAR) δ, PPARγ coactivator 1α (PGC1α), and Forkhead box protein O1 (FOXO1)] in the EDL and soleus (Fig. 3). In 48-h FD rats, the transcript levels of UCP3 and LPL were significantly increased in both the EDL and soleus. Unexpectedly, the transcript level of PGC1α in 48-h FD rats was significantly decreased in both the EDL and soleus, and the levels of myoglobin and PPARδ were significantly decreased in the soleus.

**Protein levels of fiber type related genes**

To verify the results of mRNA expressions, we measured protein expression of myoglobin, UCP3, PGC1α (Fig. 4), and FOXO1 (Fig. 5). We found a drastic decrease in the PGC1α of 48-h food deprived rats soleus in accordance with the significant mRNA decrease. We also found an increase of mean values in UCP3 protein in 48-h food deprived rats, though the difference was not statistically significant. The myoglobin protein level was similar between fed and 48-h food deprived rats. Then, we examined the total and phosphorylation levels of FOXO1. Compared to ad libitum fed rats, 48-h food deprivation attenuated the phosphorylation levels of FOXO1 in both the EDL and soleus (Fig. 5). The 48-h food deprivation showed no substantial effect on the amount of total FOXO1 protein.

**DISCUSSION**

The important finding in this study is that 48-h food deprivation did not alter the MyHC composition of the EDL, soleus or gastrocnemius muscle in rats at the protein level. More severe muscle atrophy is observed in fast-twitch muscle with fasting ([12], [13]). Goodman et al. reported that 48-h food deprivation induced a greater decrease in protein synthesis in fast-type 2X and 2B fibers compared to slow-type 1 and 2A fibers in mice ([14]). Furthermore, LC3-II, a marker protein for macroautophagy, was expressed at a notably higher level in fast-type muscle (plantaris) than in slow-type muscle (soleus) ([15]). These studies suggest the preferential atrophy of fast-type muscle in food deprivation is caused by attenuated protein synthesis and promoted protein degradation in fast-twitch fibers. Similarly, we found a smaller decrease in the weight of the slow-type soleus than in the fast-type gastrocnemius and EDL (Table 2). First, we hypothesized that the percentage of slow-type fibers would increase while fast-type MyHC decreased; however, our results showed no change in MyHC isoform composition after 48-h food deprivation. The upregulation of MyHC2B expression might negate the effect of increased degradation of MyHC2B isoforms in EDL muscle and result in keeping MyHC composition at the same level.

It is interesting to anticipate muscle fiber type change in the case of food deprivation for more than 48-h. Lewis and Sieck found that food deprivation for 90-h resulted in no differences in diaphragm cross-sectional areas for either type 1 or 2 muscle fibers in the control and food-deprived rats ([19]). The longer food deprivation for 4.5 d
(108-h) resulted in a decrease in the cross-sectional area of type 2 fibers in rats (20). There is a report that using severe food-restricted rats (~25% of the average food intake of control) for 6 wk induced significant reduction of the cross sectional area of type 2B fibers, resulting in an increase of composition of type 1 and 2A (21). Thus, long-time food deprivation or food restriction is expected to induce an obvious increase in slow or intermediate-type MyHC composition and decrease in fast-type MyHC composition by atrophy of type 2B fibers.

UCP3 and LPL expressions were drastically increased in both the EDL and soleus. UCP3 is expressed mainly in the skeletal muscle, and is thought to play an important role in thermogenesis in larger mammals, including humans. The UCP3 mRNA shows a significant positive correlation with the percentage of type 1 fibers in healthy adult humans (22). Meanwhile, LPL catalyzes the hydrolysis of triglycerides in circulating chylomi-
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Fig. 4. Effect of 48-h food deprivation on the expression of myoglobin, UCP3, PGC1α proteins in the extensor digitorum longus (EDL) and soleus (Sol) muscles of rats. The blots show the respective proteins developed on X-ray films for all rats, with densitometry quantification in a lower panel. Each protein was normalized to β-actin. Values are expressed as fold change compared with the fed group. Values are the mean±SE for 6 rats. **p<0.01, compared with fed rats by unpaired Student’s t-test. 48-h FD, 48-h food deprivation.

Fig. 5. Effect of 48-h food deprivation on the expression of phosphorylated FOXO1 (pFOXO1) and total FOXO1 proteins in the extensor digitorum longus (EDL) and soleus (Sol) muscles of rats. The blots show the respective proteins developed on X-ray films for all rats, with densitometry quantification in a lower panel. The pFOXO1 was normalized to the total FOXO1 protein. The total FOXO1 was normalized to β-actin. Values are expressed as fold change compared with the fed group. Values are the mean±SE for 6 rats. *p<0.05, **p<0.01, compared with fed rats by unpaired Student’s t-test. 48-h FD, 48-h food deprivation.
rons and very low density lipoproteins, representing the rate-limiting step in the utilization of triglyceride-derived fatty acids (10). LPL activity in slow fibers was approximately 14- to 20-fold higher than that in fast fibers (23). Our result is supported by the report by Ladu et al., showing that 1 d of fasting significantly increased LPL mRNA levels in the soleus and red and white portions of the vastus lateralis muscle in rats (24).

We found that fast-type MyHC2B mRNA was upregulated in conjunction with a decrease in PPARδ, PGC1α, and myoglobin expression. The content of PPARδ, PGC1α (25, 26), and myoglobin (27) is lower in fast fiber type muscle. In contrast, the mRNA expression levels of UCP3 and LPL were upregulated. Thus, our results showed some discrepancy because it is well recognized that factors related to fiber type are regulated in parallel, i.e., toward fast-type or slow-type in both metabolic and contractile parameters. However, it seems that 48-h food deprivation caused a change in MyHC2B, myoglobin, PPARδ, and PGC1α expression levels toward fast-type and a change in metabolism-related factors such as UCP3 and LPL toward slow-type. One potential explanation for this observation is the activation of transcription factors such as FOXO1 and PPARδ.

The transgenic mice specifically over-expressing FOXO1 in the skeletal muscle have decreased expression of many genes related to the structural proteins of type 1 fibers (28). Thus, FOXO1 is assumed to be a factor that induces slow-to-fast type transition. It is reported that food deprivation increases FOXO1 gene expression in the skeletal muscle (quadriiceps) of mice (8), although FOXO1 was not increased at either mRNA or protein level in our study. FOXO1 activity is regulated by post-translational modifications that affect primarily its subcellular localization (29). Insulin and growth factor signaling inhibit FOXO1 via Akt-dependent phosphorylation and nuclear exclusion (30), indicating that a decrease in phosphorylated FOXO1 means its activation. Our results showed 48-h food deprivation activated FOXO1 in both EDL and soleus muscle and might contribute to the upregulation of fast-type MyHC2B genes in EDL. FOXO1 also recruits the fatty acid translocase FAT/CD36 to the plasma membrane, leading to increased fatty acid uptake and oxidation in C2C12 myogenic cells (31). Moreover, ectopic expression of FOXO1 upregulates LPL expression in C2C12 cells (8). Therefore, FOXO1 seems a key factor to explain upregulation of fatty acid metabolism-relating factors and increase in fast-type MyHC2B isoforms. The reciprocal role of FOXO1 must be noted here. The conditional ablation of FOXO1 expression in the soleus muscle leads to reduced slow fiber and increased fast fiber formation, a phenotype that is similar to that seen with FOXO1 overexpression (32). It seems to be controversial how FOXO1 plays a critical role in MyHC isoform regulation.

Meanwhile, many previous reports indicate that PPARδ is a factor that induces a fast-to-slow type transition. PPARδ is a ligand-dependent nuclear receptor that belongs to the superfamily of nuclear transcription factors. Over-expression of muscle-specific PPARδ, either in the constitutively active form (26) or in the native form (33), increases the proportion of slow-oxidative fibers (i.e., slow MyHC isoforms). Fatty acids are natural endogenous ligands for the PPARs, and they function to mediate adaptive metabolic responses to changes in systemic fuel availability (34–38). During starvation, a condition that leads to elevated plasma fatty acid levels, various PPAR-responsive genes in the skeletal muscles are dramatically upregulated (39–42). In mouse skeletal muscle, PPARδ is believed to be the most abundant isoform among PPARα, δ, and γ (43–46). Therefore, it is possible that PPARδ was activated by increased fatty acids and upregulated metabolism-related genes such as UCP3 and LPL.

Surprisingly, expression of the mitochondria marker porin did not change with food deprivation in our experiments. Increased oxidative metabolism was expected in the 48-h FD rats because we observed an increase in UCP3 and LPL. Because the mitochondria are the site of oxidative phosphorylation and UCP3 is one of the proton transporters present on the inner mitochondrial membrane, we expected to see a change in the abundance of this organelle. It is possible, however, that the transcriptional regulation of mitochondria number is independent of the metabolic pathways studied here (47, 48).

In periods of limited food availability, there is central downregulation of the hypothalamic–pituitary–thyroid (HPT) axis. As the majority of the thyroid hormone T3 in rodents comes from the thyroid gland, it is thought that the serum thyroid hormone levels fall during fasting in humans (49) and rodents (50, 51). It was shown that the hypothyroid status, which was induced by a 0.05% solution of methimazole in drinking water for more than 3 mo, leads to a preferential expression of slow MyHC isoforms, while the contrary holds true for hyperthyroid rats (52). Interestingly, while fasting induces a hypothyroid status, the actual observed phenotype was an increase in fast-type MyHC2B, similar to a hyperthyroid status, in our experiment. This result suggests that other factors besides thyroid hormone play a more pivotal role in muscle MyHC isoform regulation.

In conclusion, 48-h food deprivation did not change MyHC composition at the protein level in either slow- or fast-type muscle. However, 48-h food deprivation led to upregulation of fast-type MyHC2B mRNA in fast-type dominant muscle tissues. Interestingly, oxidative metabolism-related factors such as UCP3 and LPL were upregulated, suggesting that the modulation of MyHC isoform is not always in parallel with the regulation of metabolic genes.

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