Interorgan Signaling Between Adipose Tissue Metabolism and Skeletal Muscle Uncoupling Protein Homologs

Is There a Role for Circulating Free Fatty Acids?

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Uncoupling proteins 3 and 2 (UCP3 and UCP2) are two newly cloned genes that have been implicated in the regulation of lipids as fuel substrate in skeletal muscle on the basis that their mRNA expressions are upregulated during starvation (when fat stores are being rapidly mobilized) and downregulated during the early phase of refeeding (when fat stores are being rapidly replenished). To test the hypothesis that circulating free fatty acids (FFAs) may have a physiological role as an interorgan signal linking these dynamic changes in the fat stores to skeletal muscle expression of UCP3 and UCP2, the mRNA levels of these UCP homologs were examined in fed and fasted rats treated with the antilipolytic agent nicotinic acid. In 46-h fasted rats, we observed a threefold increase in serum FFA levels and increases in UCP3 and UCP2 mRNA levels that were more marked in the gastrocnemius and tibialis anterior muscles (predominantly fast-twitch fibers) than in the soleus muscle (predominantly slow-twitch fibers). Treatment with nicotinic acid blunted the fasting-induced increase in serum FFA levels and prevented the increase in mRNA levels of UCP3 and UCP2 in the soleus muscle, but had little or no effect on the elevated mRNA levels of these UCP homologs in the gastrocnemius and tibialis anterior muscles. Furthermore, treatment of ad libitum–fed animals with nicotinic acid resulted in a twofold reduction in serum FFA levels (i.e., by a magnitude similar to that observed during early refeeding) and significant reductions in UCP3 and UCP2 mRNA levels in the soleus muscle, but not in the gastrocnemius or tibialis anterior muscles. These results revealed a muscle-type dependency in the way UCP2 and UCP3 gene expression in skeletal muscle is regulated, and suggest that the hypothesis that circulating FFAs function as an interorgan signal between fat stores and skeletal muscle UCP3 and UCP2 gene expression is adequate only for slow-twitch (oxidative) muscles. Consequently, a signal(s) other than circulating FFAs must be implicated in the link between dynamic changes in body fat stores and UCP expression in predominantly fast-twitch (glycolytic/oxidative-glycolytic) muscles, which constitute the major fiber type of the total skeletal muscle mass and which have high susceptibility to developing insulin resistance and impairment in substrate utilization in metabolic diseases.
***Diabetes* 47:1693–1698, 1998**

Uncoupling proteins 3 and 2 (UCP3 and UCP2) are two recently cloned genes with high-sequence homology to UCP1, which plays a pivotal role in the control of thermogenesis in brown adipose tissue (BAT). Whereas UCP2 is ubiquitously expressed in all tissues/organs examined (1,2), UCP3 is highly expressed only in the skeletal muscles and in BAT (3–5). By analogy to UCP1 in BAT, a role for these UCP homologs in the control of thermogenesis in other organs and tissues was initially supported by the demonstration that both UCP3 and UCP2 lower mitochondrial membrane potential when transfected into yeast (1,2,6). However, subsequent experiments in laboratory rats (6,7) and humans (8) cast doubts about their role in the modulation of regulatory thermogenesis in skeletal muscle, since mRNA expression of both UCP2 and UCP3 in this tissue was found to be unexpectedly elevated during food deprivation, a well-established condition of whole body energy conservation (9), with skeletal muscle contributing importantly to this state of energy economy (10).

In contrast, there are converging lines of evidence from studies of starvation and refeeding suggesting that skeletal muscle UCP homologs are directly associated with lipid metabolism in this tissue, which in turn is intricately linked to the state of depletion or repletion of the adipose tissue fat stores. First, the upregulation of both UCP2 and UCP3 mRNA expression in skeletal muscle during food deprivation (6–8) parallels the increased mobilization of the fat stores and the well-known increased uptake/utilization of lipids by muscles under such starvation conditions. Second, the finding that the gene expression of these skeletal muscle UCP homologs switches from a state of upregulation to one of downregulation (below control levels) after transition from food deprivation to refeeding (11) is consistent with a role for these UCP homologs in the switching of muscle substrate metabolism from a state of enhanced lipid utilization during starvation (when glucose is limiting) to one of reduced lipid utilization during refeeding, when lipids need to be "spared" for depo-

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Received for publication 2 April 1998 and accepted in revised form 31 July 1998.

ANOVA, analysis of variance; BAT, brown adipose tissue; FFA, free fatty acid; FG, fast-twitch glycolytic; FOG, fast-twitch oxidative-glycolytic; IBAT, interscapular BAT; SO, slow-twitch oxidative; SSC, sodium chloride–sodium citrate; UCP, uncoupling protein.

ition during a phase of rapid replenishment of the fat stores 12–14). Third, the observation that these changes in UCP xpression were more pronounced in the gastrocnemius predominantly fast-twitch fibers) than in the soleus muscle predominantly slow-twitch fibers) during starvation and efeeding (11) could be explained by the greater dependency f slow-twitch (oxidative) muscles on lipids as fuel substrate, ven in the fed state, and the greater capacity of fast-twitch glycolytic/oxidative-glycolytic) muscles to shift between lucose and lipids as fuel substrate (15). During starvation, the nore marked upregulation of muscle UCP gene expression n the gastrocnemius than in the soleus would be in accordance with a much greater shift in fuel substrate from glucose oward lipids in glycolytic/oxidative-glycolytic muscles than n oxidative muscles. Similarly, during refeeding on a high-carohydrate, low-fat diet, the more marked downregulation of CP gene expression in the gastrocnemius than in the soleus would also be in line with the greater capacity of the glyolytic/oxidative-glycolytic muscles than the oxidative muscles to shift substrate utilization from lipids toward glucose n a phase of accelerated fat deposition. Taken together, nese studies of starvation/refeeding have led us to propose nat skeletal muscle UCP homologs may function as regulaors of lipids as fuel substrate rather than as mediators of reglatory thermogenesis (11).

Because the increases and decreases in skeletal muscle CP gene expression during starvation and early refeeding, espectively, parallel the changes in adipose tissue lipid nobilization and hence in the release of free fatty acids FFAs) into the circulation (11,16), the possibility arises that irculating FFAs, in addition to providing fuel substrate, may lso have a physiological role as an interorgan signal linking ne dynamic changes in adipose tissue fat stores to skeletal nuscle UCP3 and UCP2 gene expressions. To test this ypothesis of an interorgan signaling role for circulating FAs, we used nicotinic acid, an inhibitor of adipose tissue at mobilization (17, 18), to study the effects of *1*) preventing ne increase in FFAs during fasting and *2*) reducing serum FAs in the fed state (thereby mimicking the reduction in erum FFAs observed during early refeeding) on skeletal nuscle expression of UCP3 and UCP2. In view of muscle-type ifferences in the magnitude of changes in these UCP omologs' expression during starvation and refeeding (11), ve investigated the extent to which such an interorgan sigaling role for circulating FFAs might be muscle-type dependent. To this end, UCP mRNA levels were measured in three keletal muscles known to differ in the relative proportions f slow-twitch oxidative (SO) fibers, fast-twitch glycolytic FG) fibers, and fast-twitch oxidative-glycolytic (FOG) fibers, 19–20): *1*) the soleus muscle, a slow-twitch muscle that consists predominantly of SO fibers (84%) and a small proportion 16%) of FOG fibers; *2*) the gastrocnemius muscle, a fastwitch muscle containing very few SO fibers (4%), but high roportions of FG (58%) and FOG (38%) fibers; and *3*) the tibalis anterior muscle, another fast-twitch muscle that also ontains few SO fibers (2%), but high proportions of FOG 56%) and FG (32%) fibers.

ESEARCH DESIGN AND METHODS

nimals and diets. In each experiment, male SD rats aged 6 weeks (Tierzucht, urich, Switzerland) were adapted to room and cage environments for 1 week: ey were caged singly in a temperature-controlled room (22°C) with a 12-h light/dark cycle. They were maintained on a commercial pelleted laboratory diet (Provimi-Lacta, Cossonay, Switzerland) consisting (by energy) of 24% protein, 66% carbohydrates, and 10% fat, and had free access to tap water. The various experiments were conducted after this 1-week period of adaptation in rats selected on the basis of body weight being within ± 5 g of the mean body weight (200 g). During fasting, all animals had access only to water containing 0.45% NaCl. Animals used in the present studies were maintained in accordance with our institute's regulations and guide for the care and use of laboratory animals.

Study design. The study was conducted in rats that were either fed ad libitum or fasted for 36 h, with subgroups (n = 5–6) receiving either sterile saline (pH 7.4) or the antilipolytic agent nicotinic acid (Fluka Biochemika, Buchs, Switzerland) administered at 100 mg/kg i.p., as described by Lowell and Goodman (21), namely every 2 h, corresponding to the 38th, 40th, 42nd, and 44th hour of fasting. All rats were killed by decapitation 2 h after the last injection (at 46 h of fasting). After blood was collected for the assays of serum FFAs and glucose, the muscles and interscapular BAT (IBAT) were rapidly dissected out, cleaned of tissue debris, immediately frozen in liquid nitrogen, and then stored at –80°C until later processing for assay of mRNA expression of the UCPs in all tissues.

Circulating metabolites. Serum FFAs were measured using an NEFA C kit (Wako Chemicals, Neuss, Germany), and serum glucose levels were determined using a Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA).

Extraction of total RNA and Northern blotting. Total RNA was isolated by the method of Chomczynski and Sacchi (22). Then 15 µg of each RNA sample was loaded onto a 1.2% formaldehyde gel, as described by Lehrach et al. (23), and electrophoresed overnight. After vacuum blotting of the gel onto a nylon membrane (Electran Nylon Blotting Membrane; BDH Laboratory Supplies, Poole, U.K.) at 60 mbar for 3 h, the RNAs were UV cross-linked onto the membrane. The coloration of the membrane with a solution containing 0.04% bromophenol blue/0.5 mol/l sodium acetate (pH 5.2) showed an equal loading.

Northern blot analysis. The UCP3 and UCP2 probes were obtained as described previously (4,7). Hybridizations were performed in QuickHyb solution (Stratagene, La Jolla, CA), with a probe random labeled with [α-^{32}P]dCTP and at a specific activity of 10^9 dpm/µg DNA. The membranes were then washed in 2 × SSC (20 × SSC is 3 mol/l sodium chloride, 0.3 mol/l sodium citrate [pH 7.0]), twice in 0.1% SDS for 5 min at 50°C, and finally once in 0.1 × SSC, 0.1% SDS at 50°C for 5 min. The blots were exposed to enhanced chemiluminescence films (Amersham, Bucks, U.K.). Equal loading and transfer were checked using an oligonucleotide specific for 18S RNA subunit labeled with [γ-^{32}P]ATP. The signals on the autoradiograms were quantified by scanning photodensitometry using ImageQuant Software Version 3.3 (Molecular Dynamics, Sunnyvale, CA). In the case of UCP3, two bands are found at the level of the UCP3 signal; the upper band is believed to represent a premessenger of that gene. However, since the quantification of each individual band indicated that they both change in the same direction, we chose to quantify the two bands together as an indicator of the level of UCP3 mRNA. To allow quantitative between-group comparisons for a given UCP in a given tissue, the samples for fed and fasted animals, whether treated with drug saline, were loaded on the same gel. It is to be noted, however, that neither between-tissue comparisons nor between-UCP comparisons in a given tissue are possible on an absolute basis because of loading limitations on a given gel and differences in the specific activity of labeled probes for the various UCPs. However, the relative changes of a given UCP across different muscles can be compared.

Data analysis and statistics. Data are presented as means ± SE, and were analyzed using a two-factor analysis of variance (ANOVA) for the main effects of group (fed versus fasted) and treatment condition (saline versus drug) as well as for the group × treatment condition interaction effect, using the computer software STATISTIX, version 4.0 (Analytical Software, St. Paul, MN).

RESULTS

Data on serum FFAs and glucose, as well as on UCP3 mRNA levels in the three muscles, are presented in Fig. 1. The results pertaining to mRNA expression for UCP3 and UCP2 in all three muscle types as well as in IBAT are presented in Table 1, together with the results of ANOVA, showing the F and P values for the main effects as well as the interaction effect. Representative Northern blots showing the levels of mRNA expression of skeletal muscle UCP3 and UCP2 homologs in fed and fasted rats in response to saline or drug treatment are shown in Fig. 2.

As shown in Fig. 1, a 46-h fast resulted in a decrease in serum glucose and a threefold increase in serum FFAs (group effect, $P < 0.001$), changes that were associated with increases in the UCP3 mRNA expression in all three muscles

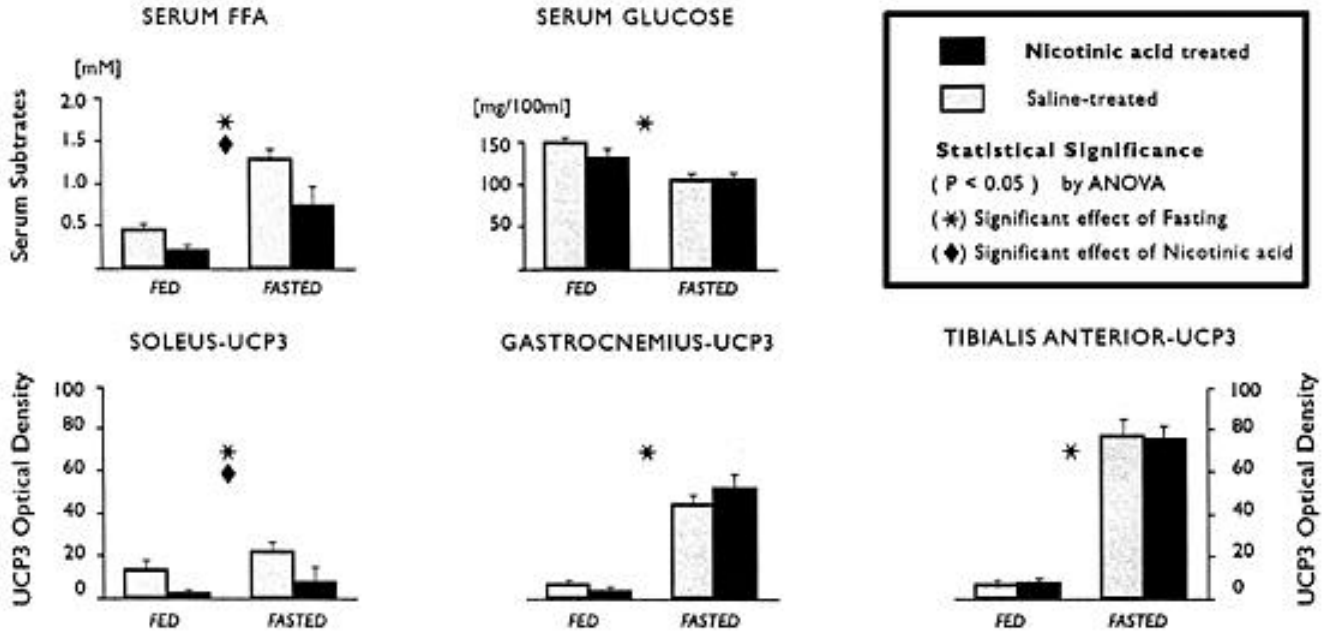


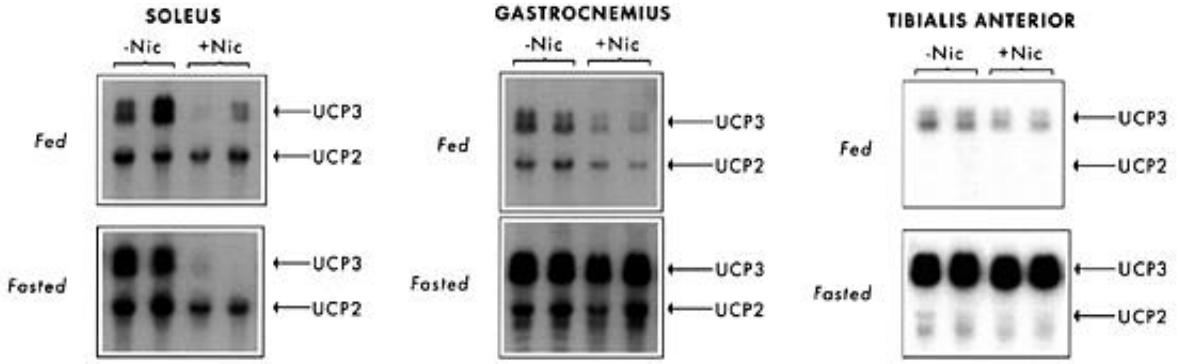
FIG. 1. Effect of nicotinic acid (antilipolytic agent) on serum substrates (FFAs and glucose) and mRNA expression of UCP3 in three skeletal muscles (soleus, gastrocnemius, tibialis anterior) of ad libitum fed and fasted rats. Data are means ± SE. The mean value of serum FFAs in the nicotinic acid–treated fasted group is not significantly different from that of the saline-treated fed group: the median values for serum FFA in these two groups were 0.47 and 0.52 mmol/l, respectively, about 33% of the value obtained in the saline-treated fasted group (1.4 mmol/l). Data for mRNA levels of UCP2 in skeletal muscles and in IBAT are shown with those for UCP3 (in skeletal muscles) and UCP1 (in IBAT) in Table 1.

studied. In line with our previously reported observations (11), the effect of 46-h fasting on UCP3 mRNA levels was more marked in the gastrocnemius and tibialis anterior muscles (6- to 12-fold increase; group effect, $P < 0.001$) than in the soleus muscle (twofold increase; $P < 0.05$). Treatment with nicotinic acid had no significant effect on serum glucose, but it markedly reduced serum FFA levels in both fed and fasted states (treatment effect, $P < 0.001$). In the fasted nicotinic acid–treated group, the magnitude of the reduction of serum FFAs was such that the apparent difference in mean values observed between this group and the fed (saline-treated) group was not statistically significant. Indeed, examination of individual data revealed that nicotinic acid was effective in completely preventing the fasting-induced surge in serum FFAs in most of the animals, with the median values for serum FFAs in the fasted nicotinic acid group and the fed saline-treated group being similar (0.47 vs. 0.52 mmol/l, respectively) and ~33% of the value found in the fasted saline-treated group (1.4 mmol/l). Examination of the data on skeletal muscle UCP3 mRNA levels in both fed and fasted states indicated that they were significantly reduced by nicotinic acid in the soleus muscle (treatment effect, $P <$

TABLE 1
Effect of the antilipolytic agent nicotinic acid on UCP mRNA expression in fed and fasted rats

	Fed		Fasted		ANOVA		
	Saline	Nicotinic acid	Saline	Nicotinic acid	Group	Treatment	Group × treatment
Soleus							
UCP3	12.5 ± 2.8	1.2 ± 0.6	22.8 ± 5.1	7.6 ± 6.7	<0.05 (4)	<0.01 (8.8)	NS (0.2)
UCP2	9.6 ± 1.5	4.6 ± 0.5	21.4 ± 2.3	11.2 ± 1.7	<0.001 (32.3)	<0.001 (22.2)	NS (2.5)
Gastrocnemius							
UCP3	6.6 ± 1.3	3.2 ± 0.9	44.1 ± 3.6	50.7 ± 7.6	<0.001 (98)	NS (0.2)	NS (1.4)
UCP2	3.7 ± 0.2	2.3 ± 0.3	17.1 ± 2.2	22.0 ± 5.0	<0.001 (36.5)	NS (0.4)	NS (1.3)
Tibialis anterior							
UCP3	6.2 ± 1.4	7.0 ± 1.8	77.9 ± 8.4	75.8 ± 5.9	<0.001 (190)	NS (0.01)	NS (0.08)
UCP2	4.9 ± 1.2	8.8 ± 1.4	72.7 ± 4.7	42.1 ± 5.8	<0.001 (173)	0.01 (12.2)	<0.001 (20.3)
IBAT							
UCP3	35.3 ± 4.0	25.3 ± 6.3	14.7 ± 7.4	5.5 ± 1.8	<0.01 (14.4)	0.09 (3.3)	NS (0.01)
UCP2	44.3 ± 4.7	32.4 ± 5.9	23.9 ± 6.4	16.4 ± 3.4	<0.01 (12.1)	0.08 (3.5)	NS (0.2)
UCP1	51.8 ± 4.3	42.5 ± 6.0	22.6 ± 3.9	11.9 ± 3.2	<0.001 (44.2)	<0.05 (4.9)	NS (0.02)

Data are means ± SE or P value (F value).



IG. 2. Northern blots of UCP2 and UCP3 mRNA expression in skeletal muscles (soleus, gastrocnemius, tibialis anterior) in fed and fasted ats treated with saline or nicotinic acid (Nic), an inhibitor of adipose tissue fat mobilization.

.01), but not in the gastrocnemius or tibialis anterior muscles. lthough the absolute mRNA level of UCP3 in the soleus nuscle of the fasted nicotinic acid group was similar to that ound in the fed control (saline-treated) group, the levels in ne gastrocnemius and tibialis anterior muscles remained as igh in the fasted nicotinic acid group as in the fasted saline roup, and hence severalfold higher than in the fed group.

Similar to the case with UCP3, changes in mRNA expression of UCP2 (Table 1) were found to be increased in all nuscle-types in response to fasting (group effect, $P < 0.001$); urther, the upregulation of these UCP homologs in skeletal nuscles contrasted with the decreases in all UCP mRNA xpression in IBAT of fasted rats (group effect, $P < 0.01$). In esponse to nicotinic acid, UCP2 mRNA levels were found to how the same pattern of changes as was observed for UCP3 n the soleus muscle (i.e., reduced expression; treatment ffect, $P < 0.01$) and gastrocnemius muscle (no significant ffect). In the tibialis anterior of fasted rats treated with nicotinic acid, UCP2 mRNA levels differed from those of UCP3 by eing significantly reduced (treatment or interaction effect, < 0.01). Nevertheless, mRNA levels of UCP2 in this muscle emained several times higher than in fed controls, indicating that nicotinic acid had only a minor effect on fasting-nduced upregulation of UCP expression in this muscle. In BAT, mRNA levels of all three UCPs were found to be educed by nicotinic acid in both fed and fasted rats, lthough these reductions were of borderline statistical significance for UCP1 and the UCP homologs (treatment effect, = 0.04–0.09).

ISCUSSION

he present work was initiated on the basis of our previous bservations that serum FFA levels and muscle UCP mRNA evels are elevated during starvation and reduced (below fed ontrol levels) on day 3 of refeeding (11). Because these hanges in serum FFA levels are the outcome of marked ncreases and decreases in the rate of FFA mobilization from dipose tissue during starvation and refeeding, respectively, is possible that, in addition to providing lipid substrate, circulating FFAs may also function as an interorgan signal etween adipose tissue fat metabolism and skeletal muscle ICP gene expression. By using nicotinic acid, a specific nhibitor of adipose tissue lipolysis (17,18) that reduces the ow of FFAs into the circulation, it was possible to gather evidence supporting the hypothesis that this signaling role for circulating FFAs is muscle-type dependent. First, the administration of nicotinic acid to fasted rats blunted the marked increase in serum FFAs and prevented the fasting-induced increases in UCP3 and UCP2 mRNA levels in the soleus (slow-twitch) muscle. By contrast, it had no effect on the fasting-induced increase in mRNA of both UCP homologs in the gastrocnemius (fast-twitch) muscle or on the increase in UCP3 mRNA levels in the tibialis anterior muscle (fast-twitch) muscle. Although UCP2 mRNA levels were significantly reduced in the tibialis anterior, this effect was quantitatively small, and the levels remained markedly elevated by more than eightfold in the fasted group treated with nicotinic acid relative to the saline-treated fed group. Second, the administration of nicotinic acid to ad libitum–fed animals also reduced serum FFAs (by a magnitude similar to that previously observed during early refeeding) and resulted in significant decreases in UCP3 and UCP2 mRNA levels in the soleus muscle, but not in the two fast-twitch muscles (gastrocnemius and tibialis anterior). Taken together, the present findings, based on the use of nicotinic acid to either prevent the elevation of circulating FFAs during fasting or mimic its reduction during early refeeding, support an interorgan signaling role for circulating FFAs in the regulation of the UCP3 and UCP2 expression in the predominantly slow-twitch muscles. By contrast, our data argue against any important interorgan signaling role of circulating FFAs in the regulation of these UCP homologs in predominantly fast-twitch muscles.

Significance of muscle-type differences in UCP regulation. It is of interest to draw a comparison between our present findings that the regulation of UCP gene expression in the soleus muscle is highly dependent on changes in circulating FFAs and the fact that even under normal fed conditions, such a predominantly slow-twitch muscle is more dependent on circulating lipids as fuel substrate than fast-twitch muscles (in which glucose is also an important fuel substrate). Under conditions of starvation and glucopenia, when substrate utilization in all muscle types shifts toward lipids, this shift from glucose toward lipids is likely to be much more striking in the fast-twitch (glycolytic/oxidative-glycolytic) muscles than in slow-twitch (oxidative) muscle. The data presented here showing that after transition from the fed to the fasted state, the increases in mRNA levels of UCP3 and UCP2 were much more marked in the two fast-twitch muscles (gastrocnemius and tibialis anterior) than in the slow-twitch soleus muscle are therefore consistent with the notion that these UCP homologs might function as regulators of lipids as fuel substrate. At the present state of knowledge, however, the

exact control points at which the UCP homologs operate in this regulation are unknown. Clues about whether they operate at the level of competition between lipid and carbohydrate utilization (Randle cycle), lipid transport systems across the cytoplasm, FFA carrier systems across the mitochondrial membrane, or at the level of mitochondrial or peroxisomal oxidation, will have to await the localization of their protein products within the cell. Similarly, the extent to which this high dependency of UCP expression in the soleus muscle on circulating FFAs might reflect a direct effect of intracellular FFAs or their metabolites (e.g., fatty acyl CoA) on the transcription of the UCP homologs is at present unknown. Studies addressing this issue of intracellular signaling of UCP transcription by FFAs and/or fatty acyl CoA would certainly be an interesting future line of investigation, particularly in light of recent findings (24), albeit in pancreatic islets, that the expression of genes encoding enzymes of FFA metabolism increases parallel to that of UCP2.

Further evidence for muscle-type differences in UCP regulation. The findings here of muscle-type dependency in the regulation of UCP3 and UCP2 expression in response to nicotinic acid–induced changes in circulating FFAs are in line with our previous findings that, beyond the early phase of refeeding, the subsequent restoration of serum FFA levels (to levels found in fed controls) paralleled the restoration of UCP homologs in the soleus muscle, but not in the gastrocnemius muscle, in which both UCP3 and UCP2 mRNA levels were found to remain downregulated even on day 10 of refeeding (11). Such dissociations between circulating FFA levels and the regulation of UCP gene expression in fast-twitch muscles, whether during refeeding or in response to nicotinic acid in fed and fasted states, might seem to conflict with the report by Weigle et al. (16), who showed that the elevation of circulating FFA levels by Intralipid plus heparin infusion in ad libitum–fed rats resulted in a threefold increase in UCP3 mRNA levels in the extensor digitorium longus (a fast-twitch muscle), changes that were similar to those found in the soleus (slow-twitch) muscle. However, because plasma FFA levels during this infusion study in fed animals were higher than those measured during fasting, the possibility arises that these results may reflect nonphysiological effects of circulating FFAs.

Regulation of UCP homologs in BAT. The data presented here also confirm previous demonstrations that in response to food deprivation, the upregulation of UCP3 and UCP2 gene expression in skeletal muscles co-exists with their marked downregulation in IBAT (11). Indeed, mRNA levels of these UCP homologs in IBAT were found to be reduced to about the same extent (~50%) as that for UCP1, the established functional uncoupler. Whereas the reduction in UCP1 gene expression is known to result primarily from diminished sympathetic neural activity in IBAT, the equally well-known role of FFAs as physiological regulators of UCP1 (25–28) (albeit secondary to the sympathetic nervous system) was also evidenced here by the finding that, in response to the reduction in serum FFA induced by nicotinic acid, UCP1 gene expression was also reduced. Furthermore, our finding that changes in the expression of the UCP homologs occurred in parallel to that of UCP1, whether in response to fasting per se or to nicotinic acid, provided additional support for our contention (11) that in IBAT, the regulation of UCP homologs are subject to the same regulation as UCP1 during starvation and refeeding.

In conclusion, the present study confirms our previous report of muscle-type dependency in the regulation of UCP3 and UCP2 gene expression (11), with fasting-induced increases in the expression of these UCP homologs being more striking in the fast-twitch (glycolytic/oxidative-glycolytic) muscles than in the slow-twitch (oxidative) muscle. Second, it suggests that the hypothesis that circulating FFAs may function as a circulating signal in the link between the dynamic changes in adipose tissue fat stores and skeletal muscle UCP3 and UCP2 gene expression is adequate only for slow-twitch muscles. Consequently a signal(s) other than circulating FFAs must be implicated in the link between the dynamic changes in adipose tissue fat stores and UCP gene expression in the predominantly fast-twitch muscles, which constitute the major muscle-type of the total skeletal muscle mass in rodents and humans (15,19,20). On the basis of our previous studies (11) showing that the UCP homologs in skeletal muscles are switched from a state of upregulation during starvation to one of downregulation during refeeding, such a candidate signal(s) or modulator(s) must operate in a way that would be compatible with a "switch" in the pattern of UCP3 and UCP2 gene expression in response to such dietary manipulation. Presumably, this would exclude known endocrine adaptations to starvation, a contention that would be in line with recent reports that leptin (16), glucocorticoids, (16), and insulin (8) cannot be implicated in fasting-induced upregulation of skeletal muscle UCP homologs. Similarly, a role for the sympathetic nervous system in the regulation of skeletal muscle UCP homologs seems unlikely, since studies using radiolabeled techniques to assess tissue norepinephrine turnover rates have indicated that sympathetic neural activity in both slow-twitch and fast-twitch skeletal muscles is unresponsive to fasting (29). Given the likely importance of skeletal muscle heterogeneity vis-à-vis fuel metabolism in the normal physiological response to starvation and refeeding, and the greater susceptibility of fast-twitch muscles to developing insulin resistance in metabolic diseases, such as those associated with obesity (30), further studies are warranted to explore the precise function of muscle UCP homologs and the mechanisms by which they may regulate lipids as fuel substrate in different muscle types.

ACKNOWLEDGMENTS

This work was supported by grant no. 31-47211.96 of the Swiss National Science Foundation.

We are grateful to Claudette Duret for excellent technical assistance, and to Raynald Schumacher and Florence Strub for the illustrations. We thank Dr. Tiziano Binzoni for stimulating discussion on skeletal muscle physiology.

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Author Queries (please see Q in margin and underlined text)

Q1: AU: Rewording of sentence beginning “By using nicotinic acid” okay?
Q2: AU: Edits to sentence beginning “Presumably” okay?>

Ref. 5: Please provide initial(s) for Nakao.>