Rapid Publication

Post-Starvation Gene Expression of Skeletal Muscle Uncoupling Protein 2 and Uncoupling Protein 3 in Response to Dietary Fat Levels and Fatty Acid Composition

A Link With Insulin Resistance

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***UCP2* and *UCP3* are two recently cloned genes with high sequence homology to the gene for uncoupling protein (UCP)-1, which regulates thermogenesis in brown adipose tissue. In the context of the current debate about whether UCP2 and UCP3 in the skeletal muscle may also function as mediators of thermogenesis or as regulators of lipids as fuel substrate, we have examined their mRNA expressions in rat gastrocnemius muscle in response to dietary manipulations known to differentially affect thermogenesis during the phase of weight recovery after starvation. Compared with ad libitum–fed control rats, the refeeding of isocaloric amounts of a low-fat (high-carbohydrate) diet resulted in lower energy expenditure and lower mRNA levels of muscle UCP2 and UCP3. This downregulation of UCP homologs was abolished by the refeeding of a high-fat diet, even though energy expenditure was significantly lower during refeeding on the high-fat than on the low-fat diet. Furthermore, major alterations in the fatty acid composition of the refeeding diet in favor of n-6 polyunsaturated or medium-chain fatty acids resulted in significant increases in energy expenditure, but with no significant changes in the expression of skeletal muscle UCP homologs. Regression analysis of gastrocnemius UCP mRNA levels against parameters that included body composition, energy expenditure, and plasma levels of free fatty acids (FFAs), insulin, and glucose as well as the increase in plasma glucose after a glucose load, revealed that only the latter (an index of insulin resistance) could explain the variability in muscle UCP2 and UCP3 mRNA expressions (r = 0.41, P < 0.02; r = 0.45, P < 0.01, respectively). Taken together, these data are at variance with a role for skeletal muscle UCP2 and UCP3 in dietary regulation (or modulation) of thermogenesis. However, they are consistent with the notion that these UCP homologs may function as regulators of lipids as fuel substrate and raise the possibility that high-fat induced upregulation of muscle UCP2 and UCP3 may be more closely linked to insulin resistance than to changes in circulating FFAs. *Diabetes* 48:436–441, 1999**

Current interests in the molecular mechanisms underlying skeletal muscle thermogenesis focus on *UCP2* and *UCP3* (1–8), two recently cloned genes with high sequence homology to the gene for uncoupling protein (UCP)-1, the uncoupling protein that plays a pivotal role in the control of thermogenesis in brown adipose tissue (BAT). By analogy to UCP1 in BAT, a role for these UCP homologs in the control of thermogenesis in skeletal muscle is supported by the reports that both UCP2 and UCP3 lower mitochondrial membrane potential when transfected into yeast (1,2,6). However, there are doubts concerning their physiological importance in the mediation of regulatory thermogenesis. First, neither the mRNA expression of UCP2 nor that of UCP3 in the skeletal muscle was found to be altered under conditions of increased thermoregulatory thermogenesis following acute or chronic exposure to cold (4,7). Second, the mRNA expressions of UCP2 and UCP3 in the skeletal muscle were found to be elevated during starvation-induced weight loss (6–8), a well-established condition of suppressed thermogenesis (9), with an important contribution by the skeletal muscle to this state of energy conservation (10). Third, in studies of fasting conducted at thermoneutrality, to prevent the increase in thermoregulatory needs consequential to food deprivation and weight loss, the gene expressions of muscle UCP homologs remained upregulated (11). Taken together, these results are at variance with a role for skeletal muscle UCPs in the mediation of thermoregulatory thermogenesis or in dietary regulation of thermogenesis.

By contrast, the pattern of changes in skeletal muscle UCP homologs during starvation and refeeding can directly be associated with changes in lipid metabolism in this tissue. This has led to an alternative hypothesis that centers on a primary role for skeletal muscle UCP2 and UCP3 in regulating lipids as fuel substrate (11). First, the upregulation of muscle UCP homologs during food deprivation occurs in parallel to the well-known shift in muscle substrate utilization (from a

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ANOVA, analysis of variance; BAT, brown adipose tissue; FFA, free fatty acid; SSC, sodium chloride–sodium citrate; UCP, uncoupling protein.

mixture of glucose and lipids) primarily toward lipids under such starvation conditions. Second, on transition from starvation to refeeding on a low-fat (high-carbohydrate) diet, the gene expressions of these skeletal muscle UCP homologs were found to be altered from a state of upregulation to one of downregulation below control levels. These findings have been interpreted (11) as being 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 as fuel during refeeding (on a low-fat diet) when lipids need to be spared for deposition during a phase of increased metabolic efficiency and accelerated fat deposition (12–14).

To explore further these two hypotheses about the functional role of muscle UCP2 and UCP3 (as mediators of thermogenesis or as regulators of lipids as fuel substrate), we have conducted two studies that examined the transcriptional levels of both genes in the gastrocnemius muscle in response to dietary manipulations known to differentially affect thermogenesis during weight recovery after starvation. The first study assessed the extent to which the downregulation of muscle UCP homologs during early refeeding on a low-fat (high-carbohydrate) diet would persist during isocaloric consumption of a high-fat diet (i.e., under conditions whereby the necessity for the sparing of lipids as fuel is obviated) and when thermogenesis was shown to be lower than during refeeding on a low-fat diet (15). A second study examined UCP2 and UCP3 mRNA expressions in the gastrocnemius muscle obtained from a previously reported study (16) in which rats refed isocalorically high-fat diets varying in fatty acid composition showed differential body fat gain, energy expenditure, plasma lipids, and insulin and glucose tolerance.

RESEARCH DESIGN AND METHODS

General study design. Five-week-old male Sprague-Dawley rats (Zurich, Tierzucht, Switzerland) were adapted to room and cage environments for 2 weeks. They were caged singly in a temperature-controlled room (22°C) with a 12-h light-dark cycle and were maintained on a commercial pelleted laboratory diet (Provimi-Lacta, Cossonay, Switzerland), consisting (by energy) of 28% protein, 60% carbohydrates, and 12% fat, and had free access to tap water. The experiments were conducted after this period of adaptation in rats selected on the basis of body weight within ±5 g of the mean body weight (230 g), and using a design similar to that previously described in establishing a rat model of weight recovery for studying adjustments in energy expenditure and energy partitioning (11,12). Groups of rats (n = 6) had food restrictions for 2 weeks at ~50% of the spontaneous food intake of ad libitum–fed rats (mean intake of 30 g daily) by providing them a fixed ration of 15 g of laboratory diet daily. At the end of this food restriction period, the animals were divided into groups (n = 5–6) of similar mean body weight. One group of rats was killed (and retained for analysis of body energy content and body composition), and the animals in the other groups were refed isocaloric amounts of the test diets for periods of either 5 or 14 days. Details about the diet preparations, diet composition, and the measurements of metabolizable energy intake have been reported previously (16). However, it should be noted that the contributions of protein, carbohydrate, and fat to energy intake were 23, 71, and 6%, respectively, in the low-fat diet, compared with 23, 24, and 53%, respectively, in the high-fat diets. 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 1: Influence of dietary fat levels. In the first study, two groups of food-restricted rats were refed with either the low-fat diet or the high-fat diet (in which a 1:1 mixture of lard and corn oil contributed to either 5 or 53% of energy intake, respectively) and at levels equal in metabolizable energy content to the spontaneous food intake of rats matched for weight at the onset of being refed the low-fat diet and fed the low-fat diet. After 2 weeks of such calorie-controlled refeeding, all the animals were killed and their carcasses analyzed for body energy and fat content. In a parallel experiment, identical in design, rats in the three groups (n = 6) were killed by decapitation on day 5 of being refed. After blood was collected for the assays of plasma glucose and free fatty acids (FFAs), the gastrocnemius muscle was 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 UCP2 and UCP3.

Study 2: Influence of dietary fat types. The second study, in which groups of food-restricted rats were refed a high-fat diet varying in fatty acid composition, has been described in detail previously (16). In brief, seven groups (n = 6) of food-restricted rats were refed the low-fat or high-fat diets varying in fat types (with each fat type contributing 53% of energy intake), and at a level equal in metabolizable energy content to the ad libitum food intake of control rats of similar weight. After 2 weeks of calorie-controlled refeeding, all animals were killed and their carcasses analyzed for body energy and fat content. On day 10 of this refeeding study, food was removed early in the morning (7:00 A.M.). At 7–8 h later, blood was drained from the tail vein and immediately followed by an intraperitoneal injection of glucose (2 g/kg body wt). At intervals of 20 min for the next 2-h period, blood samples were taken from the tail vein and transferred on ice. The blood samples were then centrifuged, and the plasma frozen and stored at –20°C for later assays of plasma glucose; the glucose tolerance index was calculated from the area under the glucose response curve. From the blood sample collected before the glucose load, the plasma levels of insulin, triglycerides, and nonesterified fatty acids were also measured. Food was resumed later in the evening on day 10, and on day 14, all animals were killed. The gastrocnemius muscle was 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 UCP2 and UCP3. The remaining carcasses were analyzed for body energy and fat content.

Determination of body composition and energy balance. Triplicate samples of the homogenized dry carcass were analyzed for energy content by bomb calorimetry (17) and for fat content by the Soxhlet extraction method (18). Body fat gain and energy gain during the 2 weeks of refeeding were obtained as the difference between the final and initial values (with the latter values estimated from values obtained from the group killed at the onset of refeeding). Energy balance was assessed, as previously described (16), by the comparative carcass technique over the 2-week period, during which the metabolizable energy intake was directly determined from the difference between the gross energy intake and the energy content of feces, urine, and spillage. Energy expenditure was determined as the difference between energy gain and the metabolizable energy intake over the 2-week period in each study.

Extraction of total RNA and Northern blot analysis. Total RNA was isolated by the method of Chomczynski and Sacci (19), and 15 µg of each RNA sample was loaded onto a 1.2% formaldehyde gel as described by Lehrach et al. (20) 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 ultraviolet (UV) cross-linked; the coloration of the membrane showed an equal loading. 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. The membranes were then washed twice in sodium chloride–sodium citrate (SSC) (2 × SSC) and 0.1% SDS for 5 min at 50°C, and finally once in 0.1 × SSC and 0.1% SDS at 50°C for 5 min. The blots were exposed to enhanced chemiluminescence films (Amersham, Bucks, U.K.). The signals on the autoradiograms were quantified by scanning photodensitometry using ImageQuant Software, version 3.3 (Molecular Dynamics, Sunnyvale, CA).

Determination of plasma glucose, insulin, and lipids. Plasma glucose was determined using a Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA). Plasma immunoreactive insulin level was measured by radioimmunoassay according to the method of Herbert et al. (21), and enzymatic colorimetric methods were used to measure the levels of plasma triglycerides and nonesterified fatty acids, as described previously (16).

Data analysis and statistics. Except where indicated, the data are presented as mean ± SE. The data were analyzed using a one-way analysis of variance (ANOVA), and post hoc comparisons between group pairs were made with the Neuman-Keuls multiple comparison test after ANOVA had established significant differences among the groups. These tests (in study 1 and 2), together with linear and stepwise regression analyses (in study 2), were performed using the computer software STATISTIX, version 4.0 (Analytical Software, St. Paul, MN).

RESULTS

Study 1: Influence of dietary fat levels. Figure 1 shows that compared with control animals fed the low-fat diet, the group refed isocaloric amounts of the same low-fat diet had markedly lower mRNA expression of UCP2 and UCP3 in the gastrocnemius muscle ($P < 0.001$) in a phase characterized by lower energy expenditure (–16%, $P < 0.001$) and by increased rate of body fat gain (approximately threefold increase, $P <$

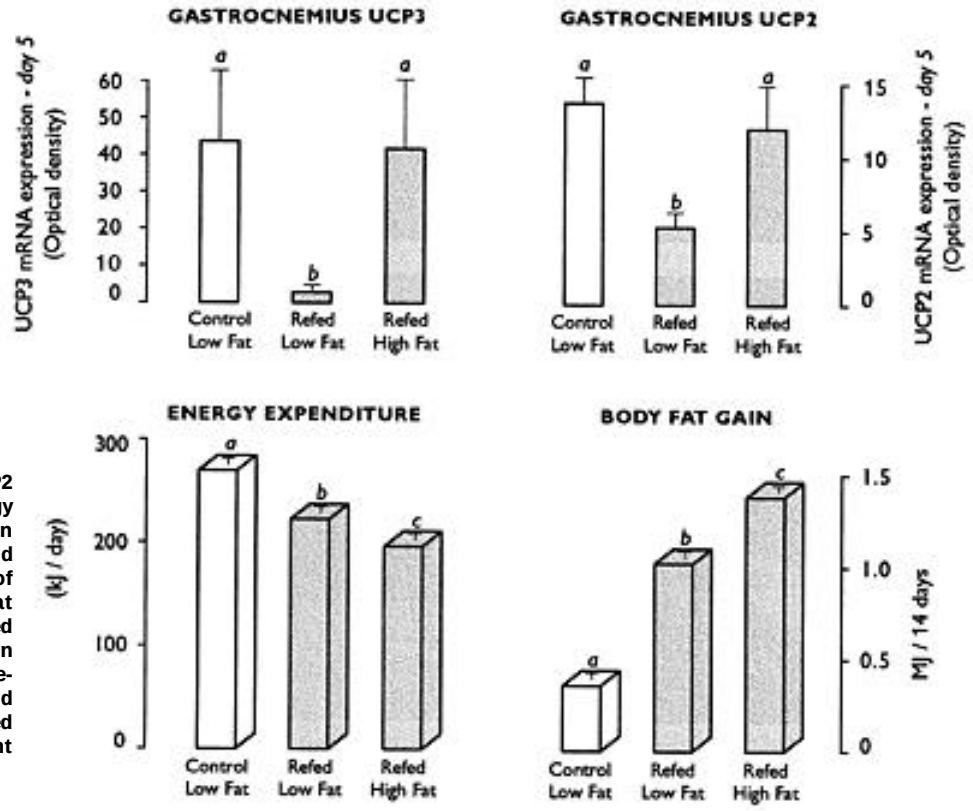

FIG. 1. Gastrocnemius muscle UCP2 and UCP3 mRNA expressions, energy expenditure, and body fat gain in control animals fed a low-fat diet and in groups refed isocaloric amounts of the same low-fat diet or a high-fat diet (with 5 or 53% of energy derived from a 1:1 mixture of lard and corn oil, respectively). All values are presented as mean ± SE (n = 6), and those sharing the same superscripted letter are not significantly different from each other.

0.001). Compared with the refed group on the low-fat diet, the group refed isocaloric amounts of the high-fat diet showed significantly higher muscle UCP2 and UCP3 mRNA levels, but lower energy expenditure (–12%) and increased body fat gain (+33%). In other words, the refeeding of the high-fat diet abolished the downregulation of muscle UCP2 and UCP3 mRNA expression observed during the refeeding of a low-fat diet, although this was accompanied by further reduction in energy expenditure and exacerbation of body fat gain. Determination of fasting plasma glucose, FFAs, and insulin on day 5 of refeeding indicates *1*) lower plasma glucose in the groups refed either the low-fat diet (124 ± 4 mg/100 ml) or high-fat diet (130 ± 4 mg/100 ml) than in the controls (144 ± 2; $P < 0.01$), *2*) higher plasma FFAs in the group refed the high-fat diet (0.45 ± 0.05 mmol/l, $P < 0.01$) than in the refed or control groups fed the low-fat diet (0.26 ± 0.02 and 0.26 ± 0.03, respectively), and *3*) no significant difference in plasma insulin across the three groups, namely, 48.1 ± 5.3, 45.1 ± 1.9, and 49.2 ± 5.9 μU/ml in the control, refed low-fat, and refed high-fat groups, respectively.

Study 2: Influence of dietary fat types. The effects of 2 weeks of refeeding to rats the isocaloric amounts of a low-fat diet and high-fat diets varying in fat types on gastrocnemius UCP2 and UCP3 mRNA levels are presented in Fig. 2. Although muscle UCP mRNA expressions in the high-fat lard group tended to be higher than in the low-fat group, no significant differences could be detected in the mean values nor in the median values (horizontal lines) across the groups. This lack of effect of fat types on muscle UCP mRNA levels contrasts with the findings in this same study that suggest that diets high in coconut oil (rich in medium-chain triglycerides) or in safflower oil (rich in n-6 polyunsaturated fatty acids) resulted in lower energetic efficiency (16) (i.e., in higher rates of energy expenditure relative to the other fat types as shown in Fig. 2). However, it is noticeable that there is a large interindividual variability in muscle UCP2 or UCP3 within each diet type. In an attempt to find determinants of this variability, we have regressed them against parameters of energy balance and energy partitioning (total energy expenditure, final body fat, final body protein, final fat:protein ratio, fat gain, protein gain, ratio fat:protein gain) as well as against blood parameters measured on day 10 of refeeding, namely, fasting plasma glucose, insulin, insulin:glucose ratio, triglycerides, FFAs and the glucose tolerance (assessed as the area under the glucose response curve after the intraperitoneal glucose load). Of these, only the glucose tolerance data were found to be significantly associated with gastrocnemius UCP2 and UCP3 mRNA levels ($r = 0.41$, $P < 0.02$; and $r = 0.45$, $P < 0.01$), respectively (Fig. 3).

DISCUSSION

Difficulties in attributing a role for skeletal muscle UCP2 and UCP3 in dietary regulation of thermogenesis have arisen after the findings that during starvation-induced weight loss and subsequent weight recovery, two phases characterized by a continuum in an adaptive suppression of thermogenesis (12–14), mRNA levels change from a state of marked upregulation during starvation to one of downregulation below control levels during refeeding. By contrast, these data seem to be more 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 on a low-fat diet, when lipids need to be 'spared' for deposition during a phase of rapid replenishment of the fat stores (11). Because it costs less energy to store fat from dietary

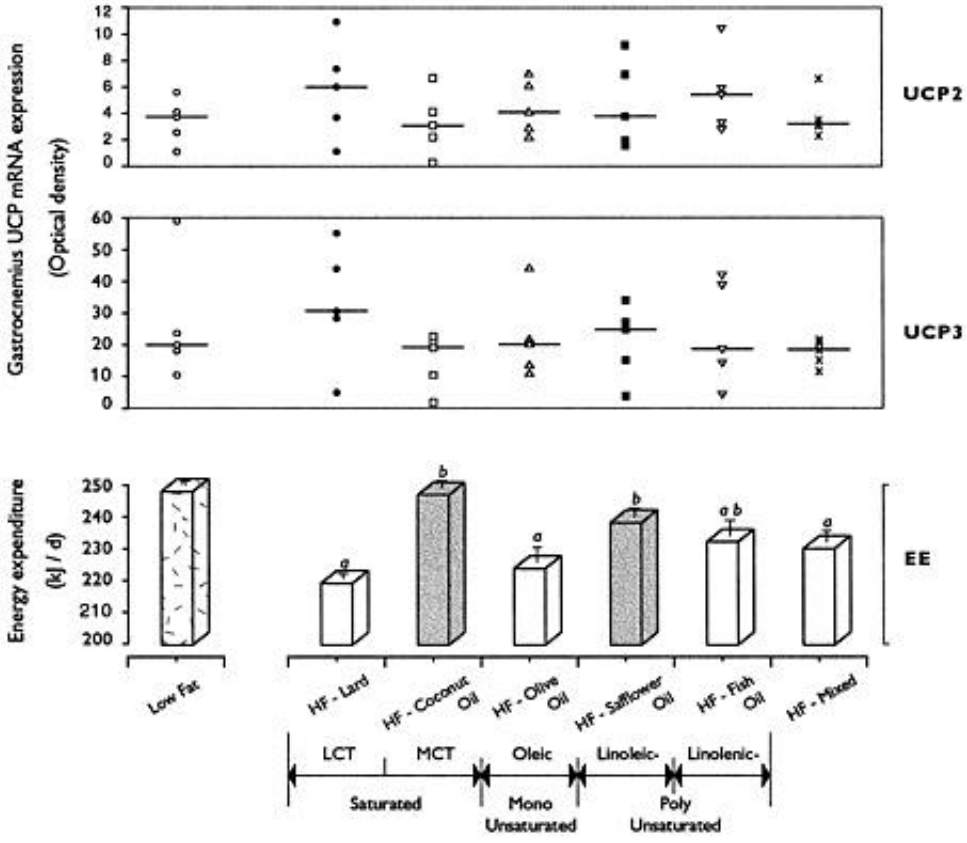

FIG. 2. Gastrocnemius muscle UCP2 and UCP3 mRNA expression and energy expenditure in response to refeeding for 2 weeks on a low-fat diet and on a high-fat diet in which 53% of the energy content was derived either from lard, coconut oil, olive oil, safflower oil, fish oil, or a mixture of all five fat types in equal parts (the high-fat–mixed diet). For muscle UCP2 mRNA levels, the individual values are provided within each diet group (n = 5), with the horizontal line passing through the median value. The data on energy expenditure are mean ± SE, and are calculated from previously published data on metabolizable energy intake and body energy gain in this study (16); values sharing the same superscript are not significantly different from each other.

lipids than to synthesize fat from carbohydrates via de novo lipogenesis, a shift in muscle substrate utilization in favor of glucose (hence sparing of dietary lipids as fuel) during consumption of a low-fat, high-carbohydrate diet would be an energetically more efficient way for depositing fat and would be in keeping with a role for these UCP homologs in the regulation of lipids as fuel substrate. Such a contention linking muscle UCP downregulation and lipid sparing is in line with our findings that suggest that the downregulation of muscle UCP2 and UCP3 mRNA expressions observed during refeeding on a low-fat diet is abolished by refeeding on a high-fat diet (i.e., when the availability of dietary lipids [for rapid fat replenishment] is no longer limited, and the need for de novo lipogenesis is obviated).

In the context of the current debate about the proposed functional role of *UCP2* and *UCP3* in the mediation of thermogenesis, it should be noted that the higher mRNA levels of muscle UCP homologs during early refeeding on the high-fat than on the low-fat diet contrast with the lower energy expenditure and diminished thermogenesis of the rats refed the high-fat diet (Fig. 1). Consequently, the early phase of weight recovery on a high-fat diet provides another situation, in addition to starvation-induced weight loss, when muscle UCP mRNA expressions are upregulated under conditions of energy conservation caused by suppressed thermogenesis. Further dissociation between dietary modulation of thermogenesis and muscle UCP expression can be derived from our present study involving isocaloric refeeding on high-fat diets with markedly different fatty acid composition. Whereas refeeding with high-fat diets high in coconut oil (rich in medium-chain triglycerides) or high in safflower oil (rich in n-6 polyunsaturated fatty acids) resulted in increased energy expenditure, neither UCP2 nor UCP3 mRNA levels in these two groups were found to differ from those rats refed the other high-fat diets. This dissociation between muscle UCP mRNA expression and energy expenditure is also observed in the lack of a significant correlation between muscle UCP2 or UCP3 mRNA and energy expenditure in a regression analysis of all diet groups pooled together.

Because both conditions of starvation and high-fat refeeding can result in elevated plasma FFA levels, the possibility arises for a cause-effect relationship between changes in circulating FFAs and UCP regulation. Although this notion may derive support from the demonstration that infusion of intralipids to elevate circulating FFA levels in normally fed rats resulted in marked upregulation of UCP3 in the hindlimb muscle (22), other lines of evidence suggest that muscle UCP regulation can be delineated from changes in circulating FFAs. First, the administration of the antilipolytic agent nicotinic acid to prevent the surge in circulating FFAs during fasting failed to prevent the marked upregulation of UCP2 and UCP3 mRNA expression in the gastrocnemius and tibialis anterior muscles (23). Second, our recent study examining the pattern of changes in muscle UCP gene expressions and circulating FFAs at various time points during the course of weight recovery indicated that beyond the first few days of refeeding on a low-fat diet, the subsequent restoration of serum FFA levels to similar levels as in controls was not accompanied by a restoration of mRNA of UCP homologs in the gastrocnemius (11). These remained significantly downregulated even on day 10 of refeeding. Similarly, as shown here on day 5 of refeeding, the markedly lower mRNA levels of UCP2 and UCP3 in the gastrocnemius muscle in the low-fat refed group than in the control group occurred in the

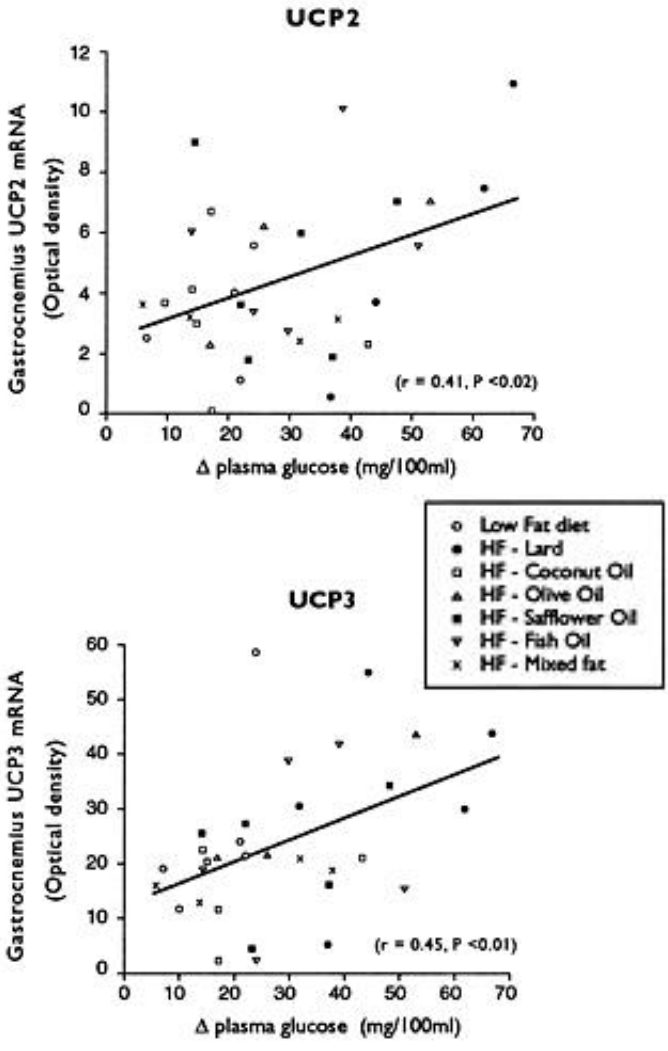

FIG. 3. Regression plot between the gastrocnemius muscle UCP2 or UCP3 mRNA levels against glucose tolerance (increase in plasma glucose over 2-h after a glucose load) in rats fed on a low-fat diet and on high-fat diets varying in fat types.

absence of differences in their plasma FFA levels. Third, although refeeding with high-fat diets varying in fat types resulted in marked differences in plasma FFA levels (16), no significant correlation is observed here between muscle UCPs and plasma FFAs.

By contrast, regression analyses reveal that out of the parameters that included body composition, energy expenditure, fasting plasma FFAs, glucose, and insulin, as well as the glucose tolerance index, only the latter could predict variability in muscle UCP2 and UCP3 mRNA expression, thereby suggesting a link between muscle UCP homologs and the increase in plasma glucose after a glucose load (an index of insulin resistance) rather than in changes in circulating FFAs. Such a positive association between muscle UCP2 or UCP3 expressions and insulin resistance would also be consistent with a role for muscle UCP in the regulation of lipid as fuel substrate, because in the early stages in the development of muscle insulin resistance (without hyperglycemia), the consequential impairment in glucose availability to muscle cells is likely to result in compensatory increases in lipid utilization to meet the fuel needs of the tissue. However, caution is necessary in the interpretation of muscle UCP data in pathophysiological conditions characterized by insulin resistance, because the pattern of glucose and fat metabolism in the skeletal muscle can also be determined by other metabolic changes consequential to insulin resistance. For example, there is evidence that in patients with NIDDM, hyperglycemia may compensate for insulin resistance (24) or be associated with decreased rates of lipid oxidation by the skeletal muscle (24), and that even in NIDDM, the skeletal muscle of obese patients may retain a greater intrinsic preference for oxidizing carbohydrates (25). In this context, the results of two recent studies involving humans that indicated a higher respiratory quotient (indicative of reduced lipid oxidation) in obese diabetic patients (26) and a lower UCP3 mRNA level in the skeletal muscle of hyperglycemic NIDDM patients (27) may be interpreted as being consistent with a link between muscle UCP regulation and the utilization of lipids as fuel substrate.

In conclusion, the present studies examining post-starvation gene transcription of muscle UCP2 and UCP3 during refeeding on diets varying in fat levels and fat types raise the possibility that high-fat induced upregulation of muscle UCP2 and UCP3 may be more closely linked to the development of insulin resistance rather than to changes in circulating FFAs. These studies also provide further data that are at variance with a role for skeletal muscle UCP2 and UCP3 in dietary regulation (or modulation) of thermogenesis, but remain consistent with the hypothesis that these UCP homologs may function as regulators of lipids as fuel substrate (11). The recent association (in obese and diabetic humans) between polymorphisms in UCP3 with marked reduction in basal lipid oxidation and not with differences in resting metabolic rate (28) would also be consistent with this proposal of a physiological role for UCP3 in regulating lipids as fuel substrate rather than in regulating thermogenesis.

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REFERENCES

1. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillard F, Seldin MF, Surwit RS, Ricquier D, Warden CH: Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinaemia. *Nat Genet* 13:269–272, 1997
2. Gimeno RE, Dembski M, Weng X, Deng N, Shyjan AW, Gimeno CJ, Iris F, Ellis SJ, Woolf EA, Tartaglia LA: Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. *Diabetes* 46:900–906, 1997
3. Vidal-Puig A, Solanes G, Grujic D, Flier JS, Lowell BB: UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem Biophy Res Commun* 235:79–82, 1997
4. Boss O, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo AG, Seydoux J, Muzzin P, Giacobino JP: Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett* 408:39–42, 1997
5. Matsuda J, Hosoda K, Itoh H, Son C, Doi K, Tanaka T, Fukunaga Y, Inoue G, Nishimura H, Yoshimasa Y, Yamori Y, Nakao K: Cloning of rat uncoupling protein-3 and uncoupling protein-2 cDNAs: their gene expression in rats fed high-fat diet. *FEBS Lett* 418:200–204, 1997
6. Gong DW, He Y, Karas M, Reitman M: Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, β3-adrenergic agonists and leptin. *J Biol Chem* 272:24129–24132, 1997

7. Boss O, Samec S, Dulloo AG, Seydoux J, Muzzin P, Giacobino JP: Tissue-dependent upregulation of rat uncoupling protein-2 expression in response to fasting or cold. *FEBS Lett* 412:111–114, 1997
8. Millet L, Vidal H, Andreelli F, Larrouy D, Riou JP, Ricquier D, Laville M, Langin D: Increased uncoupling protein-2 and –3 mRNA expression during fasting in obese and lean humans. *J Clin Invest* 100:2665–2670, 1997
9. Keesey RE: The relation between energy expenditure and the body weight setpoint: its significance to obesity. In *Handboook of Eating Disorders*. 2. Burrows GD, Beaumont PG, Casper RC, Eds. Amsterdam, Elsevier Science, 1988, p. 87–102
10. Ma SWY, Foster DO: Starvation-induced changes in metabolic rate, blood flow, and regional energy expenditure in rats. *Can J Physiol Pharmacol* 64:1252–1258, 1986
11. Samec S, Seydoux J, Dulloo AG: Role of UCP homologues in skeletal muscles and brown adipose tissue: mediators of thermogenesis or regulators of lipids as fuel substrate? *FASEB J* 12:715–724, 1998
12. Dulloo AG, Girardier L: Adaptive changes in energy expenditure during refeeding following low calorie intake: evidence for a specific metabolic component favouring fat storage. *Am J Clin Nutr* 52:415–420, 1990
13. Dulloo AG, Jacquet J: Adaptive reduction in basal metabolic rate in response to food deprivation in humans: a role for feedback signals from fat stores. *Am J Clin Nutr* 68:599–606, 1998
14. Dulloo AG: Human pattern of food intake and fuel-partitioning during weight recovery after starvation: a theory of autoregulation of body composition. *Proc Nutr Soc* 56:25–40, 1997
15. Dulloo AG, Girardier L: Influence of dietary composition on energy expenditure during weight recovery of body weight in the rat: implications for catch-up growth and obesity relapse. *Metabolism* 41:1336–1342, 1992
16. Dulloo AG, Mensi N, Seydoux J, Girardier L: Differential effects of high-fat diets varying in fatty acid composition on the efficiency of lean and fat tissue deposition during weight recovery after low food intake. *Metabolism* 44:273–279, 1990
17. Miller DS, Payne PR: A ballistic bomb calorimeter. *Br J Nutr* 13:501–508, 1959
18. Entenman C: General procedures for separating components of tissue. In *Methods in Enzymology*. Vol 3. Colowick SP, Kaplan O, Eds. New York, Academic, 1957, p. 299–317
19. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
20. Lehrach H, Diamond D, Wozney JM, Boedtker H: RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. *Biochemistry* 16:4743–4751, 1977
21. Herbert V, Lau KS, Gottlieb CW: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–1384, 1965
22. Weigle DS, Selfridge LE, Schwartz MW, Seeley RJ, Cummings DE, Havel PJ, Kuijper JL, BertrandelRio H: Elevated free fatty acids induce uncoupling protein 3 expression in muscle. *Diabetes* 47:298–302, 1998
23. Samec S, Seydoux J, Dulloo AG: Interorgan signaling between brown adipose tissue metabolism and skeletal muscle uncoupling protein homologs: is there a role for circulating free fatty acids? *Diabetes* 47:1693–1698, 1998
24. Kelley DE, Mandarino LJ: Hyperglycaemia normalizes insulin-stimulated skeletal muscle glucose oxidation and storage in non-insulin-dependent diabetes mellitus. *J Clin Invest* 86:1999–2007, 1990
25. Mandarino LJ, Consoli A, Jain A, Kelley DE: Interaction of carbohydrate and fat fuels in human skeletal muscle: impact of obesity and NIDDM. *Am J Physiol* 270:E463–E470, 1996
26. Wallner S, Smolle KH, Bahadori S, Wascher TC: Indirect calorimetry in overweight diabetic and nondiabetic subjects (Abstract). *Int J Obesity* 22 (Suppl. 3):S156, 1998
27. Krook A, Digby J, O'Rahilly S, Zierath JR, Wallberg-Henriksson H: Uncoupling protein 3 is reduced in skeletal muscle of NIDDM patients. *Diabetes* 47:1528–1531, 1998
28. Argyropoulos G, Brown AM, Willi SM, Zhu J, He Y, Reitman M, Gevao SM, Spruill I, Garvey WT: Effects of mutations in the human uncoupling protein 3 gene on the respiratory quotient and fat oxidation in severe obesity and type 2 diabetes. *J Clin Invest* 102:1345–1351, 1998