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Regulation of Gene Expression by Activation of the Peroxisome Proliferator-Activated Receptor γ with Rosiglitazone (BRL 49653) in Human Adipocytes

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To better define the mechanism of action of the thiazolidinediones, we incubated freshly isolated human adipocytes with rosiglitazone and investigated the changes in mRNA expression of genes encoding key proteins of adipose tissue functions. Rosiglitazone (10^{-6} M, 4 h) increased p85αphosphatidylinositol 3-kinase (p85αPI-3K) and uncoupling protein-2 mRNA levels and decreased leptin expression. The mRNA levels of insulin receptor, IRS-1, Glut 4, lipoprotein lipase, hormone-sensitive lipase, acylation-stimulating protein, fatty acid transport protein-1, angiotensinogen, plasminogen activator inhibitor-1, and PPARγ1 and γ2 were not modified by rosiglitazone treatment. Activation of RXR, the partner of PPARγ, in the presence of rosiglitazone, increased further p85αPI-3K and UCP2 mRNA levels and produced a significant augmentation of Glut 4 expression. Because p85αPI-3K is a major component of insulin action, the induction of its expression might explain, at least in part, the insulin-sensitizing effect of the thiazolidinediones. © 1999 Academic Press

Thiazolidinediones are oral hypoglycemic agents that reduce blood glucose, insulin and triglyceride levels in insulin resistant animal models and in type-2 (non-insulin-dependent) diabetic patients (1–3). They also increase insulin sensitivity in peripheral tissues as demonstrated in type-2 diabetic patients treated with troglitazone (1, 3). Although their mechanism of action is not entirely understood, it is well recognized that thiazolidinediones are high-affinity ligands of the peroxysome proliferator-activated receptor γ (PPARγ), a member of the nuclear receptor family (4), also identified as NR1C3 in the new nuclear receptor nomenclature (5). The relative potency of different thiazolidinediones to bind and to activate PPARγ *in vitro* correlates with their antidiabetic action *in vivo* (6, 7), indicating thus that PPARγ mediates the insulin sensitizing effect of these molecules. PPARγ is predominantly expressed in differentiated adipocytes (8–10) suggesting that adipose tissue might be one of the main target tissues of the thiazolidinediones. There are three PPARγ mRNAs (γ1, γ2 and γ3) giving rise to two proteins (γ1, γ2) (11) and that are generated from the same gene by alternative promoter usage and mRNA splicing (12, 13). The two proteins differ in their 28 N-terminus amino acids in humans (13). In subcutaneous adipose tissue and isolated adipocytes, PPARγ1 mRNA represents more than 80% of the PPARγ transcripts (10).

PPARγ plays a major role in adipocyte differentiation (14, 15). Nevertheless, the high level of expression in mature adipocytes (9, 10) indicates that PPARγ is also involved in the control of the metabolic functions of the fully differentiated adipocytes. Accordingly, most of the known PPARγ target genes encode adipose tissue proteins involved in lipid storage and metabolism, like, for example, adipose fatty acid-binding protein (16), lipoprotein lipase (17) or fatty acid transport protein-1 (18, 19). PPARγ is a nuclear receptor that heterodimerizes with the 9-*cis*-retinoic acid receptor (RXR) (20) to control the transcription of target genes. The Peroxisome Proliferator Responsive Elements (PPREs) generally consist of a hexameric nucleotide

Abbreviations used: PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator responsive element; RT-cPCR, reverse-transcription competitive polymerase chain reaction, BMI, body mass index; IRS-1, insulin receptor substrate-1; p85αPI-3K, p85α regulatory subunit of phosphatidylinositol 3-kinase; UCP-2, uncoupling protein-2; Glut 4, insulin-dependent glucose transporter; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; ASP, acylation stimulating protein; PAI-1, plasminogen activator inhibitor-1.

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direct repeat spaced by one nucleotide (DR-1) (21). The demonstration that a gene is a target of the PPARs requires thus the identification of a functional PPRE in its promoter sequence. To date, only a few number of genes have been really identified as target genes of PPARγ (16, 17, 19, 21, 22). Nevertheless, valuable indications can be also obtained by studying the changes in the expression level of specific genes upon activation of PPARγ by thiazolidinediones. Using such approach, several other putative target genes have been reported, mainly on the basis of investigations in rodents or in cell lines (17, 23–30). Moreover, because activation of PPARγ promotes important modifications of the metabolism of the animals as well as of cell functions and differentiation, it might be difficult to ascertain whether the observed changes in gene expression result from a direct transcriptional effect of PPARγ or represent secondary events of thiazolidinedione treatment (30). To overcome such problems, it may be possible to use fully differentiated cells. To date, only a limited number of studies have been performed in human mature adipocytes (31–33).

In this work, we examined whether genes encoding important proteins of adipose tissue functions and metabolism are possible targets of PPARγ in human adipocytes. Isolated mature adipocytes and short incubation periods were used to limit possible effects due to PPARγ-induced cell differentiation. The cells were incubated with rosiglitazone (BRL 49653), a potent PPARγ-selective thiazolidinedione (4), and we measured the rapid changes in the expression levels of 13 candidate genes using specific reverse transcription-competitive polymerase chain reaction (RT-cPCR) mRNA assays.

METHODS

Products. Rosiglitazone and LG 1069, a selective RXR agonist (34), were kindly provided by Dr. Dr. Heyman and Dr. M. Leibowitz (Ligand Pharmaceuticals, San Diego, CA).

Preparation of human isolated adipocytes. Abdominal subcutaneous adipose tissue (8 $\pm$ 2 g wet weight, n = 24) was obtained during surgery with the informed consent of the patients, in agreement with the Ethic Committee of the Hospices Civils of Lyon (France). None of the subjects suffered from severe systemic illness, diabetes mellitus or dyslipidemia. Their age ranged from 33 to 77 years (mean $\pm$ SD = 59 $\pm$ 11 years) and their body mass indexes (BMI) from 21 to 61 (mean $\pm$ SD = 31 $\pm$ 8 kg/m^2). Eighteen subjects (11 women, 7 men) were obese (mean age: 57 $\pm$ 9 years, mean BMI: 33 $\pm$ 8 kg/m^2. The six lean subjects (4 women, 2 men) were 71 $\pm$ 6 years old and had a BMI of 23 $\pm$ 1.3 kg/m^2. The adipose tissue sample was immediately immersed in Hanks medium kept at 4°C and cell preparation was started within 15 min after tissue sampling. Adipocytes were isolated by collagenase digestion (0.5 mg/ml; type II, Sigma, La Verpillère, France) according to the method of Rodbell (35) with the modifications previously described (36). Isolated adipocytes were incubated at 37°C in DMEM (Life Technologies, Cergy Pontoise, France) containing 4% fatty acid free bovine serum albumin in a final volume of 1 ml. The average cell number in the incubation medium varied from 50,000 to 150,000 cells per milliliter. Cells were incubated 30 min at 37°C prior to the addition of the different agents or vehicle (0.1% dimethyl sulfoxide). The viability and the metabolic integrity of the cells prepared and incubated under these conditions have been verified (36). After 4 h of incubation, infranatant was removed by aspiration and cells were immediately lysed in the denaturating buffer from the RNeasy kit (Qiagen, Courtaboeuf, France) and stored at −80°C.

Total RNA preparation and quantification of the target mRNAs. Total RNA was prepared using the RNeasy total RNA kit from Qiagen. Total RNA concentration was determined by absorbance measurement at 260 nm. The 260/280 nm absorption ratio of all preparation was between 1.8 and 2.0. The average yield of total RNA was about 1 μg/100,000 cells.

The mRNA levels of 13 different candidate genes were determined by RT-cPCR. The method consists, after specific reverse transcription reactions, in the co-amplification of the target cDNAs with known amounts of a specific competitor DNA in the same tube (37). The construction of the different competitor DNA, the sequences of the primers and the validation of the different RT-cPCR assays have been reported elsewhere (10, 37–39). The coefficient of variation of the different assays was demonstrated to be between 5 and 15% (37). The RT-cPCRs and the analysis of the PCR products were performed in the conditions previously indicated (10, 37, 39).

Presentation of the results. The results were presented either as absolute mRNA levels, in amol/μg of total RNA, or as percentage of variation induced by drug treatments. The data are shown as means $\pm$ SEM. The nonparametric Wilcoxon test for paired values was used for statistical analysis. $p < 0.05$ was the threshold of significance.

RESULTS

The mRNA levels of 13 different genes encoding proteins involved in insulin signaling, lipid metabolism and adipocyte functions were quantified by RT-cPCR in human isolated adipose cells. Under the control condition, the expression levels of the target mRNAs displayed large differences. Lipoprotein lipase (LPL, 2371 $\pm$ 308 amol/μg total RNA, n = 5 obese subjects) and hormone sensitive lipase (HSL, 574 $\pm$ 106, n = 5 obese subjects) mRNAs were expressed at the higher levels. Insulin receptor (60 $\pm$ 14, n = 4 lean subjects), insulin receptor substrate-1 (IRS-1, 18 $\pm$ 6, n = 4 lean subjects), acylation stimulating protein (ASP, 16 $\pm$ 6, n = 3 obese subjects) and uncoupling protein-2 (UCP-2, 9 $\pm$ 2, n = 5 obese subjects) mRNA were expressed at intermediary levels. Plasminogen activator inhibitor-1 (PAI-1, 3.9 $\pm$ 0.8, n = 3 obese subjects), fatty acid transport protein-1 (FATP-1, 1.5 $\pm$ 0.4, n = 5 obese subjects) and angiotensinogen (0.16 $\pm$ 0.05, n = 3 obese subjects) were expressed at very low levels. The mRNA levels of p85α regulatory subunit of phosphatidylinositol 3-kinase (p85αPI-3K), leptin, the insulin-dependent glucose transporter (Glut 4) and PPARγ were measured in 10 preparations of adipocytes from lean and obese subjects, and are presented in Table I. A significant higher expression of leptin mRNA was observed in isolated adipocytes from obese subjects when compared to cells from lean individuals. On the opposite,

TABLE I

Levels of p85αPI-3K, Leptin, Glut 4, and PPARγ mRNAs in Isolated Human Adipocytes

		Control	Rosiglitazone (10^{-6} M)	% change
p85α PI3kinase	Lean (n = 4)	105 ± 19	162 ± 11[a]	68 ± 21
	Obese (n = 6)	134 ± 18	242 ± 41[a]	79 ± 21
Leptin	Lean (n = 5)	35 ± 5	24 ± 2[a]	−29 ± 5
	Obese (n = 5)	74 ± 14[b]	53 ± 7[a]	−35 ± 5
Glut 4	Lean (n = 5)	142 ± 37	167 ± 43	29 ± 21
	Obese (n = 5)	62 ± 14[b]	66 ± 15	28 ± 24
PPARγ (total)	Lean (n = 5)	38 ± 9	34 ± 5	−5 ± 9
	Obese (n = 5)	25 ± 5	30 ± 3	12 ± 9

Note. Isolated cells were incubated for 4 h with rosiglitazone or with vehicle alone (control). Levels of the mRNAs were determined by RT-cPCR. Total (γ1 + γ2) mRNA levels were shown for PPARγ.

[a] $p < 0.05$ for rosiglitazone vs control.

[b] $p < 0.05$ for obese vs lean subjects.

Glut 4 mRNA levels were higher in adipocytes from lean subjects. There was no differences in the basal mRNA levels of p85αPI-3K and PPARγ between adipocyte preparations from lean and obese subjects (Table I).

Figure 1 shows the percentages of change in the mRNA levels of the 13 target genes after 4 h of treatment with 10^{-6} M rosiglitazone. For the PPARγ gene, effects on both total PPARγ (γ1 + γ2) and PPARγ2 mRNA levels are presented (Fig. 1). Regarding the mRNAs encoding proteins involved in the insulin signaling pathway, rosiglitazone did not affect the expression levels of insulin receptor and IRS-1 while it promoted a significant increase (p = 0.03, n = 10) in the mRNA levels of p85αPI-3K. This effect of rosiglitazone was similarly found in adipocytes from lean and from obese subjects (Table I).

After 4 h of incubation, rosiglitazone did not affect the mRNA expression levels of the insulin-dependent glucose transporter Glut 4 in human adipocytes (Fig. 1 and Table I). Similarly, the mRNA levels of LPL, HSL, ASP, and FATP-1, were not modified during incubation with rosiglitazone in cells from obese subjects (Fig. 1).

The expression of PAI-1 and angiotensinogen has been recently documented in human adipose tissue. Our results indicate that the mRNA expression levels of these two components of the regulation of blood flow and vascular functions were not acutely modified by rosiglitazone in adipocytes from obese subjects (Fig. 1).

Regulation of leptin and UCP-2 mRNA expression by thiazolidinediones has been already reported in adipose cells. The present study confirmed these results in freshly isolated human adipocytes. Rosiglitazone promoted a slight (45%) but significant (p = 0.03, n = 5) increase in UCP-2 mRNA levels in cells isolated from obese subjects (Fig. 1). On another hand, rosiglitazone decreased leptin mRNA abundance by more than 30% (p = 0.03, n = 10). Leptin mRNA levels measured in cells from obese subjects were twice the levels found in adipocytes from lean subjects, but rosiglitazone reduced leptin mRNA expression to a similar extent in both groups (Table I).

Thiazolidinediones and rosiglitazone are high affinity ligands for the nuclear receptor PPARγ. It was therefore of interest to determine whether PPARγ can auto-regulates its expression. Figure 1 and Table I clearly show that rosiglitazone did not modify the mRNA levels of PPARγ (γ1 + γ2) after 4 h of incubation in human adipocytes from both lean and obese subjects. Incubation with troglitazone (10^{-6} M, 6 h), another thiazolidinedione, also did not affect PPARγ mRNA levels (data not shown). In human adipocytes, PPARγ2 represented about 15% of the total PPARγ transcripts (3.5 ± 1.1 amol/μg total RNA, n = 3). The mRNA levels of PPARγ2 were also not modified by rosiglitazone in 3 preparations from obese subjects (Fig. 1).

To verify whether the observed effects of rosiglitazone were mediated by the heterodimer PPARγ/RXR, we investigated the effect of a combination of rosiglitazone and LG 1069, a specific agonist of RXR, on the mRNA levels of p85αPI-3K, UCP-2 and leptin that were significantly changed by rosiglitazone alone, and of Glut 4, FATP-1 and PPARγ because these 3 genes have been previously suspected to be targets of the thiazolidinediones. Figure 2 shows the percentages of change in the mRNA levels of these 6 genes after 4 h of incubation with 10^{-6} M rosiglitazone and 10^{-6} M LG 1069. The treatment with the two molecules increased p85αPI-3K mRNA levels (116 ± 24%, n = 5) to an extent that seemed more important than with rosiglitazone alone (82 ± 18%, n = 5), but the difference did not reach significance (p = 0.225). On another hand, the combination of rosiglitazone and LG 1069 clearly improved the increase in UCP-2 mRNA levels induced by rosiglitazone alone (85 ± 25% vs 44 ± 12%, p = 0.04,

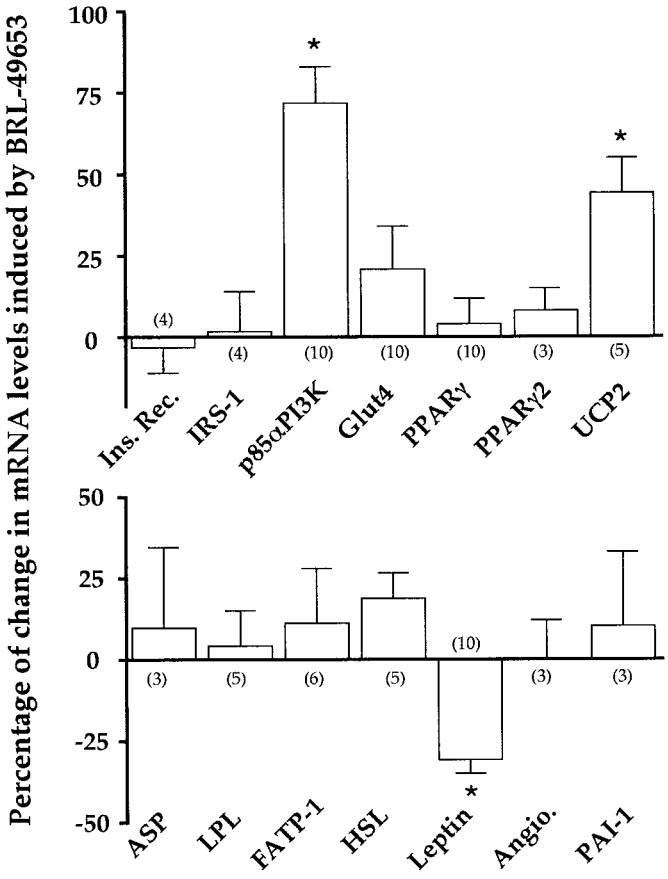

FIG. 1. Effects of rosiglitazone on the mRNA levels of selected genes in human isolated adipocytes. The levels of the 14 target mRNAs were determined by RT-cPCR in human adipocytes after 4 h of incubation with 10^{-6} M rosiglitazone. The results are presented as percentage change in mRNA levels. Values measured in cells incubated 4 h with 0.1% dimethyl sulfoxide (vehicle) were the references. The figure shows the mean ± SE for the number of independent preparations of adipocytes indicated in parentheses. *$p < 0.05$.

$n = 5$). Moreover, Glut 4 mRNA levels were significantly increased in the presence of the two agents (54 ± 14%, $p = 0.03$, $n = 6$) whereas rosiglitazone alone did not modified Glut 4 expression in human adipocytes (Fig. 1 and Table I). The effect of rosiglitazone and LG 1069 on leptin mRNA levels was similar to what was observed with rosiglitazone alone. Finally, the combination of the two agents did not modify the mRNA levels of PPARγ and FATP-1 (Fig. 2).

DISCUSSION

Thiazolidinediones are potential new pharmacological agents for the treatment of insulin resistance in type-2 diabetes mellitus (1, 3). Although they can bind and activate the nuclear receptor PPARγ (4, 6, 7), the precise mechanism of action of these drugs is still largely debated. Several possibilities have been proposed to explain their *in vivo* insulin sensitizing effect (see 40 for review). Activation of PPARγ by thiazolidinediones in adipose tissue may increase the production of small adipocytes that are more sensitive to insulin. Thiazolidinedione can also decrease the production and/or the biological activity of "adipocytokines" (i.e., tumor necrosis factor-α, leptin) that are suspected to induce insulin resistance, and finally, they can reduce circulating lipid and fatty acid concentrations by increasing their clearance by adipocytes. These hypotheses are mainly based on data obtained using animal models. Moreover, most of the human target genes of PPARγ are not yet known, limiting thus the understanding of the exact mechanism of action of these drugs.

The aim of this work was to verify whether some important genes involved in insulin action, lipid metabolism and adipocyte functions are target genes of PPARγ and rosiglitazone, a potent thiazolidinedione, in human adipocytes. Because PPARγ is highly expressed in adipose cells, we made the assumption that changes in the expression levels of putative target genes should be seen after only few hours of incubation. In addition, short incubation periods and the use of freshly isolated mature adipocytes should limit possible changes in gene expression that were secondary to PPARγ (or rosiglitazone)-induced modifications of

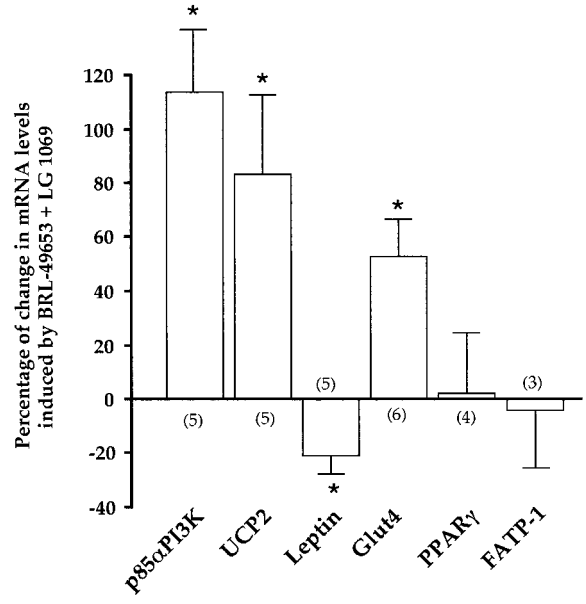

FIG. 2. Effect of rosiglitazone and LG 1069 on the mRNA levels of selected genes in human isolated adipocytes. The levels of the 6 target mRNAs were determined after 4 h of incubation with 10^{-6} M rosiglitazone and 10^{-6} M LG 1069. The results are presented as percentage change in mRNA levels. Values measured in cells incubated 4 h with 0.1% dimethyl sulfoxide (vehicle) were the references. The figure shows the mean ± SE for the number of independent preparations of adipocytes indicated in parentheses. *$p < 0.05$.

cell functions and differentiation. We found that activation of PPARγ by rosiglitazone increased the mRNA levels of the p85α subunit of phosphatidylinositol 3-kinase (p85αPI-3K) and of the uncoupling protein-2 (UCP2) genes. In addition, rosiglitazone decreased leptin mRNA levels in human adipocytes. The mRNA levels of insulin receptor, IRS-1, Glut 4, LPL, HSL, ASP, FATP-1, angiotensinogen, PAI-1, and PPARγ1 and γ2 were not modified after 4 h of incubation with rosiglitazone. The concomitant activation of RXR, the partner of PPARγ to form a transcriptionally active heterodimer, further increased the mRNA levels of p85αPI-3K and UCP2 and produced a significant augmentation in Glut 4 mRNA abundance.

UCP2 has been already reported to be target genes of PPARγ and the thiazolidinediones in different adipose cell models (29), including human adipocytes (33). As in the present work, a moderate, but significant, increase in UCP2 mRNA levels has been observed after 24 h of incubation of human adipose tissue with rosiglitazone (33). The amplified effect of rosiglitazone in the presence of LG 1069 strongly suggest that UCP2 is a target gene of the heterodimer PPARγ/RXR. Identification of an efficient PPRE in the promoter region of the human gene will be the next step to confirm this assumption.

Reduction in leptin gene expression by thiazolidinediones has been largely documented both *in vivo* and *in vitro* (23, 24, 32). Our results are in accordance with these data and extend them to freshly isolated human adipocytes. On another hand, LPL and FATP-1 have been demonstrated to be target genes of PPARγ in rodent adipocytes and PPREs have been identified in their promoter regions (17, 19). Surprisingly, we found that the mRNA levels of these two genes were not affected by rosiglitazone after 4 h of treatment in human mature adipocytes. A lack of modification of LPL mRNA levels during rosiglitazone treatment has been already observed in differentiated adipose cell lines (41). In addition, we found that activation of RXR did not improve the effect of rosiglitazone on FATP-1 mRNA levels. These results may suggest that species-related differences can exist in the regulation of these genes by PPARγ. Alternatively, longer incubations might be required to obtain an effect, suggesting that the mechanism of action of rosiglitazone on FATP-1 and LPL gene can be different from the mechanism involved in the rapid responses of p85αPI-3K and UCP2 mRNAs. For example, recruitment of additional partners or cofactors might be needed.

In human adipocytes, rosiglitazone did not modify the mRNA levels of ASP, HSL, angiotensinogen and PAI-1, suggesting that they are not targets of PPARγ. Regarding the regulation of PAI-1 expression by thiazolidinediones and PPARγ in endothelial cells, contradictory data have been recently reported (42, 43). Adipose tissue is also a possible source of PAI-1 *in vivo* and its expression is increased in obesity (44). However, it seems from our work that PAI-1 gene is not acutely regulated by PPARγ in human adipocytes.

Conflicting data have been reported regarding the effect of PPARγ activation on the expression of its own gene. Some authors have observed a decreased expression of PPARγ in cultured adipose cells treated with rosiglitazone (26, 27) whereas others have found an increased expression in rat adipose tissue *in vivo* (28) and in human muscle cells in culture (45). In human mature isolated adipocytes, we did not observed any effect of rosiglitazone without or with activator of RXR on the mRNA levels of PPARγ. The incubation of the cells for 6 h with different concentrations (10^{-10} to 10^{-6} M) (data not shown) or the use of troglitazone, another thiazolidinedione, did not change the result. It seemed therefore that thiazolidinediones do not rapidly affect PPARγ gene expression in human mature adipocytes.

In adipose cell lines, thiazolidinediones increased the expression of the glucose transporter Glut 4 and enhanced insulin stimulated glucose uptake (46). *In vivo* also, treatment of insulin resistant Zucker fa/fa rats with pioglitazone (30) or ob/ob mice with rosiglitazone (47) increase adipose tissue expression of Glut 4, suggesting that Glut 4 may be a target gene of PPARγ. Our data showed that Glut 4 expression is not modified by treatment of human adipocytes by rosiglitazone alone. However, the combination of rosiglitazone and LG 1069 significantly increased Glut 4 mRNA levels. These results suggested that Glut 4 may be a target gene of the heterodimer PPARγ/RXR in human adipocytes. Reduced expression of Glut 4 has been demonstrated in adipose tissue from insulin resistant obese and type-2 diabetic patients (48). In accordance, we found lower mRNA levels of Glut 4 in isolated cells from obese subjects. Thus, increased expression of Glut 4 and enhanced glucose transport rate in adipocytes may participate in the improvement of insulin sensitivity during thiazolidinedione therapy.

We also investigated the regulation by rosiglitazone of genes coding 3 major actors of the insulin signaling cascade, namely insulin receptor, IRS-1 and p85αPI-3K. Insulin receptor and IRS-1 mRNA levels were not affected by the treatment of isolated adipocytes, suggesting that these genes are not acutely regulated by PPARγ. On another hand, the mRNA levels of p85αPI-3K were significantly increased by rosiglitazone. The p85αPI-3K is an adaptor protein that links the catalytic subunit of phosphatidylinositol 3-kinase to upstream signaling molecules (such as IRS-1), playing thus a major role in the intracellular action of insulin (49). Therefore, the rapid induction of p85αPI-3K expression in response to rosiglitazone may have important consequences in the insulin response of the cells. We have now demonstrated that in addition

to the mRNA, the protein level of p85αPI-3K is significantly increased by rosiglitazone and that the antilipolytic response to insulin is markedly improved in human isolated adipocytes (Rieusset *et al.* manuscript in preparation). Several studies in rodents had already suggested that thiazolidinediones affect the phosphatidylinositol 3-kinase pathway of insulin action (50–52). Our data in human adipocytes indicate that among the different components of this signaling cascade, the gene encoding the p85αPI-3K may be the molecular target of the thiazolidinediones.

Activation of the phosphatidylinositol 3-kinase pathway is required for a variety of insulin effects, including the regulation of glucose uptake, glycogen synthesis and the antilipolytic action of insulin (49). Furthermore, altered expression and activation of phosphatidylinositol 3-kinase have been reported in subjects with type-2 diabetes or obesity (51–53). Therefore, the identification of p85αPI-3K as a possible target gene of the thiazolidinediones shed new light on the understanding of the insulin sensitizing action of these molecules and their beneficial effect in the treatment of type 2 diabetes mellitus.

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REFERENCES

1. Saltiel, A. R., and Olefsky, J. M. (1996) *Diabetes* **45,** 1661–1669.
2. Chaiken, R. L., Eckert-Norton, M., Pasmantier, R., Boden, G., Ryan, I., Gelfand, R. A., and Lebovitz, H. E. (1995) *Diabetologia* **38,** 1307–1312.
3. Day, C. (1999) *Diabet. Med.* **16,** 179–192.
4. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M., and Kliewer, S. A. (1995) *J. Biol. Chem.* **270,** 12953–12956.
5. A unified nomenclature system for the nuclear receptor superfamily (1999) *Cell* **97,** 161–163.
6. Wilson, T. M., Cobb, J. E., Cowan, D. J., Wiethe, R. W., Correa, I. D., Prakash, S. R., Beck, K. D., Moore, L. B., Kliewer, S. A., and Lehmann, J. M. (1996) *J. Med. Chem.* **39,** 665–668.
7. Berger, J., Bailey, P., Biswas, C., Cullinan, C. A., Doebber, T. W., Hayes, N. S., Saperstein, R., Smith, R. G., and Leibowitz, M. D. (1996) *Endocrinology* **137,** 4189–4195.
8. Braissant, O., Foufelle, F., Scotto, C., Dauça, M., and Wahli, W. (1996) *Endocrinology* **137,** 354–366.
9. Vidal-Puig, A., Considine, R. V., Jimenez-Linan, M., Werman, A., Pories, W. J., Caro, J. F., and Flier, J. S. (1997) *J. Clin. Invest.* **99,** 2416–2422.
10. Auboeuf, D., Rieusset, J., Fajas, L., Vallier, P., Freering, V., Riou, J. P., Staels, B., Auwerx, J., Laville, M., and Vidal, H. (1997) *Diabetes* **48,** 1319–1327.
11. Fajas, L., Fruchart, J. C., and Auwerx, J. (1998) *FEBS Lett.* **438,** 55–60.
12. Zhu, Y., Qi, C., Korenberg, J. R., Chen, X. N., Noya, D., Rao, M. S., and Reddy, J. K. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7921–7925.
13. Fajas, L., Auboeuf, D., Raspé, E., Schoonjans, K., Lefebvre, A. M., Saladin, R., Najib, J., Laville, M., Fruchart, J. C., Deeb, S., Vidal-Puig, A., Flier, J., Briggs, M. R., Staels, B., Vidal, H., and Auwerx, J. (1997) *J. Biol. Chem.* **272,** 18779–18789.
14. Schoonjans, K., Staels, B., and Auwerx, J. (1996) *Biochem. Biophys. Acta* **1302,** 93–109.
15. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) *Cell* **79,** 1147–1156.
16. Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M. (1994) *Genes Dev.* **8,** 1224–1234.
17. Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A. M., Heyman, R. A., Briggs, M. R., Deeb, S., Staels, B., and Auwerx, J. (1996) *EMBO J.* **15,** 5336–5348.
18. Martin, G., Schoonjans, K., Lefebvre, A. M., Staels, B., and Auwerx, J. (1997) *J. Biol. Chem.* **272,** 28210–28217.
19. Frohner, B. I., Hui, T. Y., and Bernlohr, D. A. (1999) *J. Biol. Chem.* **274,** 3970–3977.
20. Kliewer, S. A., Umesono, K., Noonan, D. J., Heyman, R. A., and Evans, R. M. (1992) *Nature* **358,** 771–774.
21. Juge-Aubry, C., Pernin, A., Favez, T., Burger, A. G., Wahli, W., Meier, C. A., and Desvergnes, B. (1997) *J. Biol. Chem.* **272,** 25252–25259.
22. Tontonoz, P., Hu, E., Devine, J., Beale, E. G., and Spiegelman, B. M. (1995) *Mol. Cell. Biol.* **15,** 351–357.
23. De Vos, P., Lefebvre, A. M., Miller, S. G., Guerre-Millo, M., Wong, K., Saladin, R., Hamann, L. G., Staels, B., Briggs, M. R., and Auwerx, J. (1996) *J. Clin. Invest.* **98,** 1004–1009.
24. Kallen, C. B., and Lazar, M. A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5793–5796.
25. Kurebayashi, S., Hirose, T., Miyashita, Y., Kasayama, S., and Kishimoto, T. (1997) *Diabetes* **46,** 2115–2218.
26. Rosenbaum, S. E., and Greenberg, A. S. (1998) *Mol. Endocrinol.* **12,** 1150–1160.
27. Camp, H. S., Whitton, A. L., and Tarufi, S. R. (1999) *FEBS Lett.* **26,** 186–190.
28. Pearson, S. L., Cawthorne, M. A., Clapham, J. C., Dunmore, S. J., Holmes, S. D., Moore, G. B., Smith, S. A., and Tadayyon, M. (1996) *Biochem. Biophys. Res. Commun.* **229,** 752–757.
29. Camirand, A., Marie, V., Rabelo, R., and Silva J. E. (1998) *Endocrinology* **139,** 428–431.
30. Hallakou, S., Doaré, L., Foufelle, F., Kergoat M., Guerre-Millo, M., Berthault M. F., Dugail, I., Morin, J., Auwerx, J., and Ferré, P. (1997) *Diabetes* **46,** 1393–1399.
31. Digby, J. E., Montague, C. T., Sewter, C. P., Sanders, L., Wilkison, W. O., O'Rahilly, S., and Prins, J. B. (1998) *Diabetes* **47,** 138–141.
32. Nolan, J. J., Olefsky J. M., Nyce M. R., Considine R. V., and Caro, J. F. (1996) *Diabetes* **45,** 1276–1278.
33. Viguerie-Bascands, N., Saulnier-Blache, J. S., Dandine, M., Dauzats, D., Daviaud, D., and Langin, D. (1999) *Biochem. Biophys. Res. Commun.* **256,** 138–141.
34. Mukherjee, R., Davies, P. J., Crombie, D. L., Bischoff, E. D., Cesario, R. W., Jow, L., Hamann, L. G., Boehm, M. F., Mondon, C. E., Nadzan, A. M., Paterniti, J. R., and Heyman, R. A. (1997) *Nature* **386,** 407–410.

35. Rodbell, M. (1964) *J. Biol. Chem.* **239,** 375–380.
36. Rieusset, J., Andreelli, F., Auboeuf, D., Roques, M., Vallier, P., Riou, J. P., Auwerx, J., Laville, M., and Vidal, H. (1999) *Diabetes* **48,** 699–705.
37. Auboeuf, D., and Vidal, H. (1997) *Anal. Biochem.* **245,** 141–148.
38. Laville, M., Auboeuf, D., Khalfallah, Y., Vega, N., Riou, J. P., and Vidal, H. (1996) *J. Clin. Invest.* **98,** 43–49.
39. 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.
40. Spiegelman, B. M. (1998) *Diabetes* **47,** 507–514.
41. Ranganathan, S., and Kern, P. A. (1998) *J. Biol. Chem.* **273,** 26117–26122.
42. Marx, N., Bourcier, T., Sukhova, G. K., Libby, P., and Plutzky, J. (1999) *Arterioscler. Thromb. Vasc. Biol.* **19,** 546–551.
43. Kato, K., Satoh, H., Endo, Y., Yamada, D., Midorikawa, S., Sato, W., Mizuno, K., Fujita, T., Tsukamoto, K., and Watanabe, T. (1999) *Biochem. Biophys. Res. Commun.* **258,** 431–435.
44. Eriksson, P., Reynisdottir, S., Lonnqvist, F., Stemme, V., Hamsten, A., and Arner, P. *Diabetologia* **41,** 65–71.
45. Park, K. S., Ciaraldi, T. P., Lindgren, K., Abrams-Carter, L., Mudaliar, S., Nikoulina, S. E., Tufari, S. R., Veerkamp, J. H., Vidal-Puig A., and Henry, R. R. (1998) *J. Clin. Endocrinol. Metab.* **83,** 2830–2835.
46. Sandouk, T., Reda, D., and Hofmann, C. (1993) *Endocrinology* **133,** 352–359.
47. Young, P. W., Cawthorne, M. A., Coyle, P. J., Holder, J. C., Holman, G. D., Kozka, I. J., Kirkham, D. M., Lister, C. A., and Smith S. A. (1995) *Diabetes* **44,** 1087–1092.
48. Garvey, W. T., Maianu, L., Hueckstead, T. P., Birnbaum, M. J., Molina, J. M., and Ciaraldi, T. P. (1991) *J. Clin. Invest.* **87,** 1072–1081.
49. Shepherd, P. R., Navé, B. T., and Rahilly, S. O. (1996) *J. Mol. Endocrinol.* **17,** 175–184.
50. Berger, J., Biswas, C., Hayes, N., Ventre, J., Wu, M., and Doebber, T. W. (1996) *Endocrinology* **137,** 1984–1990.
51. Bonini, J. A., Colca, J. R., Dailey, C., White, M., and Hofmann, C. (1995) *Am. J. Physiol.* **269,** E759–E765.
52. Zhang, B., Szalkowski, D., Diaz, E., Hayes, N., Smith, R., and Berger, J. (1994) *J. Biol. Chem.* **269,** 25735–25741.
53. Zierath, J. R., Krook A., and Wallberg-Henriksson, H. (1998) *Mol. Cell. Biochem.* **182,** 153–160.
54. Goodyear L. J., Giorgino, F., Sherman, L. A., Carey, J., Smith, R. J., and Dohm, G. L. (1995) *J. Clin. Invest.* **95,** 2195–2204.
55. Andreelli, F., Laville, M., Ducluzeau, P. H., Vega, N., Vallier, P., Khalfallah, Y., Riou, J. P., and Vidal, H. (1998) *Diabetologia* **42,** 358–364.