Original Articles

Induction of Fatty Acid Translocase/CD36, Peroxisome Proliferator-Activated Receptor-γ2, Leptin, Uncoupling Proteins 2 and 3, and Tumor Necrosis Factor-α Gene Expression in Human Subcutaneous Fat by Lipid Infusion

Enzo Nisoli, Michele O. Carruba, Cristina Tonello, Cinzia Macor, Giovanni Federspil, and Roberto Vettor

Little is known about the mechanisms involved in the preferential channeling of different fuels to fat and how the target tissue participates in this process. Dietary fatty acids have been shown to act as signaling molecules that bind and activate a new class of nuclear receptors, the peroxisome proliferator-activated receptors (PPARs). PPAR-γ is particularly interesting because it may have the potential to link particular fatty acids with a program of gene expression involved in lipid storage and metabolism. We investigated whether a nutrient-sensing pathway is activated by an increased availability of lipid fuels in nine normal weight male volunteers. Using reverse transcriptase–polymerase chain reaction analysis, the mRNA expression of fatty acid translocase (FAT)/CD36, PPAR-γ2, leptin, uncoupling protein (UCP)-2 and UCP-3, and tumor necrosis factor (TNF)-α was investigated in gluteal subcutaneous fat biopsies before and after 5 h infusions of saline or Intralipid (Pharmacia and Upjohn, Milan, Italy) plus heparin, which does not modify insulinemia. Marked increases in FAT/CD36 (724 ± 18%; $P < 0.05$), PPAR-γ2 (200 ± 8%; $P < 0.05$), leptin (110 ± 13%; $P < 0.05$), UCP-2 (120 ± 7%; $P < 0.05$), UCP-3 (80 ± 5%; $P < 0.05$), and TNF-α mRNA (130 ± 12%; $P < 0.05$) were observed in comparison with pretreatment levels, whereas there was no change after saline infusion. These data suggest that the in vivo gene expression of FAT/CD36, PPAR-γ2, leptin, UCP-2, UCP-3, and TNF-α in subcutaneous adipose tissue is regulated by circulating lipids independent of insulin and that prolonged hyperlipidemia may therefore contribute to increased fat metabolism and storage as a result of the increased expression of these proteins. *Diabetes* 49:319–324, 2000

From the Department of Preclinical Sciences (E.N., M.O.C., C.T.), the Center for Study and Research on Obesity, L. Sacco Hospital, University of Milan, Milan; and the Department of Medical and Surgical Sciences (C.M., G.F., R.V.), the Endocrine-Metabolic Laboratory, University of Padua, Padua, Italy.

Address correspondence and reprint requests to Enzo Nisoli, MD, PhD, Center for Study and Research on Obesity, Department of Preclinical Sciences, Polo Vialba, L. Sacco Hospital, via G.B. Grassi, 74, 20157 Milan, Italy. E-mail: enzo.nisoli@uniml.it.

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FAT, fatty acid translocase; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; PPAR, peroxisome proliferator-activated receptor; RT-PCR, reverse transcriptase–polymerase chain reaction; TNF, tumor necrosis factor; UCP, uncoupling protein.

The development of obesity requires the continuous new adipocyte differentiation or growth (or both) to store energy as triglycerides (1). There is increasing evidence to suggest that adipocytes may function as finely regulated endocrine cells containing several signaling molecules and other factors that play an active part in regulating energy homeostasis (2). Many of the lipids stored in adipocytes are derived from the uptake of the nonesterified fatty acids (NEFAs) released by the hydrolysis of circulating triglyceride-rich lipoproteins or from circulating fatty acids bound to serum albumin. The key regulatory reaction in this metabolic pathway includes lipoprotein lipase (LPL), which is related to body fat mass and fat cell size (3). Furthermore, fatty acid translocase (FAT)/CD36 has recently been suggested as a transmembrane transporter of long-chain fatty acids (4), which are activated by fatty acids themselves in all of the tissues active in fatty acid metabolism, including adipose tissue (5,6). This particular role of FAT/CD36 is controversial, since NEFAs are rapidly and extensively transported across membranes in the absence of FAT/CD36 (7) and no reconstitution experiments clearly demonstrating a transporter role for FAT/CD36 have been reported.

Once dietary fatty acids enter the cells, they act as signaling molecules by binding and activating a new class of nuclear receptors: the peroxisome proliferator-activated receptor (PPAR) family (8). These receptors have the potential to link particular fatty acids with a program of gene expression, including the genes that encode for the proteins involved in adipogenesis and lipid storage and metabolism (9). Of particular interest is PPAR-γ, which can be activated by a number of mono- and polyunsaturated fatty acids (10) and is also the receptor for the thiazolidinedione class of antidiabetic drugs known to improve insulin resistance (8). PPAR-γ also controls the expression of leptin, an adipocyte-derived satiety peptide hormone (11), and regulates the brown adipocyte differentiation-dependent expression of uncoupling protein

(UCP)-1 (12), the inner mitochondrial membrane protein involved in heat production and energy expenditure in brown fat. Newly described members of the uncoupling protein family, UCP-2 and UCP-3, which are expressed in adipose tissues and skeletal muscle, have been shown to lower mitochondrial membrane potential when transfected into yeast (13–16). It has been suggested that UCP-2 and UCP-3 also act as retrograde anion transporters and may be implicated in the regulation of lipids as a fuel substrate in skeletal muscle and adipose tissue because, in the absence of ingested nutrients (i.e., fasting), the mobilization of stored fat provides a major source of fuel to meet ongoing energy requirements, especially in skeletal muscle. The onset of lipolysis leads to a significant increase in circulating free fatty acid levels within 24 h of fasting, which is accompanied by a marked increase in UCP-3 in adipose tissue and muscle (17), casting doubt on the role of this protein as a mediator of increased thermogenesis. It has recently been shown that UCP-2 and UCP-3 gene expression can be induced by tumor necrosis factor (TNF)-α in rodent adipose tissues and skeletal muscle (18). TNF-α, which is actually overexpressed in the adipose tissue of obese subjects, has been also demonstrated to stimulate lipolysis (20) and decrease the activity of adipose tissue LPL (20), thus decreasing the ability of white adipocytes to accumulate fatty acids.

To investigate how adipocytes respond to increased NEFA plasma levels, the mRNA expression of FAT/CD36, PPAR-γ2, leptin, UCP-2, UCP-3, and TNF-α was studied in gluteal subcutaneous fat biopsies before and after 5-h infusions of saline or Intralipid plus heparin. These experimental conditions ensure a large supply of fatty acids and therefore are an adequate substrate for triglyceride synthesis, without inducing significant changes in plasma insulin levels. Our findings suggest that plasma NEFAs are directly involved in modulating the accumulation and metabolism of fatty acids in a fat depot.

RESEARCH DESIGN AND METHODS

Subjects and Intralipid infusion. Gluteal subcutaneous fat from nine male volunteers of normal weight (age 28–43 years; mean BMI 22.6 ± 0.9 kg/m^2) was obtained by means of needle liposuction after an overnight fast and after infusion of Intralipid (Pharmacia and Upjohn, Milan, Italy) plus heparin (21). The tissue was immediately frozen in liquid nitrogen. Intralipid 20%, a commercial emulsion of soybean oil, was infused at 1.5 ml/min (0.3 g/min) with heparin sodium (200 U in bolus and 0.4 U · min^{-1} · kg^{-1} i.v.) for 5 h. The same subjects were also administered a 5-h saline infusion on a different day after an otherwise similar experimental protocol. Protocols involving adipose biopsies have been approved by an internal ethics committee of the University of Padua, and all of the subjects gave their informed consent.

Plasma assays. Plasma glucose concentrations were measured using the glucose oxidase method (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA), and plasma free fatty acids were assayed enzymatically using the NEFA Quick "BMY" kit (Boehringer Mannheim Yamanouki KK, Tokyo). Insulin levels were determined using a commercial radioimmunoassay kit (INSIK-5; Sorin, Saluggia, Italy), and serum leptin was measured by means of a specific radioimmunoassay with minor modifications (Linco Research, St. Charles, MO). Maximal tracer binding was 35–45%, and half-maximal binding occurred at 6.0 ± 1.0 ng/ml of unlabeled leptin. The sensitivity was 0.5 ng/ml, and the intra- and interassay coefficients of variation were 0.8 and 8.5%, respectively. The percentage recovery and linearity calculated on the observed versus expected values was 4 ± 1 and 1.2 ± 0.5%.

Energy expenditure. During the test in the basal state and during the 5-h Intralipid or saline infusion, indirect calorimetry was performed to estimate rates of glucose and lipid oxidation at each hour. Continuous gaseous exchange measurements were performed with a ventilated hood system (MMC Horizon System Sensor Medics, Anaheim, CA). Moreover, O_2 consumption, measured by a thermomagnetic analyzer, and CO_2 production, measured by an infrared analyzer, were used to compute the substrate oxidation according to standard stoichiometric equations (22,23).

Reverse transcriptase–polymerase chain reaction analysis. Total RNA was isolated from the adipose tissue biopsies of the treated or untreated subjects using the RNAzol method (Cinna Scientific, Friendwood, TX). The RNAs were treated for 1 h at 37°C with 6 U RNAse-free DNAse I per µg RNA in 100 mmol per Tris-HCl, pH 7.5, and 50 mmol/l $MgCl_2$ in the presence of 2 U/µl placenta RNAse inhibitor. All of the RNAs (~200 ng from each biopsy) were reverse-transcribed with 200 U of Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) in 20 µl buffer containing 0.4 mmol/l dNTP, 2 U/ml RNAse inhibitor, and 0.8 µg oligo$(dT)_{15}$ primer (Promega). The reverse transcriptase–polymerase chain reaction (RT-PCR) was performed using *Taq* DNA polymerase (Promega) in 25 µl standard buffer (10 mmol/l Tris-HCl, pH 9, 50 mmol/l KCl, 0.1% Triton X-100, 2.5 mmol/l $MgCl_2$, and 200 µmol/l dNTPs) and 40 pmol of each sense- and antisense specific oligonucleotide primer. The primer sequences were chosen using the GeneWorks program 2.0 (IntelliGenetics, Mountain View, CA) and are shown in Table 1. The primers for β-actin were added at the tenth cycle of each PCR amplification to avoid a plateau situation. All of the genes were amplified using 30 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, followed by a 5-min final extension at 72°C. After amplification, 10 µl of the reaction mixture was separated by electrophoresis (1.2% agarose gel in Tris-acetate-EDTA buffer), visualized using ethidium bromide staining and a QuickImage-D system (Camberra Packard, Milan, Italy), and densitometrically analyzed using Phoretix 1D version 3.0. The number of cycles for the semiquantitative RT-PCR assay and the conditions of the reaction temperature were estimated to be optimal for a linear relationship between the amount of input template and the amounts of PCR product generated over a significant concentration range: 20–100 ng from total RNA. In particular, the linearity of the RT-PCR amplifications for all of the tested genes was measured at 15, 30, and 40 cycles (data not shown).

TABLE 1
PCR primers used to amplify human genes

Genes	Primer sequences	Size of amplified PCR product (bp)	Reference
FAT/CD36	Sense: 5′-AAG AGA GAT GAG GAA CCA GAG C-3′		
	Antisense: 5′-AGT GAA GGT TCG AAG ATG GC-3′	479	47
PPAR-γ2	Sense: 5′-AAC TGC GGG GAA ACT TGG GAG ATT CTC C-3′		
	Antisense: 5′-AAT AAT AAG GTG GAG ATG CGA GCT CC-3′	341	24
Leptin	Sense: 5′-CAC CAA AAC CCT CAT CAA GCA-3′		
	Antisense: 5′-AGC CTG CTC AGG GCC ACC ACC-3′	360	48
UCP-2	Sense: 5′-CTA CTG CCA CTg TGA AGT TTC-3′		
	Antisense: 5′-TCG GGC AAT GGT CTT GTA GG-3′	472	13
UCP-3	Sense: 5′-ACA GAT GTG GTG AAG GTC CG-3′		
	Antisense: 5′-TAC GAA CAT CAC CAC GTT CC-3′	467	16
TNF-α	Sense: 5′-ATG AGG ACT GAA AGC ATG ATC CGG GAC GTG G-3′		
	Antisense: 5′-CAA TGA TCC CAA AGT AGA CCT GCC CAG ACT C-3′	694	49
β-Actin	Sense: 5′-CAA AGA CCT GTA CGC CAA CAC AGT-3′		
	Antisense: 5′-CAC GAT GGA GGG GCC GGA CTC GTC-3′	241	50

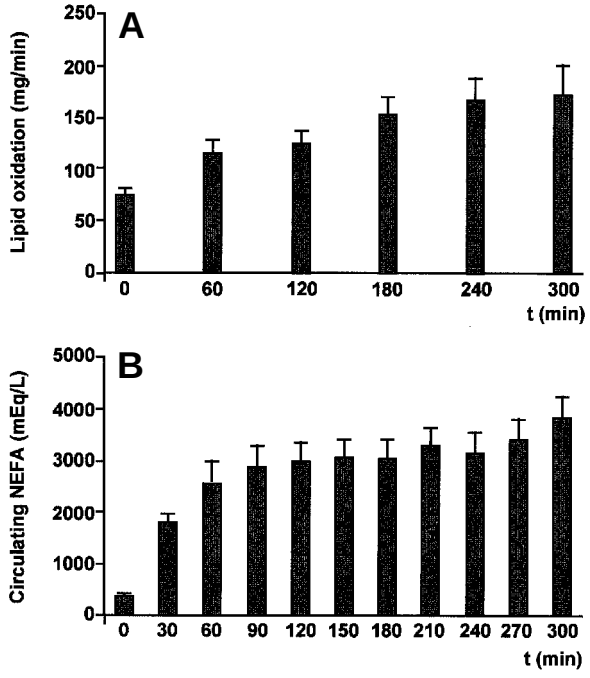

FIG. 1. Lipid oxidation and circulating NEFA measurements before and after Intralipid infusion. The progressive increases in NEFA concentrations and lipid oxidation were statistically significant after 30 and 60 min, respectively.

Statistical analysis. RT-PCR analysis was done three times on the same sample from each of the nine subjects. The results are presented as mean values ± SE of the nine subjects, each number representing the mean of three different determinations of the same sample. The intra-assay coefficient of variation was <5%. The statistical significance was assessed by means of analysis of variance. All of the analyses were made using the Statview statistical package. The level of significance was set at $P < 0.05$.

RESULTS

Substrate oxidation, plasma insulin, and NEFA measurements before and after Intralipid infusion. As shown in Fig. 1*A*, lipid infusion induces a progressive increase in lipid oxidation (baseline vs. 300 min lipid oxidation: 73.6 ± 7.3 vs. 173.6 ± 29.5 mg/min; $P < 0.001$), but the glucose oxidation rate is blunted (147.6 ± 18.7 vs. 36.7 ± 21.6 mg/min; $P < 0.01$). The continuous infusion of fat emulsion plus heparin at the chosen times and dosages led to an increased supply of fatty acids by increasing baseline NEFA concentrations from 350 ± 42 to 3,899 ± 360 mEq/l ($P < 0.001$) (Fig. 1*B*). A slight but not significant increase in plasma insulin concentrations was observed after 5 h (48.8 ± 3.0 vs. 62.4 ± 4 pmol/l). No significant changes in the lipid and glucose oxidation rate or in plasma NEFAs and insulin levels were observed after saline infusion under similar experimental conditions (data not shown).

Increased gene expression in subcutaneous fat before and after Intralipid infusion. Analysis of RT-PCR after the infusion by means of specific primers of the RNA isolated from subcutaneous fat showed a marked increase in FAT/CD36 mRNA in comparison with pretreatment concentrations (724 ± 18%, $P < 0.05$, $n = 9$) (Fig. 2). This analysis suggests that fatty acids are able to stimulate the gene expression of their transporter at the adipose cell membrane and thus modulate their cytosol concentrations.

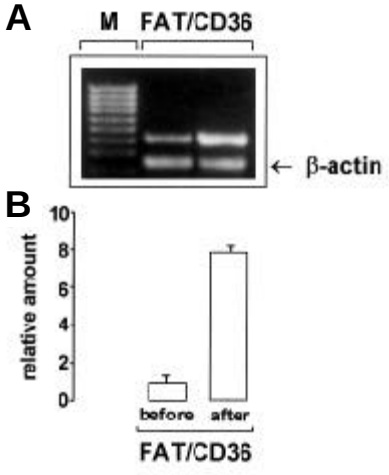

FIG. 2. FAT/CD36 expression before and after Intralipid plus heparin infusion in the subcutaneous fat of healthy volunteers. *A*: Representative agarose gel showing RT-PCR analysis of FAT/CD36 and β-actin mRNA content in a fat sample of one subject. *B*: Densitometric analysis of the ratios of CD36/β-actin mRNA abundance normalized to arbitrary units by assigning the value of 1 to the CD36/β-actin mRNA ratio before Intralipid plus heparin infusion. Bars represent the mean ± SE of the nine subjects ($P < 0.05$ vs. before infusion). The FAT/CD36 mRNA levels did not change after saline infusion. M, molecular weight marker.

Because the expression of PPAR-γ2, but not PPAR-γ1, protein appears to be increased by a low-calorie diet and downregulated in fat of obese subjects (24) (and is also the major activator of fat cell differentiation), we investigated whether the increased levels of plasma fatty acids modulate PPAR-γ2 mRNA levels and found that there was a marked increase (Fig. 3; 200 ± 8%, $P < 0.05$, $n = 9$), as previously described (25). This increase of PPAR-γ2 mRNA levels implied that fatty acids may be directly involved in modulating the expression of a gene relevant for fat cell differentiation; therefore, because it has been demonstrated that the transcriptional activation of PPAR-γ2 triggers the process of fat cell differentiation by transactivating adipose-specific genes (including those that encode proteins involved in lipid storage and metabolism), we examined the effects of fatty acids on the expression of four other fat cell genes induced during differentiation: leptin, UCP-2, UCP-3, and TNF-α.

Although high-fat feeding has been shown to lead to increased leptin gene expression in rats, it is not certain that an acute increase in NEFA availability has any direct effect on leptin production in humans. Figure 3 shows that the Intralipid infusion markedly increased the level of leptin mRNA (110 ± 13%, $P < 0.05$, $n = 9$), thus suggesting that NEFAs also modulate leptin gene expression in humans. The duration of the infusion suggests that this may be due to direct gene transactivation, probably by PPAR-γ2. Interestingly, mean plasma leptin levels were unaffected by the 5-h Intralipid plus heparin (2.8 ± 0.4 ng/ml) or saline infusion (2.9 ± 0.9 ng/ml) in comparison with baseline (3.2 ± 0.6 and 3.2 ±1.0 ng/ml, respectively), thus confirming the results obtained by Samra et al. (26).

A number of proteins with a high degree of homology to UCP-1 have recently been identified, including the widely expressed UCP-2 (13,14) and UCP-3 (15,16), which are primarily expressed in skeletal muscle. Functional studies of the various UCPs in yeast or mammalian cell expression systems

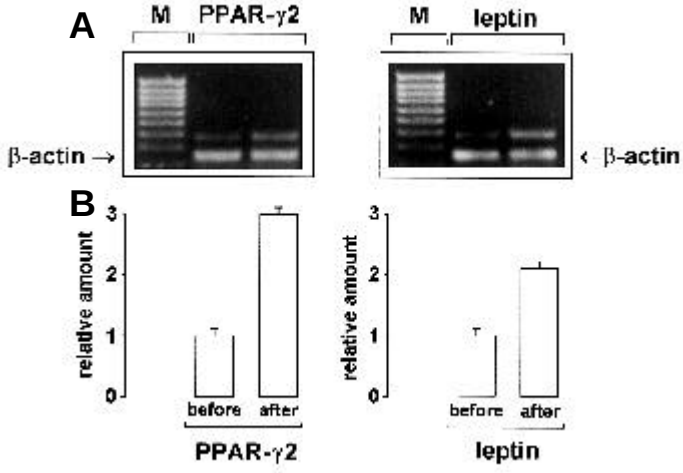

FIG. 3. PPAR-γ2 and leptin expression before and after Intralipid plus heparin infusion in the subcutaneous fat of healthy volunteers. *A*: Representative agarose gel showing RT-PCR analysis of PPAR-γ2, leptin, and β-actin mRNA content in a fat sample of one subject. *B*: Densitometric analysis of the ratios of PPAR-γ2 and leptin/β-actin mRNA abundance normalized to arbitrary units by assigning the value of 1 to the PPAR-γ2 and leptin/β-actin mRNA ratios before Intralipid plus heparin infusion. Bars represent the mean ± SE ($P < 0.05$ vs. before infusion). The PPAR-γ2 and leptin mRNA levels did not change after saline infusion. M, molecular weight marker.

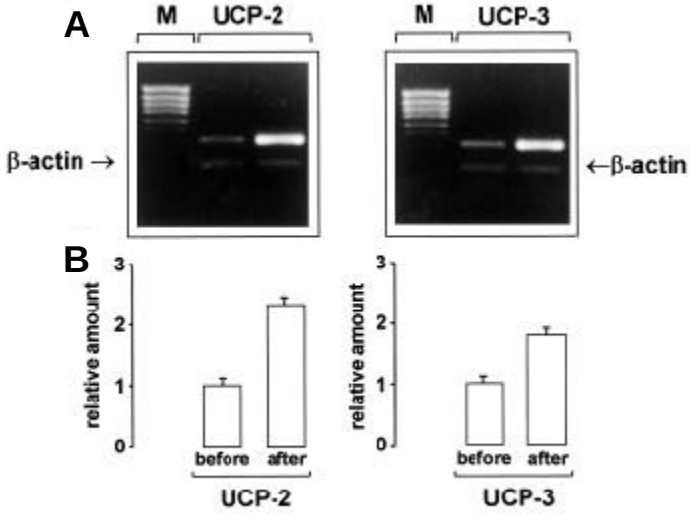

FIG. 4. UCP 2 and 3 expression before and after Intralipid plus heparin infusion in the subcutaneous fat of healthy volunteers. *A*: Representative agarose gel showing RT-PCR analysis of UCP-2, UCP-3, and β-actin mRNA content in a fat sample of one subject. *B*: Densitometric analysis of the ratios of UCP-2 and UCP-3/β-actin mRNA abundance normalized to arbitrary units by assigning the value of 1 to the UCP-2 and UCP-3/β-actin mRNA ratios before Intralipid plus heparin infusion. Bars represent the mean ± SE ($P < 0.05$ vs. before infusion). The UCP-2 and UCP-3 mRNA levels did not change after saline infusion. M, molecular weight marker.

have indicated that UCP-2 and UCP-3 both have uncoupling activity (27). Because adipose UCP-2 has been found to be upregulated by a high-fat diet (28), we investigated whether increased plasma fatty acid levels could affect the expression of these genes in the subcutaneous adipose tissue of normal subjects. The human UCP-3 gene generates two mRNA transcripts: UCP-3L (encoding a protein that is similar in length to UCP-1 and UCP-2) and UCP-3S (encoding a protein lacking the last 37 COOH-terminal residues) (27). UCP-3L and UCP-3S have been reported to have qualitatively similar effects in whole yeast and isolated mitochondria (27). The UCP-3 primers used in our RT-PCR experiments recognize UCP-3L, and Fig. 4 shows that both UCP-2 and UCP-3 mRNA levels increased after Intralipid plus heparin infusion by 120 ± 7 and 80 ± 5%, respectively ($P < 0.05$, $n = 9$).

Finally, Fig. 5 shows that the level of TNF-α mRNA markedly increased (130 ± 12%, $P < 0.05$, $n = 9$) in comparison with pretreatment levels. It has been widely reported that TNF-α can affect adipose tissue metabolism at various levels, and our findings imply that fatty acids can modulate the expression of this cytokine in fat. Saline infusion did not affect FAT/CD36, PPAR-γ2, leptin, UCP-2, UCP-3, and TNF-α mRNA levels (data not shown).

DISCUSSION

High systemic concentrations of NEFAs are known to be associated with impaired insulin sensitivity. They decrease insulin-stimulated glucose uptake and glycogen synthase activity in skeletal muscle, whereas liver gluconeogenesis is enhanced, and there is an increase in hepatic glucose output (29,30). Furthermore, β-cell insulin secretion is also affected by circulating NEFAs, and long-chain fatty acids induce apoptosis of cultured β-cells (31). In addition to skeletal muscle and liver, adipocytes may be involved in this event because their functional activity can be influenced by both insulin and NEFAs. To evaluate the acute effects of increased circulating NEFAs on adipocyte gene expression, the experimental protocol of the present study was designed to avoid any significant change in plasma insulin levels.

Because local high fatty acid concentrations may have toxic effects on cells (32), a particularly interesting result of

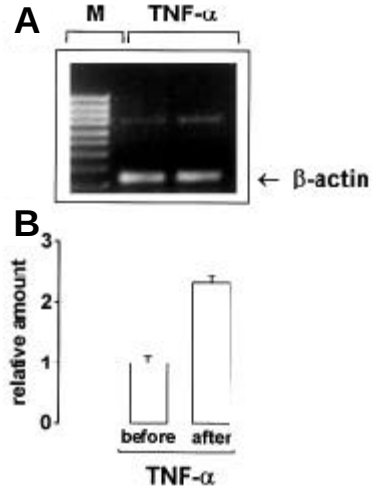

FIG. 5. TNF-α expression before and after Intralipid plus heparin infusion in the subcutaneous fat of healthy volunteers. *A*: Representative agarose gel showing RT-PCR analysis of TNF-α and β-actin mRNA content in a fat sample of one subject. *B*: Densitometric analysis of the ratios of TNF-α/β-actin mRNA abundance normalized to arbitrary units by assigning the value of 1 to the TNF-α/β-actin mRNA ratio before Intralipid plus heparin infusion. Bars represent the mean ± SE ($P < 0.05$ vs. before infusion). The TNF-α levels did not change after saline infusion. M, molecular weight marker.

our study is the finding that NEFAs are able to increase FAT/CD36 mRNA levels after a 5-h infusion. FAT/CD36 has been suggested to be a fatty acid receptor/transporter by means of labeling with reactive derivatives of long-chain fatty acids (4,5). Its expression favors the tissues that are active in fatty acid metabolism, including adipose tissue, heart muscle, and oxidative skeletal muscle. FAT/CD36 function seems to be activated by fatty acids (5,6) and it may be a transmembrane transporter of long-chain fatty acids, even if this particular role of FAT/CD36 is still controversial. If this is the case, ours and others' findings would suggest that increased circulating NEFAs can induce the expression and function of a protein that would oppose circulating and local high fatty acid levels by allowing transport into adipose cells.

It has been reported that the expression of FAT/CD36 may be directly induced by the PPAR-γ nuclear transcription factor (33), which in turn may play a role in adipogenesis and insulin action (8). It is worth noting that our present and previous results show that Intralipid plus heparin infusion markedly increases PPAR-γ2 mRNA levels in subcutaneous fat, thus implying that high concentrations of circulating NEFA in normal weight subjects induce the expression of a nuclear transcription factor known to induce the differentiation of immature cells into mature fat storage cells, probably by modulating the expression of the genes encoding the proteins involved in regulating these processes. Furthermore, fatty acids seem to increase the ability of mature adipose cells to take up circulating fatty acids by enhancing FAT/CD36 gene expression, and PPAR-γ has been demonstrated to stimulate fatty acid uptake in adipocytes (34).

Because PPAR-γ gene expression can be modulated by leptin (35) and leptin has been reported to modulate the expression of genes coding for UCP-2 and TNF-α either directly or through PPAR-γ (35), it is interesting that, as also previously reported (25,36), a 5-h Intralipid plus heparin infusion increases leptin mRNA levels in subcutaneous fat without leading to any significant changes in plasma leptin levels in normal weight individuals. Under similar experimental conditions, a 5-h exogenous triacylglycerol emulsion infusion failed to increase plasma leptin concentrations or leptin release from adipose tissue (26). The reason why leptin mRNA is affected by NEFA infusion whereas circulating leptin levels are not remains to be explained. However, it is possible to speculate that an increase in leptin gene expression that is not paralleled by increased circulating leptin levels suggests that increased local concentrations of leptin protein could have a paracrine function in adipocytes. Alternatively, one can speculate also that 5 h is not a sufficient period of time to allow a detection of increased circulating leptin levels.

Different groups have recently demonstrated that leptin increases the rate of lipolysis in white fat pads ex vivo (37,38) and decreases in vivo tissue triglyceride stores compared with pair-fed control subjects. Under our experimental conditions, it is unlikely that NEFA-induced leptin gene expression influenced the rate of lipolysis, since it has been shown that increased NEFA availability from an extracellular source inhibits intracellular lipolysis (26,39). The intracellular role of NEFA-induced leptin gene expression may rather be that of modulating the expression of other genes involved in the regulation of cell metabolism.

Evidence has recently been found to indicate that leptin and PPAR-γ agonists are able to induce UCP-2 gene expression in adipocytes and muscle cells (35,40). Unlike the situation in skeletal muscle in which a fasting-induced increase in plasma NEFA levels stimulates UCP-3 but not UCP-2 gene expression (17), the increased circulating NEFA levels due to Intralipid plus heparin infusion increased both UCP-2 and UCP-3 in subcutaneous fat, thus indicating that the functional roles of UCP-2 and UCP-3 may be different in various tissues. However, it must be emphasized that the increase in circulating NEFA levels induced by fasting or Intralipid infusion represents two completely different experimental conditions in terms of the metabolic fate of free fatty acids. Under fasting conditions, NEFA levels increase as a consequence of their release from adipocytes to supply muscle energy requirements, whereas the circulating NEFA levels induced by Intralipid infusion are preferentially channeled to fat stores.

TNF-α mRNA levels also increase in subcutaneous fat after Intralipid plus heparin infusion. TNF-α stimulates lipolysis (41) partially by downregulating the production and activity of LPL (42) and reduces lipogenesis. Furthermore, TNF-α induces insulin resistance in adipocytes (and skeletal muscle cells) in vitro by inhibiting insulin receptor tyrosine kinase activity as a result of the production of a serine phosphorilated (and hence inhibitory) insulin receptor substrate 1 (43) and downregulating mRNA for the insulin-sensitive glucose transporter GLUT4 (44). Finally, TNF-α impairs human preadipocyte differentiation, induces the dedifferentiation of mature adipocytes (45), and increases the apoptosis of both human preadipocytes and adipocytes (46). NEFA-induced TNF-α gene expression in subcutaneous fat may therefore be seen as a mechanism that tries to compensate for excessive cell lipid accumulation and fat mass by limiting a further increase in adipocyte volume and number.

Taken together, our findings suggest that NEFAs are involved in the partitioning of calories to fat in humans mainly by inducing the genes that code factors that enhance fatty acid flux into adipocytes (FAT/CD36), promote fat cell differentiation and fat storage (PPAR-γ2), and control energy metabolism (leptin and UCPs). The net effect of the rise in TNF-α mRNA levels could be to reduce or prevent an increase in lipid accumulation and adipose mass by mediating a decrease in both adipocyte volume (insulin resistance and lipolytic effects) and adipocyte number (antidifferentiation, dedifferentiation, and apoptotic effects). The relevance of the acute induction of the expression of each of these genes as a result of increased circulating NEFA levels can be seen if we consider that chronic exposure to high circulating fatty acid levels is known to cause an increase in fat mass as well as insulin resistance.

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