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Increase in Uncoupling Protein-2 mRNA Expression by BRL49653 and Bromopalmitate in Human Adipocytes

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Uncoupling protein-2 (UCP2) is a novel mitochondrial protein that may be involved in the control of energy expenditure. We have previously reported an upregulation of adipose tissue UCP2 mRNA expression during fasting in humans. Analysis of changes in metabolic parameters suggested that fatty acids may be associated with the increased UCP2 mRNA level. Culture of human adipose tissue explants was used to study *in vitro* regulation of adipocyte UCP2 gene expression. A 48-h treatment with BRL49653 and bromopalmitate, two potent activators of PPARγ, resulted in a dose-dependent increase in UCP2 mRNA levels. The induction by BRL49653 was rapid (from 6 h) and maintained up to 5 days. TNFα provoked a 2-fold decrease in UCP2 mRNA levels. Human recombinant leptin did not affect UCP2 mRNA expression. The data support the hypothesis that fatty acids are involved in the control of adipocyte UCP2 mRNA expression in humans.

Uncoupling protein-2 (UCP2) is a novel member of the mitochondrial carrier family expressed in a wide range of tissues (1, 2). The postulated role of the protein is to contribute to the partial coupling of respiration to ADP phosphorylation that exists in mitochondria from most tissues. UCP2 could therefore play a role in the control of energy expenditure. Recently, we have investigated the effect of a 5-day severe calorie restriction in humans (3). Dieting resulted in a 2-fold induction of UCP2 mRNA in adipose tissue. Fasting provokes a complex physiological adaptation with numerous hormonal and metabolic changes that could explain the increase in UCP2 mRNA levels. Among the hormones that have been shown to upregulate UCP2 gene expression in rodents (4, 5), leptin and triiodothyronine are not likely to play a role because their plasma levels decrease during fasting. Moreover, we have shown that insulin does not acutely regulate UCP2 mRNA levels in humans (3). Calorie restriction induces an increase in adipose tissue lipolysis (6), resulting in an important fatty acid release from body fat stores. Thiazolidinediones (TZD) and fatty acids stimulate UCP2 gene expression in murine adipocyte cell lines (7, 8). Therefore, fatty acids may be positive regulators of UCP2 mRNA expression in human adipose tissue.

To study the regulation of UCP2 mRNA expression in human adipocytes, we used a system based on culture of adipose tissue explants and subsequent isolation of mature adipocytes. In the present work, the effects of a stable analog of fatty acid (bromopalmitate), TZD (BRL49653 and ciglitazone) and cytokines (leptin and TNFα) on UCP2 mRNA levels are reported.

MATERIALS AND METHODS

Materials. Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum (FCS) were from Life Technology (Gaithersburg, MD), human recombinant leptin from Calbiochem-Novabiochem (Nothingham, UK), collagenase A from Boehringer Mannheim (Mannheim, Germany), penicillin, streptomycin, gentamycin, 2-bromopalmitate, TNFα and bovine serum albumin (fraction V) from Sigma Aldrich Chemicals (St-Louis, MO). BRL 49653 was a kind gift from Smith-Kline Beecham Pharmaceuticals (Harlow, UK).

Tissue culture. Human adipose tissue was obtained from the subcutaneous fat depots of female subjects with the agreement of the Ethics Committee of Toulouse University Hospitals. Subjects were from 28 to 64 years old and their body mass indexes ranged from 19 to 32 kg/m^2. Surgical samples were dissected out from skin and vessels, rinsed once in phosphate buffer saline (PBS) and transferred into a sterile environment. Part of the samples was used for adipocyte isolation from fresh adipose tissue. Then, fat pads were cut into small pieces ranging from 100 to 400 mg, then placed in DMEM supplemented with 5% FCS, penicillin (200U/ml), streptomycin (50 μg/ml) and gentamycin (200 μg/ml). Fat pieces corresponding to 6 to 9 g of adipose tissue were distributed into medium-containing 25 cm^2 polystyrene flasks (Falcon, Becton Dickinson, Meylan, France) and maintained at 37°C in a 7% CO_2 chamber. One day later, the medium was replaced by 1% FCS-enriched DMEM containing the drug tested. The medium was changed every day during the culture. At the end of the treatment, the medium was removed and the fat pieces were immediately digested for 1 h with 0.5 mg/ml collagenase A in DMEM containing 3% (w/v) bovine serum albumin at 37°C under gentle agitation. Isolated adipocytes were filtered through a nylon

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TABLE 1

Effects of Thiazolidinediones, Bromopalmitate, and Cytokines on UCP2 mRNA Levels in Human Adipocytes

Drugs	Mean ± SD	n
BRL49653 (1 μM)	191 ± 18	23
Ciglitazone (10 μM)	165	2
Bromopalmitate (250 μM)	238 ± 20	6
Leptin (1 nM)	107 ± 32	9
TNFα (3 nM)	57 ± 8	3

Note. Values are expressed as percentages of the ratio of UCP2 to β-actin mRNA levels obtained without treatment. mRNA levels were measured on isolated adipocyte total RNA from adipose tissue explants treated for 12 h (TNFα) for 2 days (other drugs). n, number of independent experiments.

mesh and washed twice with PBS. The infranatant was removed and the resulting packed cells (about 5 ml) were lysed with an equivalent amount of denaturing buffer from RNeasy kit (Qiagen, Hilden, Germany) then stored at −80°C. For each time point, control flasks containing untreated explants were prepared.

RNA analysis. Total RNA was extracted using the Qiagen RNeasy kit and stored at −80°C. Total RNA was electrophoresed in a 1% agarose, 2.2 M formaldehyde gel, transferred onto a nylon membrane (Schleicher and Schuell, Dassell, Germany) and UV-crosslinked. Hybridization was carried out for 1 hour at 68°C in Express Hyb solution (Clontech, Palo Alto, CA) with ^{32}P-labelled cDNA probes. Blots were washed at a final stringency of 15 mM NaCl, 1.5 mM citric acid, 0.1% SDS at 65°C. The blots were hybridized with a 290 bp human UCP2 probe then stripwashed and reprobed with a 388 bp human β-actin probe. The UCP2 hybridization signals were analysed with a Phosphorimager (Molecular Dynamics, Sunnyvale, CA) and quantified using ImageQuant Software.

RESULTS

Our initial attempts to study the regulation of UCP2 mRNA expression in cultured human adipocytes were unsuccessful because of a decrease in gene expression over time and large differences in the viability of cells from various individuals. To alleviate this problem, we set up a culture system based on drug treatment of adipose tissue explants and measurements of mRNA levels in isolated adipocytes. Compared to freshly isolated adipocytes, UCP2 mRNA levels did not change after 3 days of culture (95 ± 22%, n = 4). Explants were treated for 48 h in the presence or the absence of TZD (Table 1). BRL49653 is a TZD that binds to PPARγ, an important transcriptional regulator of gene expression in adipocytes. BRL49653 induced a dose-dependent increase in UCP2 mRNA levels (Fig. 1). The compound was active in the low μM range and induced a more than 2-fold increase at 10 μM. A study of the time course of induction with 1 μM of BRL49653 (n = 2) showed that the effect was rapid with a 2.3-fold increase after 6h of treatment. The level of induction was maintained for 2 days, and slightly decreased after 3 days (1.8-fold) and 5 days (1.6-fold). Ciglitazone, another PPARγ agonist, also induced an increase in UCP2 mRNA levels. To evaluate the effect of fatty acids, we used bromopalmitate which is a slowly metabolized analog of palmitate (9). A dose-dependent induction of UCP2 mRNA expression was obtained with a 2.4-fold increase at 250 μM (Fig. 2). Leptin and TNFα are cytokines that provoke, *in vivo,* an increase and a decrease in rodent adipose tissue UCP2 mRNA levels, respectively (5, 10). Human recombinant leptin did not modify UCP2 mRNA levels (Table 1). A marked decrease was observed with TNFα.

DISCUSSION

In vivo studies in humans and in rodents have pointed to a role for fatty acids, TZD and cytokines in the regulation of white adipose tissue UCP2 gene expression. To our knowledge, the effect of TZD and fatty acids on gene expression has not been studied in human mature adipocytes. Maintenance of isolated human adipocytes for several days is difficult because of the fragility of the cells and marked decrease in gene expression ((11) and present work). An alternative approach is the use of human adipose tissue explants that partly maintain the *in vivo* structure of the tissue and

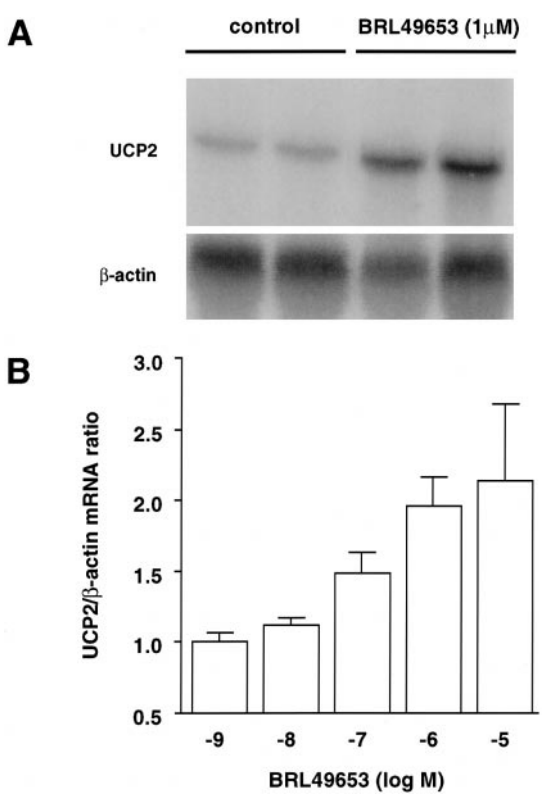

FIG. 1. The effect of BRL49653 on UCP2 mRNA levels in human adipocytes. (A) Northern blot showing adipocyte UCP2 and β-actin mRNA levels from adipose tissue explants treated or not (control) for 2 days. (B) Dose-response effect of BRL49653. Values (means ± SD of 4 experiments) are expressed as percentages of the ratio of UCP2 to β-actin mRNA levels obtained without treatment.

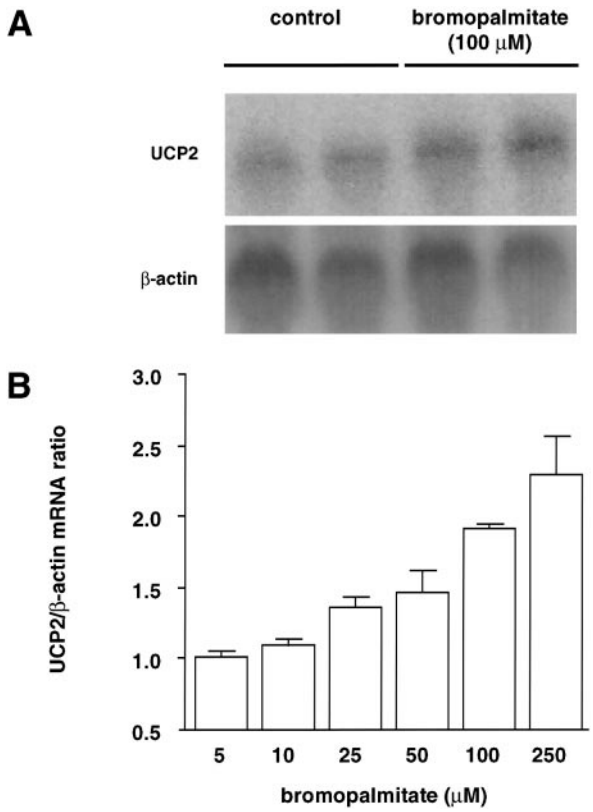

FIG. 2. The effect of 2-bromopalmitate on UCP2 mRNA levels in human adipocytes. (A) Northern blot showing adipocyte UCP2 and β-actin mRNA levels from adipose tissue explants treated or not (control) for 2 days. (B) Dose-response effect of 2-bromopalmitate. Values (means $\pm$ SD of 3 experiments) are expressed as percentages of the ratio of UCP2 to β-actin mRNA levels obtained without treatment.

permits long-term culture (12, 13). Because UCP2 is expressed in adipose tissue cell types other than adipocytes, we combined the culture of explants to the measurement of mRNA levels on isolated mature adipocytes. The present report shows that fatty acids and TZD are direct regulators of UCP2 mRNA levels. The concentrations of compounds required to increase gene expression are compatible with an activation of PPARγ, which is the main form of PPAR expressed in human adipocytes (14). The data are also consistent with the effect of TZD and fatty acids in murine adipocyte cell lines (7, 8). A positive regulation of UCP2 gene expression by fatty acids could explain the upregulation observed during fasting in human adipose tissue (3). An upregulation of UCP2 by fatty acids is also of interest in view of recent data obtained in mice after high fat feeding (15). High fat diet increases white adipose tissue UCP2 gene expression in the obesity-resistant A/J and C57BL/KsJ strains but not in the obesity-prone C57BL/6J mice. Interestingly, the diet does not affect UCP2 and UCP3 mRNA expression in skeletal muscle and brown adipose tissue. Therefore, an early induction of adipose tissue UCP2 expression by high fat diet is associated with resistance to obesity in rodents.

The effect of cytokines was also investigated. Injection of bacterial lipopolysaccharide (LPS) increases UCP2 mRNA expression in liver, skeletal muscle and white adipose tissue in mice (10). The effect could partly be mediated by TNFα. We therefore examined whether the proinflammatory cytokine had an effect on UCP2 mRNA level in human adipose tissue. TNFα induced a marked decrease in UCP2 mRNA expression. A downregulation of the level of UCP2 mRNA by LPS has recently been observed *in vitro* in rat peritoneal macrophages whereas TNFα induces UCP2 mRNA expression in primary hepatocytes (16). It seems therefore that the effect of TNFα on UCP2 gene expression is cell-specific. In adipocytes, TNFα decreases the expression of C/EBPα and PPARγ, that are transcription factors crucial for adipogenesis (17, 18). Whether C/EBPα and PPARγ participate in the transcriptional control of the UCP2 gene remains to be demonstrated. Interestingly, as observed for other genes expressed in adipocytes (18), BRL49653 and TNFα have opposite effects on UCP2 gene expression. Intracerebroventricular leptin infusion with no change in peripheral plasma levels favors UCP2 mRNA expression in rat white adipose tissue (5). An *in vitro* induction of UCP2 mRNA was observed in rat pancreatic islets in the presence of leptin (19). A similar concentration was used to study the effect of leptin on UCP2 mRNA levels in human adipocytes. No variation in UCP2 mRNA expression was observed. The data suggest that the *in vivo* induction of adipose tissue UCP2 expression is mediated by a central effect of leptin.

In conclusion, UCP2 mRNA levels are induced by fatty acid and TZD in human adipocytes. These results support the hypothesis that the *in vivo* induction of adipose tissue UCP2 mRNA during fasting is mediated by fatty acids.

REFERENCES

1. Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., Ricquier, D., and Warden, C. H. (1997) *Nat. Genet.* **15,** 269–272.
2. Gimeno, R. E., Dembski, M., Weng, X., Deng, N., Shyjan, A. W., Gimeno, C. J., Iris, F., Ellis, S. J., Woolf, E. A., and Tartaglia, L. A. (1997) *Diabetes* **46,** 900–906.
3. Millet, L., Vidal, H., Andreelli, F., Larrouy, D., Riou, J. P., Ricquier, D., Laville, M., and Langin, D. (1997) *J. Clin. Invest.* **100,** 2665–2670.
4. Masaki, T., Yoshimatsu, H., Kakuma, T., Hidaka, S., Kurokawa, M., and Sakata, T. (1997) *FEBS Lett.* **418,** 323–326.
5. Cusin, I., Zakrzewska, K. E., Boss, O., Muzzin, P., Giacobino, J. P., Ricquier, D., Jeanrenaud, B., and Rohner-Jeanrenaud, F. (1998) *Diabetes* **47,** 1014–1019.
6. Stich, V., Harant, I., De Glizesinski, I., Crampes, F., Berlan, M., Kunesova, M., Hainer, V., Dauzats, M., Rivière, D., Garrigues,

M., Holm, C., Lafontan, M., and Langin, D. (1997) *J. Clin. Endocrinol. Metab.* **82,** 739–744.

7. Aubert, J., Champigny, O., Saint-Marc, P., Négrel, R., Collins, S., Ricquier, D., and Ailhaud, G. (1997) *Biochem. Biophys. Res. Commun.* **238,** 606–611.
8. Camirand, A., Marie, V., Rabelo, R. and Silva, J. E. (1998) *Endocrinology* **139,** 428–431.
9. Grimaldi, P. A., Knobel, S. M., Whitesell, R. R., and Abumrad, N. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10930–10934.
10. Faggioni, R., Shigenaga, J., Moser, A., Feingold, K. R., and Grunfeld, C. (1998) *Biochem. Biophys. Res. Commun.* **244,** 75–78.
11. Plée-Gauthier, E., Grober, J., Duplus, E., Langin, D., and Forest, C. (1996) *Biochem. J.* **318,** 1057–1063.
12. Smith, U. (1970) *Anat. Rec.* **169,** 97–104.
13. Fried, S. K., Russell, C. D., Grauso, N. L. and Brolin, R. E. (1993) *J. Clin. Invest.* **92,** 2191–2198.
14. Auboeuf, D., Rieusset, J., Fajas, L., Vallier, P., Frering, V., Riou, J. P., Staels, B., Auwerx, J., Laville, M., and Vidal, H. (1997) *Diabetes* **48,** 1319–1327.
15. Surwit, R. S., Wang, S., Petro, A. E., Sanchis, D., Raimbault, S., Ricquier, D., and Collins, S. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 4061–4065.
16. Cortez-Pinto, H., Yang, S. Q., Lin, H. Z., Costa, S., Hwang, C. S., Lane, M. D., Bagby, G., and Diehl, A. M. (1998) *Biochem. Biophys. Res. Commun.* **251,** 313–319.
17. Stephens, J. M., and Pekala, P. H. (1991) *J. Biol. Chem.* **266,** 21839–21845.
18. Rosenbaum, S. E., and Greenberg, A. S. (1998) *Mol. Endocrinol.* **12,** 1150–1160.
19. Zhou, Y. T., Shimabukuro, M., Koyama, K., Lee, Y., Wang, M. Y., Trieu, F., Newgard, C. B., and Unger, R. H. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 6386–6390.