Regulation of Uncoupling Protein-2 and Uncoupling Protein-3 mRNA Expression During Lipid Infusion in Human Skeletal Muscle and Subcutaneous Adipose Tissue

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To study the effect of nonesterified fatty acids (NEFAs) on uncoupling protein-2 (UCP-2) and uncoupling protein-3 (UCP-3) gene expression, a triglyceride emulsion was infused for 5 h in 14 healthy volunteers. A euglycemic-hyperinsulinemic clamp was administered concomitantly in 7 of the 14 subjects. The mRNA levels of UCP-2 and of the short (UCP-3S) and long (UCP-3L) isoforms of UCP-3 were quantified by reverse transcription–competitive polymerase chain reaction in tissue biopsies taken before and at the end of the infusion periods. Plasma NEFA concentrations increased from 362 ± 52 to 989 ± 157 μmol/l (P = 0.018) during triglyceride infusion. UCP-3L (8 ± 1 vs. 19 ± 2 amol/μg total RNA, P = 0.018) and UCP-3S (11 ± 2 vs. 17 ± 3 amol/μg total RNA, P = 0.027) mRNA levels increased in skeletal muscle during triglyceride infusion. UCP-3L mRNA levels were positively correlated with plasma NEFA concentrations (r = 0.53, P = 0.005) and with lipid oxidation rates (r = 0.56, P = 0.004) determined by indirect calorimetry. In contrast, the expression of UCP-2 was not affected by lipid infusion in skeletal muscle or in subcutaneous adipose tissue. During the hyperinsulinemic clamp (plasma insulin concentrations 202 ± 12 pmol/l), NEFA levels (405 ± 49 vs. 648 ± 77 μmol/l, P = 0.063) and lipid oxidation rates (0.67 ± 0.09 vs. 0.84 ± 0.10 mg · kg^{-1} · min^{-1}, P = 0.091) were not significantly increased during triglyceride infusion. Under such conditions, the induction of UCP-3L and UCP-3S mRNA expression was totally prevented (8 ± 2 vs. 8 ± 1 and 8 ± 2 vs. 9 ± 2 amol/μg total RNA, respectively). We conclude that increased plasma NEFA levels by lipid infusion for 5 h induces the expression of UCP-3 but not UCP-2 in humans. During triglyceride infusion, physiological hyperinsulinemia appears to prevent the induction of UCP-3 mRNA levels. *Diabetes* 49:25–31, 2000

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Received for publication 12 July 1999 and accepted in revised form 6 October 1999.

cPCR, competitive polymerase chain reaction; +INS, with the hyperinsulinemic clamp; –INS, without the hyperinsulinemic clamp; NEFA, nonesterified fatty acid; PPAR, peroxisome proliferator-activated receptor; RT, reverse transcription; UCP, uncoupling protein.

Uncoupling protein-2 (UCP-2) and uncoupling protein-3 (UCP-3) are two newly identified members of the mitochondrial carrier family with high sequence homology to UCP-1, the brown adipose tissue–specific UCP that plays a crucial role in the control of rodent thermogenesis (1–5). Because little brown fat is evident in adults, UCP-1 activity does not likely contribute to a large extent to energy expenditure in humans. In contrast to the restricted tissue distribution of UCP-1, UCP-2 mRNA is expressed in all tissues examined, including adipose tissue and skeletal muscle (1,3), whereas UCP-3 mRNA is preferentially expressed in skeletal muscle (2,4). Overexpression of UCP-2 and UCP-3 reduces the mitochondrial membrane potential in yeast, which strongly suggests that they possess, as does UCP-1, respiratory chain uncoupling activity (1,3,5). UCP-2 and UCP-3 are therefore candidates to explain the mitochondrial proton leak in tissues devoid of UCP-1. However, the pathophysiological significance of UCP-2 and UCP-3 remains to be elucidated. The genomic location (11q13) of human UCP-2 and UCP-3 genes is coincident with several independently mapped quantitative trait loci for obesity and hyperinsulinemia (1), and markers in the vicinity of the genes are linked to the resting metabolic rate (6). A mutation in the exon-6 splice junction of the UCP-3 gene has been found in an African-American population with a high prevalence of obesity (7). However, other studies with mutation screening indicated that UCP-2 or UCP-3 gene polymorphisms are not closely associated with obesity in Caucasians (8,9). In addition, no major abnormality is evident in the basal steady-state mRNA levels of UCP-2 and UCP-3 in the skeletal muscle of lean and obese subjects (10–13).

Positive correlations have been observed between adipose tissue UCP-2 mRNA levels and resting metabolic rate adjusted for lean body mass in obese women after diet standardization (14) and between muscle UCP-3 mRNA and sleeping metabolic rate in Pima Indians (15), which support a role for the UCPs in energy expenditure. However, and contrary to what was expected, we have found that severe calorie restriction, a condition associated with reduced resting metabolic rate, was characterized by increased expression of UCP-2 and UCP-3 mRNA in the skeletal muscle of lean and obese individuals (10,16). Increased expression of UCP-2

and UCP-3 mRNA was also observed in rodent tissues after food deprivation (5,17), which suggests that the UCPs could be involved in functions other than the control of energy expenditure. The changes 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 (18). Moreover, patients who are heterozygous for the mutation in the exon-6 splice junction of the UCP-3 gene showed a 50% reduction in fat oxidation compared with wild-type subjects (7). Therefore, skeletal muscle UCP-2 and UCP-3 may play a role in fuel partitioning and in the preferential utilization of fatty acids as oxidative substrates (7,18,19).

Several lines of evidence implicate fatty acids as regulators of UCP-2 and UCP-3 gene expression. 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 (20,21). The induction of UCP-3 gene expression in mouse muscle just after birth correlates with the increase in plasma levels of nonesterified fatty acid (NEFA) resulting from the initiation of suckling (22). In rats, an increase in plasma levels of NEFA induced by intralipid plus heparin infusion causes an increase in skeletal muscle UCP-3 mRNA levels (23). The antilipolytic agent nicotinic acid has been shown to suppress the induction of UCP-2 and UCP-3 mRNA during fasting in the soleus muscle but not in the gastrocnemius muscle in rats (17). In humans, in vivo data also support a role for fatty acids in the regulation of UCP-2 and UCP-3 gene expression. A positive correlation was reported between plasma NEFA levels and total UCP-3 mRNA levels in skeletal muscle of obese subjects (24). We also observed positive relationships between variations in plasma NEFA levels during calorie restriction and changes in UCP-3 mRNA abundance in the vastus lateralis muscle of obese nondiabetic and type 2 diabetic patients (25). Finally, UCP-2 and UCP-3 mRNA levels were shown to be increased in the muscle of type 2 diabetic patients (25,26), who are characterized by higher plasma NEFA and triglyceride levels than lean and obese nondiabetic individuals. Although these observations are consistent with a role of fatty acids, a direct demonstration that plasma NEFA levels regulate the expression of UCP-2 and UCP-3 in vivo in humans has never been reported.

Therefore, the aim of the present study was to verify whether an experimental increase in plasma NEFA levels by triglyceride infusion in healthy volunteers may induce UCP-2 and UCP-3 gene expression in skeletal muscle and in abdominal subcutaneous adipose tissue in vivo. In addition, because most of the circumstances of increased UCP expression (i.e., fasting, exercise, birth) are characterized by an increase in fatty acid levels and concomitantly by a decrease in plasma insulin concentrations, we also investigated the action of insulin and fat metabolism on UCP gene expression. UCP-2 and UCP-3 mRNA levels were quantified by reverse transcription (RT)–competitive polymerase chain reaction (cPCR) (10,27) in tissue biopsies taken before and at the end of 5 h of lipid infusion.

RESEARCH DESIGN AND METHODS

Subjects. A total of 14 healthy men gave their written informed consent to participate in the study, which was approved by the ethics committee of Hospices Civils de Lyon and was performed according to the Helsinki guidelines. The subjects' mean age was 22 ± 1 years, and their mean BMI was 22.6 ± 0.8 kg/m^2. None of these subjects had a familial or personal history of diabetes or obesity, and no subjects were taking any medications. Subjects were asked to avoid a high-fat meal and unusual physical activity during the 2 days that preceded the study.

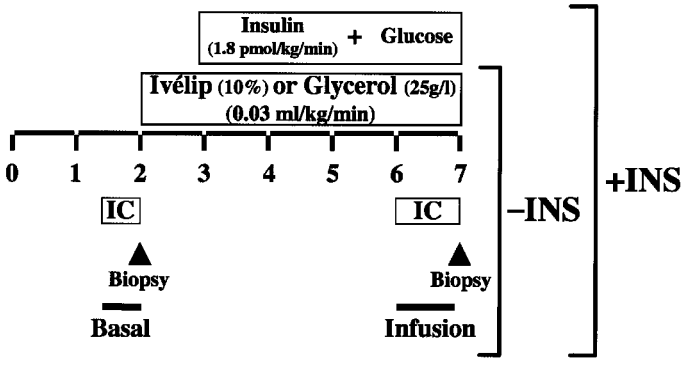

FIG. 1. Schematic representation of the study design. IC, indirect calorimetrie.

Design of the studies. All studies began in the morning between 0700 and 0800 after an overnight fast. The first protocol, which did not involve the hyperinsulinemic clamp (–INS protocol), involved seven subjects and was performed on two occasions in random order (Fig. 1). On one occasion, after 2 h of a basal period, the subjects received an infusion of triglyceride emulsion (10% Ivélip; Clintec, Sèvre, France) at the rate of 0.03 ml · min^{-1} · kg^{-1} for 5 h. On the second day, the subjects received an infusion of glycerol solution (25 g/l) at the rate of 0.03 ml · min^{-1} · kg^{-1} for 5 h. This glycerol infusion study was performed as a control study because the triglyceride emulsion contained 25 g/l of glycerol. Its infusion produced a marked rise in plasma glycerol concentrations (Table 1). The second study was carried out for each subject 4–6 weeks after the first study. On both days, vastus lateralis muscle biopsies and subcutaneous abdominal adipose tissue biopsies were performed after the 2-h basal period and after the 5-h triglyceride or glycerol infusions (Fig. 1). The studies were performed in combination with indirect calorimetry to estimate glucose and lipid oxidation rates. The second protocol, which involved the hyperinsulinemic clamp (+INS protocol), was carried out with seven other subjects and was designed exactly as the first one, except that a euglycemic-hyperinsulinemic clamp was performed during the infusions of triglycerides and glycerol (Fig. 1). Heparin, which is classically associated with triglyceride infusion to increase plasma NEFA by activation of lipoprotein lipase (28), was not given in these studies to avoid coagulation problems during the biopsies.

Indirect calorimetry. To estimate lipid and glucose oxidation rates, respiratory exchange measurements were carried out during the last 30 min of the basal period and during the last hour of the infusion periods in both the –INS and +INS protocols (Fig. 1). Measurements were made by using a computerized flow-through canopy gas analyzer system (Deltatrac Metabolic Monitor; Datex, Helsinki, Finland) as previously described (29).

Tissue biopsies. Muscle samples (70 ± 3 mg wet weight [n = 56]) were obtained by using local anesthesia via percutaneous biopsies of the vastus lateralis muscle by using Weil Blakesley pliers as previously described (30). Abdominal subcutaneous adipose tissue was aspirated from the periumbilical area through a 2.3-mm (13-gauge) needle under local anesthesia (31). Tissue samples were immediately frozen in liquid nitrogen and stored at –80°C for further analysis of UCP-2 and UCP-3 mRNA levels.

Euglycemic-hyperinsulinemic clamp. During the +INS protocol, the subjects were submitted to a 4-h euglycemic-hyperinsulinemic clamp that began 1 h after triglyceride or glycerol infusion (Fig. 1). Insulin (Actrapid; Novo-Nordisk, Copenhagen) was infused at a constant rate of 1.8 pmol (0.3 mU) · min^{-1} · kg^{-1} together with an adapted infusion of a 10% glucose solution (Aguettant, Lyon, France) to maintain euglycemia (29). The rate of insulin infusion was chosen to obtain an approximately threefold increase in insulin concentrations.

Analytical procedures. For the determination of metabolite (glucose, triglyceride, NEFA, glycerol, ketone bodies) and insulin concentrations, blood samples were drawn during the last 30 min of the basal period and during the last hour of the infusion periods. Metabolite and insulin concentrations were measured by using enzymatic methods and radioimmunoassay, respectively (29).

Total RNA preparation. Frozen muscle samples were powdered in liquid nitrogen, and total RNA was prepared by using guanidium thiocyanate and phenol/chloroform extractions followed by alcohol precipitations (32). Yields in total RNA (34 ± 1 µg/100 mg of tissue [n = 56]) were similar, with muscle samples taken before and after infusions of triglycerides or glycerol. Preparation of total RNA from adipose tissue was performed by using the RNeasy Mini Kit (QIAGEN, Courtaboeuf, France). The mean size of fat tissue that was used for total RNA preparation was

TABLE 1
Metabolic and hormonal values during the basal period and the last hour of either glycerol or triglyceride infusion during the protocols without (–INS) and with (+INS) the euglycemic-hyperinsulinemic clamp

	–INS		+INS	
	Glycerol	Triglyceride	Glycerol	Triglyceride
Triglycerides (mmol/l)				
Basal	0.76 ± 0.15	0.95 ± 0.24	0.80 ± 0.60	0.86 ± 0.20
Infusion	0.70 ± 0.94	5.7 ± 0.7*‡	0.76 ± 0.10	5.3 ± 1.7*‡
Glycerol (μmol/l)				
Basal	55 ± 7	47 ± 8	47 ± 6	41 ± 7
Infusion	571 ± 64*	452 ± 38*	430 ± 37*	394 ± 37*
Glucose (mmol/l)				
Basal	5.2 ± 0.1	5.2 ± 0.2	5.5 ± 0.2	5.2 ± 0.2
Infusion	5.1 ± 0.1	5.1 ± 0.2	5.5 ± 0.1	5.7 ± 0.2*§
Insulin (pmol/l)				
Basal	42 ± 5	54 ± 6	49 ± 7	50 ± 6
Infusion	37 ± 6	56 ± 4	170 ± 14*¶	202 ± 12*‖
Glucose oxidation (mg · kg^{-1} · min^{-1})				
Basal	2.0 ± 0.4	2.4 ± 0.4	1.8 ± 0.3	1.8 ± 0.3
Infusion	1.4 ± 0.3*	1.3 ± 0.3*	3.1 ± 0.2*¶	1.6 ± 0.2‡
Lipid oxidation (mg · kg^{-1} · min^{-1})				
Basal	0.65 ± 0.14	0.54 ± 0.16	0.68 ± 0.05	0.67 ± 0.09
Infusion	0.87 ± 0.12†	0.89 ± 0.14*	0.20 ± 0.07*¶	0.84 ± 0.10‡

Data are means ± SE. *P < 0.02 and †P < 0.05 for infusion vs. basal; ‡P < 0.02 for triglyceride vs. glycerol; §P < 0.05 and ‖P < 0.01 for triglyceride +INS vs. triglyceride; ¶P < 0.01 for glycerol +INS vs. glycerol.

87 ± 3 mg wet weight (n = 28). Yields in total RNA (1.2 ± 0.2 μg/100 mg of adipose tissue [n = 28]) were similar with samples taken before and after infusions of triglycerides or glycerol. The 260–280 nm absorption ratios were always between 1.7 and 2. Total RNA solutions were stored at –80°C.

Quantification of UCP mRNA. The mRNA levels of UCP-2 and UCP-3 long and short variants were quantified by RT-cPCR (27). The conditions of the assays, the sequences of the primers, and the validation of the method have been previously presented (10,16). To determine accurately the effect of triglyceride infusion, the total RNA of the four muscle biopsies from the same individual (before and after triglyceride infusion and before and after glycerol infusion) were prepared simultaneously, and the assays of the UCP mRNAs were always made in parallel in the same run of PCR and with the same working solutions of competitors. UCP-2 mRNA levels were measured during the –INS protocol only. The results are expressed in attomoles per microgram of total RNA.

Statistical analysis. Nonparametric Wilcoxon's test for paired values was used when comparing mRNA levels before and after triglyceride or glycerol infusion in the same protocol. Correlations were analyzed with Spearman's rank correlation test. The threshold for significance was P < 0.05. Data in text and figures are means ± SE.

RESULTS

Hormone and metabolite data. Table 1 presents the metabolic and hormonal values that were measured during the basal period and during the last hour of either glycerol or triglyceride infusion in the two protocols (–INS and +INS). Triglyceride infusion produced a marked rise in plasma glycerol concentrations. The glycerol infusion study was therefore a relevant control situation because, as shown in Table 1, the plasma glycerol concentrations achieved during the glycerol infusion study were similar to those obtained during the triglyceride study in both the –INS and +INS protocols. During both protocols, triglyceride infusion increased plasma triglyceride concentrations significantly, but this parameter remained at basal levels during the glycerol infusion studies. In the –INS protocol, glucose oxidation rates decreased and lipid oxidation rates increased during both glycerol and triglyceride infusions (Table 1). The changes in whole-body glucose and lipid oxidation rates were similar in the triglyceride and glycerol infusions. As expected from the choice of the insulin infusion rates, plasma insulin concentrations increased three- to fourfold when using the clamp in the +INS protocol. Lipid oxidation rates were significantly reduced and glucose oxidation rates were increased in the glycerol infusion study of this protocol. Importantly, the effects of insulin on glucose and lipid oxidation rates were completely prevented during the infusion of triglycerides (Table 1).

In the –INS protocol, plasma NEFA concentrations increased from 362 ± 52 to 989 ± 157 μmol/l (P = 0.018) during the infusion of triglycerides and remained unchanged during the infusion of glycerol (318 ± 63 vs. 400 ± 62 μmol/l in the basal period vs. during the glycerol infusion periods, respectively) as shown in Fig. 2. The infusion of insulin markedly reduced plasma NEFA concentrations (388 ± 30 vs. 43 ± 13 μmol/l in the basal period vs. when using the clamp during glycerol infusion, respectively; P = 0.018). In contrast, plasma NEFA concentrations tended to increase when the triglyceride emulsion was infused concomitantly to insulin (405 ± 49 vs. 648 ± 77 μmol/l in the basal period vs. when using the clamp during the triglyceride infusion, respectively; P = 0.063). Figure 2 also shows that the changes in plasma β-hydroxybutyrate concentrations were similar to the changes in plasma NEFA levels. The plasma concentrations of β-hydroxybutyrate increased during triglyceride infusion (78 ± 9 vs. 451 ± 81 μmol/l in the basal period vs. during triglyceride infusion, respectively; P = 0.018), whereas, during the +INS protocol, these concentrations decreased during glycerol infusion (74 ± 14 vs. 35 ± 5 μmol/l in the basal period vs. when using the clamp during the glycerol infusion, respectively; P = 0.018) and increased during triglyceride infusion (82 ± 9 vs. 173 ± 38 μmol/l in the basal period vs. when using the clamp during the triglyceride infusion, respectively; P = 0.018).

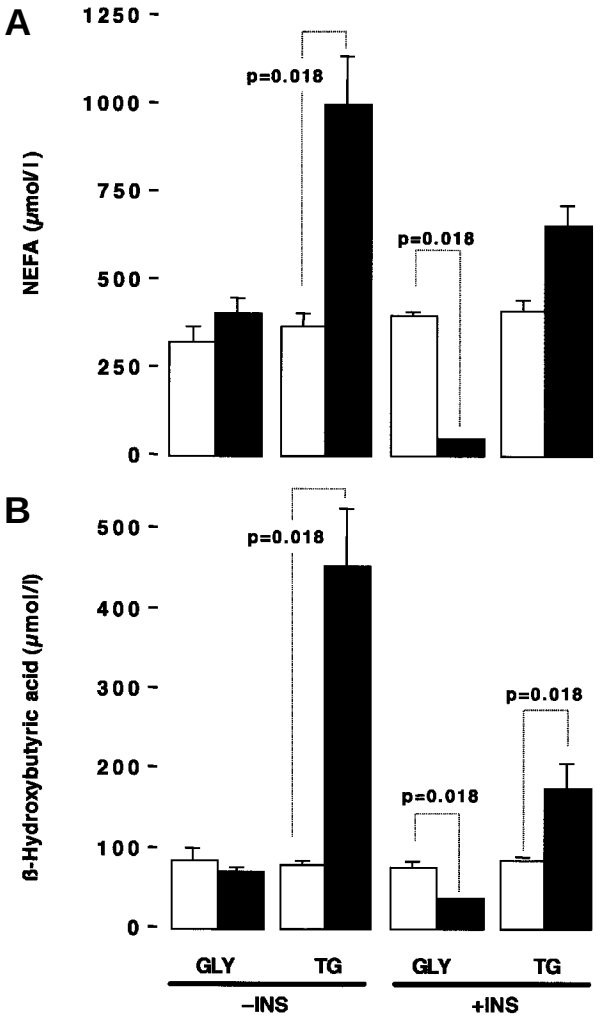

FIG. 2. Plasma NEFA (*A*) and β-hydroxybutyrate (*B*) concentrations in the basal state (□) and after 5 h of either glycerol (GLY) or triglyceride (TG) infusion (■) during the protocols without (–INS) and with (+INS) the hyperinsulinemic clamp. Data are means ± SE.

Changes in UCP mRNA levels. To assess the effect of an increase in plasma NEFA levels on the expression of the UCPs, we first compared the changes in UCP-2 and UCP-3 mRNA levels during triglyceride versus glycerol infusion in the absence of exogenous infused insulin (–INS protocol). Figure 3 shows the mean values of UCP-2 mRNA levels in skeletal muscle and in adipose tissue. UCP-2 mRNA expression was not affected by triglyceride infusion for 5 h, neither in muscle nor in adipose tissue. In contrast, Fig. 4*A* clearly shows that UCP-3L mRNA levels were increased in the skeletal muscle in all subjects during the triglyceride infusion (8 ± 1 vs. 19 ± 2 amol/μg total RNA before vs. during triglyceride infusion, respectively; $P = 0.018$). UCP-3L mRNA levels did not change significantly during glycerol infusion ($P = 0.53$). Regarding the short isoform of UCP-3 (Fig. 5*A*), triglyceride infusion also increased UCP-3S mRNA levels significantly (11 ± 2 vs. 17 ± 3 amol/μg total RNA before vs. during triglyceride infusion, respectively; $P = 0.027$). During glycerol infusion, UCP-3S mRNA levels did not change significantly ($P = 0.18$), although an increase was evident in the expression of UCP-3S mRNA in the muscle of five subjects and a decrease in the two others. The UCP-3S to UCP-3L mRNA ratio significantly decreased during triglyceride infusion (1.6 ± 0.4 vs. 0.95 ± 0.14 before vs. during infusion, respectively; $P = 0.018$), which indicates a preferential induction of the mRNA variant encoding the long UCP-3 form.

Figure 6 shows the correlations between the mRNA levels of UCP-3L and the plasma NEFA concentrations (6*A*) and the rates of lipid oxidation determined by indirect calorimetry (6*B*) when analyzing the 28 values of UCP-3L mRNA determined during the –INS protocol (during the basal period and during glycerol and triglyceride infusions). The levels of UCP-3L mRNA in skeletal muscle were significantly and positively correlated with plasma NEFA concentrations ($r = 0.53$, $P = 0.005$) and lipid oxidation rates ($r = 0.56$, $P = 0.004$) (Fig. 6). In addition to these relationships, UCP-3L mRNA levels were also positively correlated with the plasma concentrations of β-hydroxybutyrate ($r = 0.48$, $P = 0.013$) and were negatively correlated with the rates of glucose oxidation ($r = –0.61$, $P = 0.001$). Regarding the short form of UCP-3, we found that UCP-3S mRNA levels were positively correlated with lipid oxidation rates ($r = 0.41$, $P = 0.035$) and negatively correlated with glucose oxidation rates ($r = –0.51$, $P = 0.008$), but no correlation was evident with plasma concentrations of NEFA ($P = 0.17$). Finally, no correlation was found between muscle or adipose tissue UCP-2 mRNA levels and plasma concentrations of NEFA ($P = 0.61$ and $P = 0.86$, respectively).

Seven additional subjects participated in a similar protocol that included a euglycemic-hyperinsulinemic clamp (+INS protocol). As shown in Table 1, insulin infusion significantly reduced lipid oxidation rates and the plasma NEFA concentrations during the glycerol infusion study. In contrast, the infusion of triglycerides maintained whole-body lipid oxida-

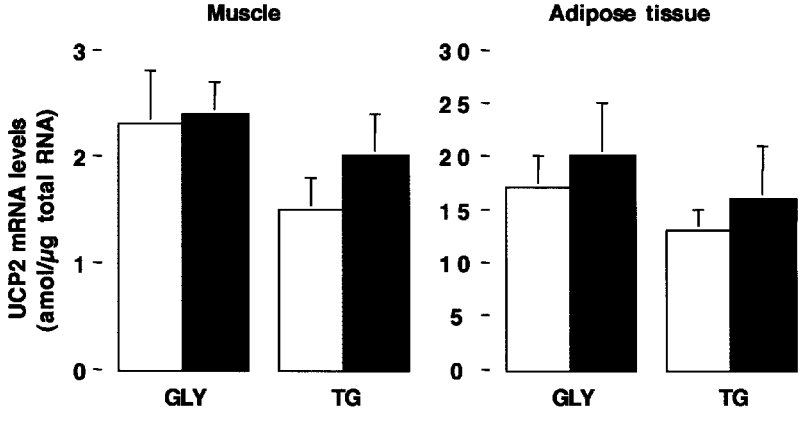

FIG. 3. UCP-2 mRNA levels in skeletal muscle and in subcutaneous adipose tissue in the basal state (□) and after 5 h of either glycerol (GLY) or triglyceride (TG) infusion (■). mRNA levels were determined by RT-cPCR as described in RESEARCH DESIGN AND METHODS. Data are means ± SE.

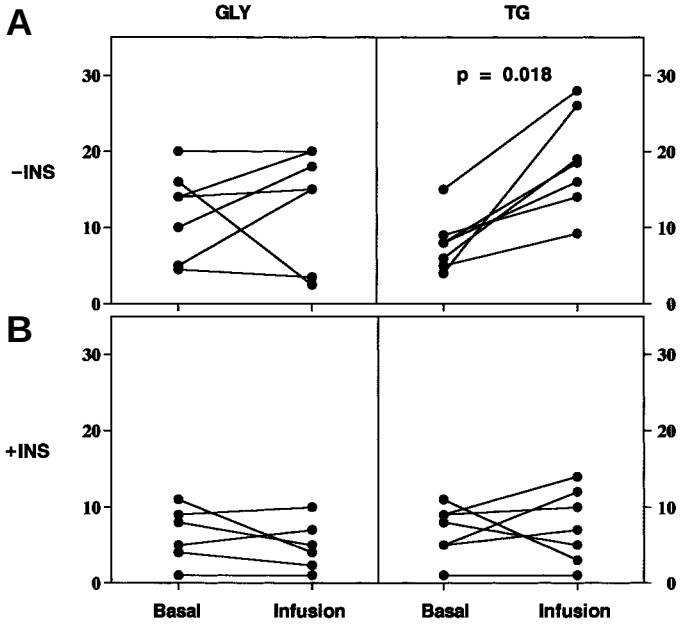

FIG. 4. Changes in the expression levels of the long form of UCP-3 (amol/μg total RNA) in skeletal muscle induced by 5 h of either glycerol (GLY) or triglyceride (TG) infusion during the protocols without (–INS; *A*) and with (+INS; *B*) the hyperinsulinemic clamp.

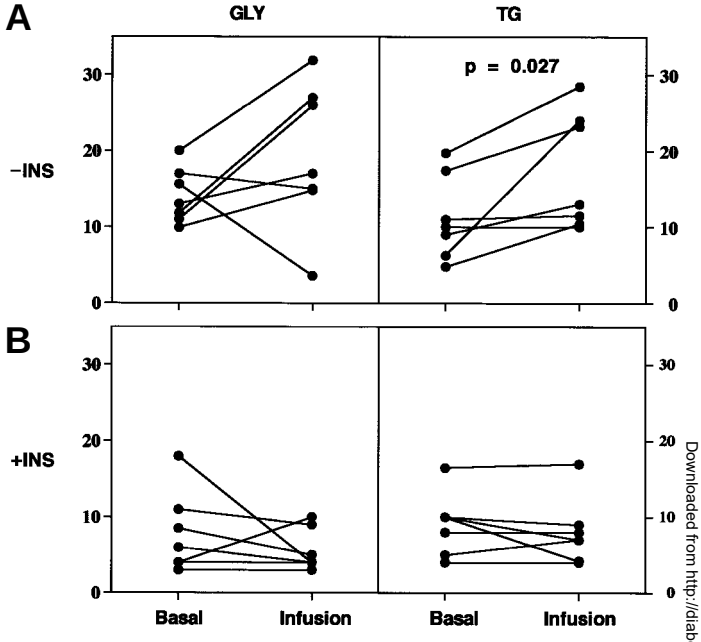

FIG. 5. Changes in the expression levels of the short form of UCP-3 (amol/μg total RNA) in skeletal muscle induced by 5 h of either glycerol (GLY) or triglyceride (TG) infusion during the protocols without (–INS; *A*) and with (+INS; *B*) the hyperinsulinemic clamp.

tion rates at values (0.84 ± 0.1 mg · kg^{-1} · min^{-1}) similar to those observed during the –INS protocol. Under these conditions, UCP-3L and UCP-3S mRNA concentrations did not change significantly, either during the infusion of glycerol or during the infusion of triglycerides (Figs. 4*B* and 5*B*). No significant correlation was evident between UCP-3L or UCP-3S mRNA levels and plasma NEFA concentrations during the +INS protocol (P = 0.20 and P = 0.09, respectively). Insulin infusion therefore counteracted the effect of triglyceride infusion on UCP-3 mRNA expression in skeletal muscle.

DISCUSSION

This study demonstrates for the first time in vivo in humans that increased plasma NEFA concentrations and lipid oxidation rates resulting from infusing triglycerides induce the mRNA expression of the two variants of UCP-3 in skeletal muscle. Moreover, an increase in plasma insulin levels to high physiological range completely prevents the effect of lipids on UCP-3 mRNA expression. In contrast to UCP-3, UCP-2 mRNA levels are not affected during the 5-h lipid infusion, neither in skeletal muscle nor in subcutaneous adipose tissue.

Several lines of evidence have already suggested a role of fatty acid in the regulation of UCP-2 and UCP-3 gene expression in vivo, both in rodents (17,20,21,23) and in humans (10,16,24,25). In the present study, we show that triglyceride infusion increased UCP-3 but not UCP-2 mRNA expression, which suggests that different mechanisms may participate in the control of the two genes. Differences in the regulation of UCP-2 and UCP-3 during fasting have been observed in rodents (23). In humans, the association of plasma NEFA concentrations with muscle UCP-3 mRNA levels was not found with UCP-2 (24,25). During fasting, however, we have previously found a similar induction of the two UCP mRNAs during a 5-day severe calorie restriction (10,16). A period of

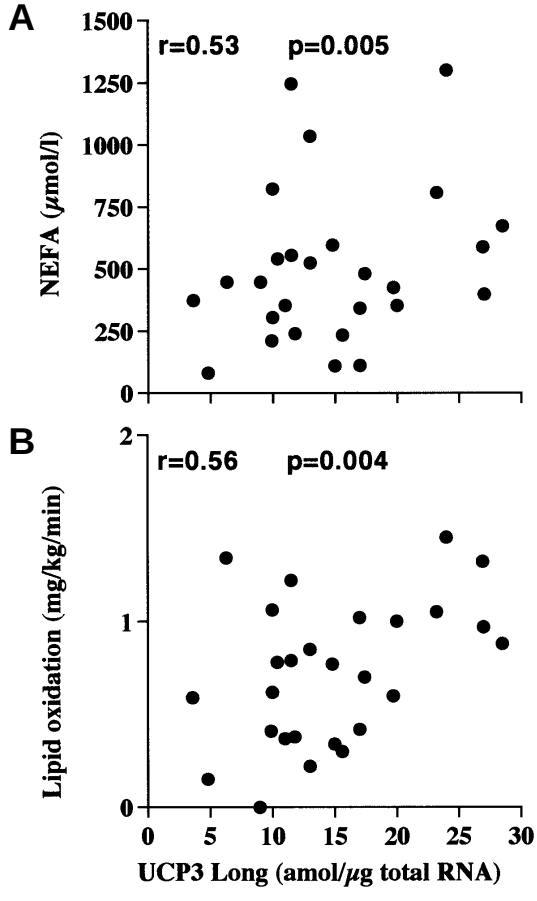

FIG. 6. Nonparametric Spearman correlations between UCP-3L mRNA levels and plasma NEFA concentrations (*A*) and lipid oxidation rates (*B*). Data are from the protocol without the hyperinsulinemic clamp (–INS).

exposure to lipids of >5 h may be required to induce UCP-2 gene expression. The triglyceride emulsion that was used in the present study may not have provided a sufficient amount of a specific type of fatty acids, which is important for UCP-2 gene induction. The expression of both UCP-3L and UCP-3S mRNA variants was increased during triglyceride infusion, but the effect was more pronounced for the long form. Because the structure difference between UCP-3L and UCP-3S is related to an alternative splicing in the 3′ region of the pre-mRNAs (33), the transcriptional regulation is likely similar for the two forms. Different stability and/or regulation of the UCP-3L and UCP-3S mRNAs may thus explain the slight difference observed in their expression levels during triglyceride infusion.

UCP-3 mRNA levels were associated with plasma NEFA concentrations, plasma β-hydroxybutyrate levels, and lipid oxidation rates, which suggests that lipid oxidation may be an important parameter of UCP-3 gene induction in skeletal muscle. Several reports have indicated, however, that lipid oxidation is probably not involved in the upregulation of the UCP genes during fasting. In rats, methyl palmoxirate, an inhibitor of lipid oxidation, did not prevent the induction of UCP-3 gene expression during fasting (34). In the peroxisome proliferator-activated receptor-α (PPAR-α) knockout mice, lipid oxidation, as assessed by β-hydroxybutyrate levels, did not increase during fasting, whereas the mRNA levels of UCP-2 and UCP-3 were normally induced in skeletal muscle (35). In this model, plasma NEFA levels increased dramatically during fasting (35). Thus, these data suggest that, in situations in which lipid oxidation is inhibited, the rise in plasma NEFA levels per se is probably responsible for the induction of the UCP genes. Our data were also consistent with this conclusion. In the absence of insulin, the infusion of glycerol was associated with an increase in lipid oxidation rates without significant changes in either UCP-3 mRNA levels or NEFA concentrations. In addition, insulin infusion dramatically decreased lipid oxidation during the control experiment (glycerol infusion) without affecting UCP-3 mRNA levels. This result confirmed our previous demonstration that supraphysiological hyperinsulinemia did not affect muscle UCP-2 and UCP-3 mRNA levels (10).

Experiments conducted in vitro also support a direct effect of fatty acids or their derivatives on UCP gene expression without involvement of lipid oxidation. In rodent preadipose cells, α-bromopalmitate (a stable analog of palmitate) increased UCP-2 mRNA expression (36), and this effect was recently confirmed in human adipocytes (37). In muscle cell lines, linoleic acid upregulated UCP-2 mRNA levels in rat L6 cells (38), and oleic acid was recently found to induce the expression of UCP-3 in mouse C2C12 myotubes in a time- and concentration-dependent manner (34). The mechanism of action of fatty acids on UCP gene expression likely implicates PPARs, which are nuclear receptors known to be activated by fatty acids and fatty acid derivatives (39). Putative PPAR responsive elements have been recently identified in the promoter region of the human UCP-3 gene (40), and several data support a role of the PPARs in the regulation of UCP-2 and UCP-3 gene expression (22,34,36–38,41). In adult skeletal muscle, PPAR-α and PPAR-β are the predominant PPAR subtypes in humans (42) and therefore are potential candidates to mediate the effect of fatty acids on UCP gene expression in this tissue.

As for the mechanism involved in the effect of insulin to prevent the induction of UCP-3 gene expression during triglyceride infusion, the lack of a significant increase in plasma NEFA levels in this situation may explain the observed result. We cannot exclude the possibility, however, that insulin also interacts directly with the regulation by fatty acids of UCP-3 gene expression. For example, insulin phosphorylates PPAR-γ (43) and PPAR-α (44), and this modifies their transcriptional activity. Further in vitro works are thus needed to define the precise mechanism of action of insulin.

In conclusion, this study demonstrates that fatty acids are direct regulators of UCP-3 mRNA expression in human skeletal muscle in vivo. After 5 h of triglyceride infusion, an approximately threefold increase in plasma NEFA concentrations occurs that upregulates UCP-3 mRNA levels. The mRNA variant encoding the long form of UCP-3 is more induced than UCP-3S, whereas muscle and adipose tissue UCP-2 mRNA levels are not affected by lipid infusion. Physiological hyperinsulinemia completely prevents the effect of lipids, which suggests a possible role of insulin on the regulation of UCP expression. Although the precise mechanism of action of fatty acids remains to be determined, our results are consistent with the idea that the induction of UCP-3 expression may not be because of fatty acid uptake and oxidation in the mitochondria but rather, because of the activation of specific transcription signals, may be mediated by the PPARs.

ACKNOWLEDGMENTS

This work was supported by research grants from Institut National de la Recherche Agronomique (INRA), INSERM (Progres n°4P020D), and Association de Langue Française pour l'Étude du diabète et des Maladies Métaboliques (ALFEDIAM)–Novo/Nordisk.

We thank Corinne Urbain, Michèle Odéon, Nathalie Vega, and Jocelyne Peyrat for excellent technical assistance, and we thank all of the volunteers for their contributions.

The laboratories involved in this study are members of the Concerted Action FATLINK (FAIR-CT98–4141), which is supported by the European Commission. Y.K. is Chargé de Recherche from INRA.

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