Lack of skeletal muscle uncoupling protein 2 and 3 mRNA induction during fasting in type-2 diabetic subjects

HUBERT VIDAL,1 DOMINIQUE LANGIN,2 FABRIZIO ANDREELLI,1,3 LAURENCE MILLET,2 DOMINIQUE LARROUY,2 AND MARTINE LAVILLE1,3
1Institut National de la Santé et de la Recherche Médicale Unit 449 and Centre de Recherche en Nutrition Humaine de Lyon, Faculté de Médecine René Laennec, 69372 Lyon; 2Institut National de la Santé et de la Recherche Médicale Unit 317, Institut Louis Bugnard, Université Paul Sabatier, Hôpital Rangueil, 31403 Toulouse; and 3Service d’Endocrinologie, Diabétologie et Nutrition, Hôpital E. Herriot, 69437 Lyon, France

Vidal, Hubert, Dominique Langin, Fabrizio Andreelli, Laurence Millet, Dominique Larrouy, and Martine Laville. Lack of skeletal muscle uncoupling protein 2 and 3 mRNA induction during fasting in type-2 diabetic subjects. Am. J. Physiol. 277 (Endocrinol. Metab. 40); E830–E837, 1999.—Skeletal muscle uncoupling protein 2 and 3 (UCP-2 and UCP-3) mRNA levels are increased during calorie restriction in lean and nondiabetic obese subjects. In this work, we have investigated the effect of a 5-day hypocaloric diet (1,045 kJ/day) on UCP-2 and UCP-3 gene expression in the skeletal muscle of type-2 diabetic obese patients. Before the diet, UCP-2 and UCP-3 mRNA levels were more abundant in diabetic than in nondiabetic obese subjects. The long (UCP-3L) and short (UCP-3S) forms of UCP-3 transcripts were expressed at similar levels in nondiabetic subjects, but UCP-3S transcripts were twofold more abundant than UCP-3L transcripts in the muscle of diabetic patients. Calorie restriction induced a two- to threefold increase in UCP-2 and UCP-3 mRNA levels in nondiabetic patients. No change was observed in type-2 diabetic patients. Variations in plasma nonesterified fatty acid level were positively correlated with changes in skeletal muscle UCP-3 (r = 0.6, P < 0.05) and adipose tissue hormone-sensitive lipase (r = 0.9, P < 0.001) mRNA levels. Lack of increase in plasma nonesterified fatty acid level and in hormone-sensitive lipase upregulation in diabetic patients during the diet strengthens the hypothesis that fatty acids are associated with the upregulation of uncoupling proteins during calorie restriction.

obesity; calorie restriction; nonesterified fatty acid; lipolysis

uncoupling proteins 2 and 3 (UCP-2 and UCP-3) are novel members of the mitochondrial carrier family that have been postulated to play a role in the control of energy expenditure (7, 16, 18, 19, 42). The two proteins show sequence identity with UCP-1, an uncoupling protein known to dissipate the mitochondrial proton gradient generated by the respiratory chain, producing heat instead of ATP (32). Like UCP-1, UCP-2 and UCP-3 are predicted to contain six transmembrane domains and mitochondrial carrier motifs, and their overexpression in yeast causes a decrease in mitochondrial membrane potential (16, 18, 19). UCP-1 is expressed in brown adipose tissue, a site of adaptive thermogenesis that plays an important role in the control of body weight and body temperature in rodents. Because there is little brown fat in adult humans, UCP-1 activity may not contribute to a large extent to energy expenditure. UCP-2 mRNA is present in many tissues, including adipose tissue and skeletal muscle (16, 18), whereas UCP-3 mRNA is preferentially expressed in skeletal muscle (7, 42). UCP-2 and UCP-3 are therefore candidates to explain the mitochondrial proton leak in tissues devoid of UCP-1. Two human UCP-3 transcripts have been characterized (7). They are produced from a single gene through alternative splicing and use of polyadenylation signals (37). The short-form transcript encodes a putative protein, designated UCP-3s, that does not contain the COOH-terminal region present in the long-form UCP-3 (UCP-3L) in UCP-2 and in UCP-1. Because this region is predicted to be critical for uncoupling activity, the activity of UCP-3s is likely to differ markedly from the activity of the other uncoupling proteins. Consequently, variation in the respective amounts of UCP-3L and UCP-3S could modulate skeletal muscle uncoupling activity.

Human UCP-2 and UCP-3 genes are located adjacent to one another on chromosome 11 (37). This location (11q13) is coincident with several independently mapped quantitative trait loci for obesity and hyperinsulinemia (16). Strong evidence of linkage was found between markers in the vicinity of the genes and resting metabolic rate adjusted for lean body mass (8). We have recently reported a positive correlation between adipose tissue UCP-2 mRNA levels and resting metabolic rate adjusted for lean body mass in obese women after diet standardization (5). In addition to a possible involvement in the control of energy expenditure, recent data support a role for skeletal muscle UCP-2 and UCP-3 in fuel partitioning. In humans, mutations in the UCP-3 gene are associated with decreased lipid oxidation and increased respiratory quotient in an African-American population with high prevalence for obesity (1). Moreover, modifications in skeletal muscle UCP-2 and UCP-3 expression in rodents parallel the switch from enhanced lipid utilization in muscle during fasting to reduced lipid utilization during refeeding, leading to the suggestion that the muscle uncoupling proteins may function as regulators of lipids as fuel substrate rather than as mediators of regulatory thermogenesis (33). It has been proposed that UCP-1 participates in the mitochondrial transport of fatty acid.
anions (17, 36). Whether UCP-2 and UCP-3 have such a role remains to be shown. Obesity and type-2 (non-insulin-dependent) diabetes mellitus are pathologies characterized by abnormalities of lipid metabolism, lipid utilization by skeletal muscle, and fuel partitioning associated with insulin resistance (13, 20, 21, 39). Therefore, altered regulation or expression of the uncoupling proteins might participate in the development of these pathologies. We have recently found that the expression levels of UCP-2, UCP-3, and UCP-3S mRNAs do not differ in vastus lateralis muscle from lean and obese Caucasian subjects (28, 29). Others have observed a 28% reduction in UCP-2 mRNA levels in muscle from morbidly obese subjects, without alteration in the total UCP-3 mRNA expression (30). To determine whether the regulation of UCP-2 and UCP-3 gene expression is altered during obesity, we investigated the effect of a 5-day severe calorie restriction in lean and obese individuals (28, 29). Dieting resulted in a two- to threefold induction of UCP-2, UCP-3, and UCP-3S mRNAs in skeletal muscle, and of UCP-2 mRNA in adipose tissue. However, no difference was observed between lean and obese subjects. Analysis of changes in metabolic parameters that occurred during fasting led us to propose that the increased nonesterified fatty acid (NEFA) levels in the two groups of subjects might contribute to the upregulation of UCP-2 and UCP-3 mRNA levels (28, 29). This hypothesis is now strongly supported by experiments in rodents showing that lipid infusion (44) induces modifications in UCP-2 and UCP-3 mRNA levels in rat skeletal muscle that parallel the changes in NEFA concentrations.

Type-2 diabetic patients are characterized by several alterations in lipid metabolism, particularly increased plasma levels and impaired clearance of NEFA (20, 39). In the postabsorptive state, utilization and oxidation of NEFA are reduced in the leg muscles of type-2 diabetic patients (21). Therefore, if lipid metabolism and uncoupling proteins are closely linked in muscle, an altered expression and/or regulation of the UCP-2 and/or UCP-3 might occur in the diabetic muscles. In the present study, we determined the mRNA levels of the three muscle uncoupling proteins in obese type-2 diabetic patients. In addition, to get more insight into the relationship between lipid metabolism and uncoupling protein expression, we investigated the regulation of UCP-2 and UCP-3 mRNAs during calorie restriction in a group of type-2 diabetic subjects. A group of obese nondiabetic patients that were matched for body mass index (BMI) with the type-2 diabetic subjects was studied as controls. To assess the possible relationship between the regulation of adipose tissue lipolysis and skeletal muscle uncoupling protein gene expression, we determined the mRNA levels of hormone-sensitive lipase (HSL), the rate-limiting enzyme of lipolysis.

SUBJECTS, MATERIALS, AND METHODS

Subjects. The subjects comprised eight obese type-2 diabetic patients (1 man and 7 women, age 51 ± 3 yr, BMI 31 ± 1 kg/m², blood type HbA1c, 10.7 ± 0.6%, duration of diabetes 6.3 ± 1.3 yr) and seven nondiabetic obese individuals (2 men and 5 women, age 43 ± 4 yr, BMI 35 ± 2 kg/m²). Subjects had maintained a stable body weight for 2 ± 0 mo before the beginning of the protocol. Diabetic patients had interrupted their usual treatment of oral antidiabetic drugs ≥1 wk before the beginning of the study. The obese subjects were not on regular medication and did not show known complications of obesity, such as established hypertension, diabetes, or dyslipidemia. All subjects were Caucasians. The subjects participated in a 7-day study protocol (41). During the first 2 days, the diet was standardized according to the body weight of the subjects (calorie intake of 104 kJ·kg⁻¹·day⁻¹). For the next 5 days, they received a 1,045 kJ/day diet (45% carbohydrate, 25% fat, and 30% protein). Biopsies of the vastus lateralis muscle and of subcutaneous abdominal adipose tissue were performed as previously reported (25, 41). The first biopsies were performed after an overnight fast before the beginning of the calorie restriction. The second biopsies were performed the morning of the 6th day of calorie restriction. Samples were immediately frozen in liquid nitrogen and stored at −180°C. Venous blood sampling was performed before the biopsies. The data from the diet-induced reduction of target mRNA expression in muscle from the nondiabetic obese subjects have been previously reported (28, 29). All subjects had given written consent, and the experimental protocols were approved by the ethics committee of Hospices Civils de Lyon.

Analytical procedures. Plasma glucose, insulin, triglyceride, NEFA, and β-hydroxybutyrate levels were determined using previously described methods (26). Plasma leptin levels were determined using a commercial kit (Linco Research).

Total RNA preparation. Total RNA from skeletal muscle was prepared using guanidinium thiocyanate-phenol-chloroform extraction (12). The yield of total RNA was 0.27 ± 0.02 µg/mg muscle tissue (wet weight) and was not significantly different in tissues from diabetic and nondiabetic subjects. The absorption ratios 260 to 280 nm were between 1.7 and 2.0. RNA integrity was verified on agarose gel electrophoresis with ethidium bromide staining. Total RNA was stored at −80°C until quantification of the target mRNAs.

Quantification of mRNAs. Human UCP-2 and UCP-3 mRNAs were quantified by RT-competitive PCR. Human UCP-2 mRNA levels were determined using a specific competitor DNA obtained by the deletion of 55 bp from a 290-nt-long UCP-2 cDNA fragment, as previously described (28). Quantitative RT-PCR was performed with 5′-GACCTATGACCTCATCAAGG-3′ as sense primer and 5′-ATAGGTTGACCGACATCACCAGG-3′ as antisense primer. UCP-3 (390 nt long) and UCP-3 (436 nt long) cDNA fragments were obtained by RT-PCR on human skeletal muscle total RNA using UCP-3S (5′-ATGGACGCTTACAGAACACC-3′) and antisense primer UCP-3 (5′-CTGGGCCACCATCTTTATCA-3′), respectively, as antisense primers. The two competitor DNAs were obtained by a deletion of 40 bp (29). Each competitor could be used to quantify total UCP-3 mRNA levels (i.e., levels of the long plus the short-form transcripts) by use of UCP-3S as sense primer and UCP-3AS (5′-CTGGGCCACCATCTTTATCA-3′) as antisense primer. Alternatively, another UCP-3 competitor DNA (28) can be used with UCP-3S and UCP-3AS primers to measure total UCP-3 mRNA levels. For the assay, the reverse transcription reaction was performed from 0.1 µg of skeletal muscle total RNA in the presence of a thermostable reverse transcriptase (Tth, Promega) by use of one of the specific antisense primers. The competitive PCR assays were performed as previously described (28, 29). HSL mRNA levels were determined in adipose tissue biopsies from 12 subjects.
obtained before and during calorie restriction (28, 41) by use of the competitor DNA described elsewhere (25). To improve the quantitation of the amplified products, fluorescent dye-labeled sense oligonucleotides were used. The PCR products were separated and analyzed on an ALFexpress DNA sequencer (Pharmacia) with the Fragment Manager software. The RT-competitive PCR assay used in this study allowed us to determine the absolute levels of target mRNAs expressed as attomoles per microgram (amol/µg) of total RNA. To accurately determine the effect of calorie restriction, total RNA preparations and RT-competitive PCR assays of the two skeletal muscle samples from the same individual (before and during fasting) were performed simultaneously.

Data analysis. Values are given as means ± SE. The nonparametric Wilcoxon test for paired values was used for comparisons before and during fasting. The nonparametric U Mann-Whitney test for unpaired values was used for comparisons between groups of subjects. Statistical calculations were performed using Statview (Abacus Concepts). P < 0.05 was the threshold of significance.

RESULTS

The nondiabetic obese and type-2 diabetic subjects did not show significant differences in metabolic and hormonal parameters before the diet, except glycemia (P < 0.01) and triglyceridemia (P < 0.05), which were lower, and leptinemia (P < 0.01), which was higher in nondiabetic subjects (Table 1). During the 5 days of calorie restriction, the nondiabetic and type-2 diabetic subjects lost 2.8 ± 0.5 and 2.4 ± 0.7 kg, respectively. The diet produced a significant decrease in basal glucose, triglyceride, and leptin plasma levels (P < 0.05). The decrease in insulinemia was significant in nondiabetic obese patients (P < 0.05). Plasma β-hydroxybutyrate level was increased in both groups (P < 0.05). Plasma NEFA level was increased in nondiabetic (P < 0.05) but not in type-2 diabetic patients, suggesting a lack of induction of lipolysis in diabetic subjects. Interestingly, adipose tissue HSL mRNA levels were increased during calorie restriction in nondiabetic (P < 0.05) but not in type-2 diabetic patients (Table 1).

The levels of UCP-2, UCP-3L, and UCP-3S mRNAs were first compared before the diet. Diabetic subjects showed higher UCP-2 (11 ± 2 vs. 5 ± 1 amol/µg total RNA, P < 0.01) and total UCP-3 (34 ± 3 vs. 9 ± 2 amol/µg total RNA, P < 0.01) mRNA levels than nondiabetic subjects. UCP-3S mRNA level was sevenfold higher in diabetic patients than in nondiabetic subjects (24 ± 2 vs. 3 ± 1 amol/µg total RNA, P < 0.005). A threefold difference was seen for UCP-3L mRNA levels (14 ± 2 and 4 ± 1 amol/µg total RNA in type-2 diabetic and nondiabetic subjects, P < 0.02). UCP-3L and UCP-3S mRNA levels were not significantly different in nondiabetic subjects, but UCP-3S transcripts were twofold more abundant than UCP-3L transcripts in skeletal muscle of diabetic patients (P = 0.05).

Uncoupling protein mRNA levels were next compared before and at the end of a 5-day calorie restriction period. The diet induced a 2.5-fold increase in UCP-2 (12 ± 2 vs. 5 ± 1 amol/µg total RNA, P < 0.02) and total UCP-3 (23 ± 4 vs. 9 ± 2 amol/µg total RNA, P < 0.02) mRNA levels in nondiabetic subjects (Fig. 1). No significant difference was observed in diabetic patients for UCP-2 (14 ± 2 vs. 11 ± 2 amol/µg total RNA) and UCP-3 (37 ± 4 vs. 34 ± 3 amol/µg total RNA) mRNA levels. UCP-3L and UCP-3S mRNA levels were measured in samples from seven nondiabetic and six type-2 diabetic patients (Fig. 2). As previously reported, calorie restriction induced UCP-2 and UCP-3 mRNA expression in nondiabetic subjects (28). There was no increase in the amounts of the transcripts during the diet in the diabetic subjects. The higher expression of UCP-3S mRNA compared with UCP-3L mRNA persisted in type-2 diabetic subjects during calorie restriction (P < 0.05).

The relationship between calorie restriction-induced variations in plasma NEFA levels and changes in uncoupling protein mRNA expression was studied in the whole population. During the diet, changes in plasma NEFA levels were positively and significantly associated with variations in UCP-3L (r = 0.6, P < 0.05) mRNA levels (Fig. 3). The relationship was also found for the changes in total UCP-3 mRNA levels (r = 0.5, P < 0.05) but not with the changes in the UCP-3S mRNA variant (data not shown). In addition, we observed a strong positive correlation between the changes in plasma NEFA levels and the variations in adipose tissue HSL mRNA levels (r = 0.9, P < 0.001; Fig. 3). Variations in the levels of the other plasma parameters (Table 1) were not correlated with changes in uncoupling protein mRNA levels (data not shown).

DISCUSSION

Because of the potential importance of UCP-2 and UCP-3 in energy expenditure, body weight regulation, and lipid metabolism, we sought to determine the factors controlling uncoupling protein gene expression in tissues of patients in which these parameters might be altered. In previous studies, we have shown that the mRNA levels of UCP-2 and of the long form (UCP-3L) and short form (UCP-3S) of UCP-3 were not different

<table>
<thead>
<tr>
<th>Table 1. Characteristics of 7 nondiabetic obese and 8 obese type-2 diabetic patients before and during severe calorie restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Obese</strong></td>
</tr>
<tr>
<td><strong>Before</strong></td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
</tr>
<tr>
<td><strong>Glucose, mM</strong></td>
</tr>
<tr>
<td><strong>Insulin, pm</strong></td>
</tr>
<tr>
<td><strong>Leptin, ng/ml</strong></td>
</tr>
<tr>
<td><strong>Triglycerides, mM</strong></td>
</tr>
<tr>
<td><strong>β-Hydroxybutyrate, mM</strong></td>
</tr>
<tr>
<td><strong>NEFA, mM</strong></td>
</tr>
<tr>
<td><strong>HSL mRNA, amol/µg total RNA</strong></td>
</tr>
</tbody>
</table>

Values are means ± SE. Calorie restriction consisted of 5 days of 1,045 kcal/day. BMI, body mass index; NEFA, nonesterified fatty acid; HSL, hormone-sensitive lipase. *P < 0.05, during vs. before diet; †P < 0.05 and ‡P < 0.01, type-2 diabetic vs. nondiabetic patients.
between obese and lean subjects in skeletal muscle (28, 29). Calorie restriction resulted in an increase of the three transcript levels that was similar in lean and obese subjects. The data suggested that there is no major alteration of UCP-2 and UCP-3 gene expression and regulation at the level of transcription and alternative splicing in skeletal muscle of obese nondiabetic subjects (28, 29). The present study was carried out to identify potential alteration in the expression and the regulation by restrictive diet of uncoupling protein mRNAs in skeletal muscle of type-2 diabetic obese subjects.

Compared with nondiabetic obese subjects, type-2 diabetic patients showed higher levels of UCP-2 and UCP-3 mRNAs in skeletal muscle. The difference was more pronounced for UCP-3S than for UCP-3L. In type-2 diabetic patients, UCP-3S mRNA represents two-thirds of UCP-3 transcripts. This differs from the data in lean and nondiabetic obese patients, whose UCP-3L and UCP-3S mRNAs are expressed in equal amounts (29). UCP-3S lacks a putative purine nucleotide binding domain that is involved in the guanosine diphosphate-mediated inhibition of UCP-1 activity (9). Hence, the protein could show an absence of control by

---

**Fig. 1.** Effect of 5 days of calorie restriction on uncoupling protein (UCP)-2 and UCP-3 mRNA levels in skeletal muscle of 7 nondiabetic obese and 8 type-2 diabetic obese subjects. Amounts of UCP-2 and total UCP-3 mRNA were determined for each subject using RT-competitive PCR before and after a 5-day calorie restriction period.

**Fig. 2.** Effect of 5 days of calorie restriction on short- and long-form (UCP-3S and UCP-3L) mRNA levels, respectively, in skeletal muscle of 7 nondiabetic obese and 6 type-2 diabetic obese subjects. Open bars, values before diet; hatched bars, values during calorie restriction. Data are means ± SE. *P < 0.05, during vs. before diet; $P < 0.05$, obese vs. type-2 diabetic subjects.
purine nucleotides compared with UCP-3. It must, however, be mentioned that, to date, there is no direct evidence that UCP-3 activity is modulated by purine nucleotides. UCP-3S also lacks the predicted sixth transmembrane domain, which might be crucial for the insertion of the protein in the mitochondrial inner membrane. Therefore, UCP-3S may not be correctly expressed or fully functional. The effect of mutations recently found in the human UCP-3 gene (1) is in accordance with a defective function of UCP-3S. A mutation at the exon 6-splice donor site detected in African-Americans results in a premature termination of the protein product that is identical to UCP-3S. Heterozygotes for the mutation showed a 50% reduction in fat oxidation and an elevation of the nonprotein respiratory quotient compared with wild-type subjects (1). The present study shows that the ratio between the long- and short-form transcripts can be modulated in a pathological state and suggests that the higher expression of UCP-3 mRNA in diabetic patients may influence skeletal muscle uncoupling activity. During the course of this study, increased UCP-3S and UCP-3L mRNA levels were reported in the skeletal muscle of type-2 diabetic subjects, with, as in our work, a greater difference in the short compared with the long UCP-3 transcript (4). In agreement with our study, the same authors also reported an increased level of UCP-2 mRNA in the muscle of diabetic patients (4). In contrast to the results of Bao et al. (4) and to our present data, a moderate but significant reduction in total UCP-3 mRNA levels in the muscle of diabetic patients compared with lean controls was recently reported by other investigators (24). The difference in the degree of obesity of the subjects is unlikely to explain the discrepancy, because we and others have previously observed similar expression levels for UCP-3 mRNA in skeletal muscle of lean and obese subjects (4, 28, 29). The fact that similar results were found using different RT-PCR methods to quantify uncoupling protein mRNAs (Ref. 4 and this study) strongly suggests that type-2 diabetic patients are characterized by higher expression levels of uncoupling protein in skeletal muscle. However, because of the heterogeneity of the diabetic population, additional studies are needed to definitively clarify this important issue.

Severe calorie restriction resulted in an upregulation of UCP-2 and UCP-3 gene expression in nondiabetic but not in type-2 diabetic patients. We have previously hypothesized that fatty acids may contribute to the upregulation of UCP-2 and UCP-3 gene expression during calorie restriction (28, 29). In the rat, an increase in plasma level of NEFA induced by Intralipid plus heparin infusion causes a rise in skeletal muscle UCP-3 mRNA level (44). Acute exercise, a condition known to be associated with elevated fatty acid levels, results in an increase of rodent skeletal muscle UCP-3 mRNA levels (14, 40). Furthermore, a positive correlation was recently reported between plasma NEFA levels and total UCP-3 mRNA levels in skeletal muscle of obese subjects (6). Accordingly, we found positive relationships between changes in UCP-3S and total UCP-3 mRNA levels and variations in plasma NEFA level during calorie restriction. The lack of significant relationship between UCP-3S mRNAs and NEFA, however, suggests that factors other than fatty acids may contribute to the upregulation of uncoupling protein gene expression during fasting. Recently, Samec et al. (34) showed that the antilipolytic agent nicotinic acid suppresses the induction of UCP-2 and UCP-3 mRNA during fasting in the soleus muscle but not in the gastrocnemius muscle. The authors suggested that circulating NEFA act, during fasting, as modulators of uncoupling protein gene expression in oxidative muscles mainly constituted of type I fibers, such as the soleus muscle, but not in type IIb fiber-rich muscles, such as the gastrocnemius muscle (34, 35). In humans, the vastus lateralis muscle is composed of several fiber types with a predominance of type I fibers (23). The twofold increase in UCP-2 and UCP-3 mRNA levels observed during fasting in rat soleus muscle, in which fatty acids appear as the mediator of UCP upregulation, is similar to the increase reported in our studies in human vastus lateralis muscle, whereas in gastrocnemius muscle, in which fatty acids do not seem to play a major role in UCP upregulation, a much higher (6- to 12-fold) increase was observed (35). Decreased insulin
sensitivity is characterized by an increased number of type Iib fibers (31). In our diabetic population, a possible increase in type Iib fibers is therefore unlikely to explain the lack of uncoupling protein upregulation during fasting. Leptin was shown to be a positive regulator of UCP-2 and UCP-3 mRNA expression in rodents (19). During calorie restriction, leptinemia decreased in the two groups of subjects investigated in the present work. Therefore, leptin is not a likely candidate to underlie the upregulation of uncoupling protein gene expression during fasting in obese subjects and to explain the differences between diabetic and nondiabetic patients.

Calorie restriction promotes the hydrolysis of triglycerides stored in adipose tissue. Released fatty acids become, with ketone bodies, the preferred fuels of the body. The enhanced rate of lipolysis is associated with an increased activity and protein level of HSL, the rate-limiting enzyme of the catabolic pathway (38). A strong correlation was found between changes in HSL mRNA levels and variations in plasma NEFA levels, suggesting that the levels of HSL gene expression in adipose tissue and plasma NEFA concentration are tightly linked during calorie restriction. In nondiabetic obese subjects, adipose tissue HSL mRNA level was increased during calorie restriction, and circulating NEFA concentration was significantly enhanced. In the diabetic subjects, the diet did not modify HSL mRNA abundance and did not induce a rise in plasma NEFA levels, suggesting, therefore, an altered induction of lipolysis. Similar lack of induction of lipolysis in type-2 diabetic subjects during short calorie restriction has been reported in some (22) but not all studies (15). It should be noted, however, that triglyceride levels decreased markedly, whereas ketone bodies rose, indicating that the use of lipids as energy fuel was increased during the diet in the diabetic subjects. Nevertheless, in the group of diabetic patients studied, a defect in lipolysis associated with an impaired activation of HSL gene transcription could contribute to the lack of change in plasma NEFA levels. Interestingly, then, there was a lack of upregulation of uncoupling protein expression in the muscle of type-2 diabetic patients that paralleled the absence of increase in plasma NEFA levels. These results therefore strengthen the hypothesis that changes in the circulating levels of NEFA contribute to the regulation of the uncoupling protein gene expression in muscle.

The mechanism of action of fatty acids on uncoupling protein gene expression may implicate the peroxisome proliferator-activated receptors (PPAR), nuclear receptors known to be activated by fatty acids and fatty acid derivatives. In preadipose cells, α-bromopalmitate, a stable analog of palmitate, increases UCP-2 mRNA expression potentially via activation of PPAR-β (2). We recently showed that BRL-49653, a thiazolidinedione activator of PPAR-γ, and α-bromopalmitate induce UCP-2 gene expression in human adipocytes (43). Thiazolidinediones also increase UCP-2 and UCP-3 mRNA levels in rodent adipocytes (2, 11, 27), whereas linoleic acid upregulates UCP-2 mRNA expression in the rat L6 muscle cell line (11). Recently, PPAR-α has been implicated in the regulation of UCP-3 gene expression in neonatal muscle in mice (10). In human skeletal muscle, PPAR-α and -β are the predominant PPAR subtypes (3) and constitute, therefore, potential candidates to mediate the effect of fatty acids on uncoupling protein gene expression. If the regulation of the uncoupling protein gene expression is mediated by one of the PPAR isoforms, the changes observed in vivo should depend on changes in the plasma levels and muscle uptake of specific fatty acids, most probably polyunsaturated, that can activate the PPARs. Alternatively, the regulation of UCP-2 and UCP-3 gene expression could be controlled by the intracellular metabolism and oxidation of lipids, without recruitment of specific nuclear receptors.

In conclusion, UCP-2 and UCP-3 mRNA levels are higher in skeletal muscle of type-2 diabetic patients. The induction of uncoupling protein gene expression during fasting in nondiabetic subjects was not observed in diabetic patients. The lack of increase in NEFA levels in diabetic subjects and the positive relationship found between the variations in plasma NEFA levels and the changes in UCP-3 mRNA levels further suggest that fatty acids play a role, in vivo in humans, in the regulation of uncoupling protein gene expression.

H. Vidal and D. Langin contributed equally to this work.
All of the authors appreciate the technical assistance of J. Peyrat, C. Louche-Pellissier, S. Perche, and N. Vega.

The laboratories involved in this study are members of the FAIR Concerted Action “FATLINK” supported by the European Commission. The work was also supported in part by a grant from Institut National de la Santé et de la Recherche Médicale (Progres no. 4P020D).

Addresses for correspondence and reprint requests: H. Vidal, INSERM U449, Faculté de Médecine, Rue Guillaume Paradin, 69372 Lyon Cedex 08, France (E-mail: vidal@laennec.univ-lyon1.fr) or D. Langin, INSERM U317, Institut Louis Buguin, Bâtiment L3, CHU Rangueil, 31403 Toulouse Cedex 4, France (E-mail: langin@angelu.inserm.fr).

Received 1 February 1999; accepted in final form 23 June 1999.

REFERENCES


