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Effects of fenofibrate on lipid metabolism in adipose tissue of rats

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Abstract

The effect of fenofibrate, a peroxisome proliferator–activated receptor α agonist, on body weight gain and on reduction of adipose tissue pads has been ascribed to increased fat catabolism in liver mainly through the induction of target enzymes involved in hepatic lipid metabolism. The aim of this study was to investigate whether peroxisome proliferator–activated receptor α activation also affects metabolic pathways in adipose tissue of rats treated with fenofibrate (100 mg/kg body weight) for 9 days. Fenofibrate lowered body weight gain and plasma triglyceride, total cholesterol, and high-density lipoprotein cholesterol but had no influence on food intake and on plasma glucose levels. The activity of lipoprotein lipase of treated animals decreased 50% in epididymal, 29% in retroperitoneal, and was not affected in the mesenteric fat pads. In this study, we show a 34% decrease in epididymal adipose tissue de novo lipogenesis by fenofibrate. The results demonstrate that insulin sensitivity of lipolysis is decreased in fenofibrate-treated rats which resulted in 30% higher rate of glycerol release when compared to the control group. These findings suggest that besides its effects on liver, fenofibrate exerts effects on lipid metabolism in adipose tissue which may contribute to decreasing adiposity.

1. Introduction

Lipid deposition in adipose tissue is dependent on the availability and uptake of exogenous nonesterified fatty acids (FAs) released from plasma lipoproteins by lipoprotein lipase (LPL) [1] and the rate of de novo synthesis (lipogenesis) of FA within the tissue [2]. The amount of FA released to the bloodstream from stored triglyceride is mainly dependent on the rate of lipolysis, mediated by hormone-sensitive lipase [3]. Lipogenesis and lipoprotein uptake are both stimulated by insulin [4,5], whereas lipolysis is inhibited by insulin and stimulated by cathecolamines [6].

Several aspects of intracellular lipid and FA metabolism in cells are subjected to transcriptional control by the peroxisome proliferator–activated receptor (PPAR) family. Three receptor subtypes of PPAR termed α, β, and γ have been identified [7]. These receptors heterodimerize with the retinoid X receptor and alter the transcription of target genes after binding to peroxisome proliferator response elements located in the promoter region of target genes [8]. The transcriptional activity of PPAR subtypes is enhanced by 2 classes of drugs, fibrates and glitazones.

Fibrates and their derivatives constitute a group of hypolipidemic agents that are widely used in the treatment of hypertriglyceridemia and combined hyperlipidemia, being particularly effective in lowering the plasma triglyceride and increasing plasma high-density lipoprotein (HDL) [2,9-12]. The pharmacological actions of fibrates have been found to be mediated through activation of the PPARα [7], leading to expression of genes involved in lipid and lipoprotein metabolism [13]. PPARα is expressed predominantly in liver and, to a lesser extent, in heart and muscle, where it has a crucial role in controlling FA oxidation [14]. It has been reported that fenofibrate can reduce body weight gain in animal models of diabetes [15], obesity [16], and insulin resistance such as fatty *fa/fa* Zucker rats and high-fat–fed C57B1/6 mice [17]. This effect of fenofibrate on body weight gain and on reduction

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of adipose tissue pads has been ascribed to increased fat catabolism in liver mainly through the induction of target enzymes involved in hepatic lipid metabolism [16,17]. However, recent works indicate that PPARα is expressed in adipose tissue and upregulates genes involved in FA oxidation [18,19]. Therefore, the aim of the present study was to investigate the effect of fenofibrate on adipose tissue metabolism.

2. Material and methods

2.1. Materials

Bovine serum albumin (Bovuminar Reagent Pure Powder) was obtained from Intergen (Purchase, NY); collagenase was from Worthington (Freehold, NJ). All other chemicals were of analytical grade and were obtained commercially.

2.2. Animals and treatment

Wistar male rats (8-10 weeks) were obtained from Centro de Bioterismo da Universidade Federal de Minas Gerais (CEBIO-UFMG) and kept in individual cages in an environmentally controlled room with a 14/10-hour light/dark cycle and had free access to tap water and food. For 9 days, the animals were fed either a balanced diet (control) consisting of 66% carbohydrate (33% starch and 33% sucrose), 8% corn oil, 17% casein, 5% salts, and 1% vitamins [20], or the same diet supplemented with 100 mg/kg body weight per day of fenofibrate [21]. The food intake of the animals was carefully monitored by weighing special food containers at regular intervals. The animals were maintained according to the ethical guidelines of our institution, and the experimental protocol was approved by the Ethics Committee in Animal Experimentation of the Federal University of Minas Gerais.

The experiments were carried out on fed rats between 8:30 and 10:30 AM, and the animals were killed and tissues collected and weighed. Plasma was isolated and stored at −20°C until further analysis.

2.3. Adipocyte isolation and lipolysis measurements

Adipocytes were isolated from epididymal fat pads by the method of Rodbell [22]. Digestion was carried out at 37°C with constant shaking for 45 minutes. Cells were filtered through nylon mesh and washed 3 times with buffer containing (mmol/L) the following: 137 NaCl, 5 KCl, 4.2 $NaHCO_3$, 1.3 $CaCl_2$, 0.5 $MgCl_2$, 0.5 $MgSO_4$, 0.5 KH_2PO_4, 20 mmol/L HEPES (pH 7.4), plus 1% bovine serum albumin.

Lipolysis was measured by following the rate of glycerol release, as previously described [23]. After washing, adipocytes were incubated at 37°C in a water bath for 60 minutes, in the presence or absence of isoproterenol (ISO, 0.1 μmol/L), and the effects of insulin on ISO-stimulated lipolysis were determined by constructing dose-response curves using 0 to 12.5 ng/mL. At the end of the incubation period, an aliquot of the infranatant was removed for enzymatic determination of glycerol released into the incubation medium (LABTEST, Lagoa Santa, MG, Brazil).

2.4. Lipoprotein lipase activity

Samples of epididymal (EPI), retroperitoneal (RP), and mesenteric (MES) adipose tissue (50 mg) were homogenized in buffer containing heparin and detergents [24], and total LPL activity was measured using a [9,10-^{3}H] triolein-containing substrate emulsified with lecithin [25] and contained 24-hour fasted rat plasma as a source of apo C_{II}. The reaction was stopped with extraction mixture [26], and liberated ^{3}H-free fatty acids (FFAs) were quantified by liquid scintillation. The enzyme activity is expressed as nanomoles of FA released per minute.

2.5. In vitro lipogenesis

Portions of epididymal adipose tissue (200 mg) were incubated at 37°C for 2 hours in the buffer described above, to which had been added 5 mmol/L of glucose and 150 μCi/mL of ^3H_2O as a radioisotopic tracer. At the end of the incubation period, ^{3}H-total lipids were extracted with chloroform-methanol (2:1) [27] and counting of the lipids was done by adding a toluene-diphenyloxazole scintillation fluid (5 g/L). Rates of lipid synthesis were calculated as previously described [28].

2.6. Plasma analysis

The levels of plasma glucose, triglycerides, total cholesterol, and HDL cholesterol (HDL-C) were measured using enzymatic methods (LABTEST).

2.7. Statistical analysis

All values shown are expressed as mean ± SE. The area under the curve was analyzed by using the trapezoid rule. Statistical analysis was, where appropriate, by paired Student *t* test or by 1-way analysis of variance followed by the Tukey test for pairwise comparisons of treatment groups with the use of SigmaStat 2.0 (Jandel, San Rafael,

Table 1
Effects of fenofibrate treatment on metabolic parameters

	Control	Fenofibrate
Body weight (g)	255 ± 8	221 ± 8*
Body weight gain (g/9 d)	61 ± 7	38 ± 6*
Food intake (g/d)	22 ± 0.6	18 ± 2
Food intake (g/100 g body weight)	9.1 ± 0.4	8.7 ± 0.5
Epididymal adipose tissue weight (g)	1.1 ± 0.05	0.74 ± 0.02*
Retroperitoneal adipose tissue weight (g)	0.67 ± 0.02	0.34 ± 0.06*
Plasma triglyceride (mg/dL)	171 ± 19	70 ± 8*
Plasma total cholesterol (mg/dL)	112 ± 8	76 ± 10*
Plasma HDL-C (mg/dL)	71 ± 5	34 ± 2*
Plasma glucose (mg/dL)	122 ± 2	117 ± 11

Data are means ± SEM for n = 7 to 9 rats.
* $P < .05$ vs control.

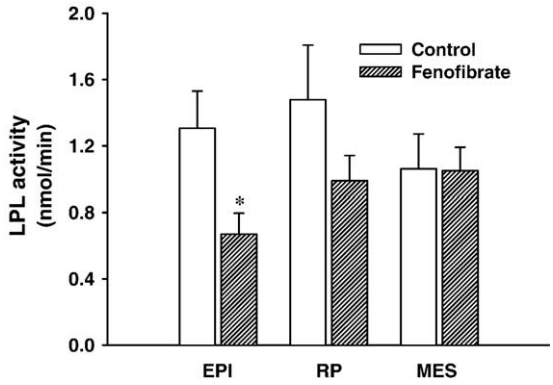

Fig. 1. Effect of fenofibrate on LPL activity of epididymal, retroperitoneal, and mesenteric fat pads. Data are reported as means ± SEM for n = 6 to 7 rats. *$P < .05$.

Calif). Differences were considered statistically significant at the $P < .05$ levels.

3. Results

The administration of fenofibrate to rats for 9 days induced the expected modification of plasma lipid concentration (Table 1) with significant decreases in plasma triglyceride concentration (59%), total cholesterol (32%), and HDL-C (52%). By contrast, treatment with fenofibrate did not influence plasma glucose concentration (Table 1). Animals fed fenofibrate also had markedly lower gain in body weight (40%) than controls.

This was accompanied by 30% and 49% lower epididymal and retroperitoneal fat pads weight (Table 1). Moreover, the results showed similar food consumption in both groups of animals throughout the study, suggesting the effects of fenofibrate on body weight and adipose tissue mass are not driven by a reduction in caloric intake.

Circulating triglylceride-FA uptake was estimated by measuring the LPL activity, the enzyme that hydrolyzes the core of triglyceride-rich lipoproteins into FFAs and monoglyceride in epididymal, mesenteric, and retroperitoneal fat pads. The results showed 50% lower LPL activity in epididymal and 29% lower (trend, $P = .0662$) retroperitoneal fat pads, but was not affected in the mesenteric fat pads (Fig. 1).

Table 2
Values of glycerol release by epididymal adipocytes isolated from control and fenofibrate-treated rats

	Glycerol release (μmol/h)	
	Basal	ISO (0.1 μmol/L)
Control	0.73 ± 0.08	3.45 ± 0.36*
Fenofibrate	0.81 ± 0.09	3.97 ± 0.38*

Data are means ± SEM for n = 6 to 7 rats.
* $P < .05$ vs basal.

To determine whether de novo lipogenesis was altered by fenofibrate treatment, the incorporation of ^3H_2O in total lipids was measured in epididymal fat pads. The data in Fig. 2 show a 34% decrease in the lipogenic rates in adipose tissue from fenofibrate-treated rats.

To determine the effect of fenofibrate on lipolysis, adipocytes were incubated in basal or ISO-stimulated conditions and the insulin sensitivity of lipolysis was also measured. Because basal lipolysis is low, the antilipolytic action of insulin was tested against ISO-stimulated lipolysis. As can be seen in Table 2, the presence of ISO produced a significant increase in lipolysis in both groups, but no difference between control and fenofibrate-treated rats was detected. Insulin action was calculated as the difference between 0.1 μmol/L ISO-stimulated lipolysis in the presence of various concentrations of insulin. Therefore, to compare the antilipolytic action of insulin, inhibition of lipolysis was presented as a percentage of 0.1 μmol/L ISO-stimulated lipolysis (Fig. 3). The results demonstrate that insulin sensitivity of lipolysis is lower in fenofibrate-treated rats which resulted in 30% higher rate of glycerol release (calculated

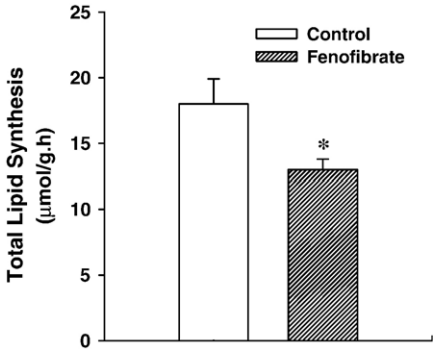

Fig. 2. Rates of ^3H incorporation from tritiated water into epididymal adipose tissue total lipids by rats fed a balanced diet supplemented or not with fenofibrate. Data are reported as means ± SEM for n = 5 rats. *$P < .05$.

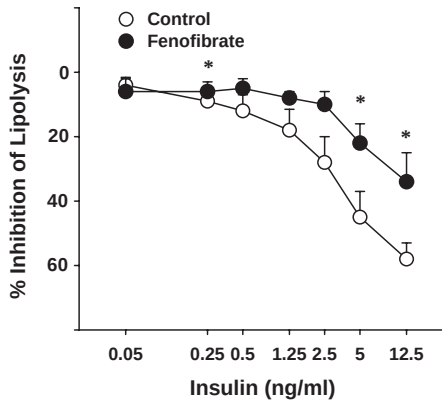

Fig. 3. Antilipolytic action of insulin, presented as percent inhibition of 0.1 μmol/L ISO-stimulated lipolysis in the absence and presence of increasing amounts of insulin. Data are reported as means ± SEM for n = 6 to 7 rats. *$P < .05$.

by area under the curve analysis) when compared to the control group (Fig. 3).

4. Discussion

The present experiments demonstrate that fenofibrate treatment prevents hypertriglyceridemia, hypercholesterolemia, body weight gain, and adiposity, consistent with previous reports. The results also demonstrate that fenofibrate decreased LPL activities in epididymal and retroperitoneal, but not mesenteric, fat pads, suggesting site-specific regulation. Lipoprotein lipase is synthesized as an inactive monomer, and acquisition of catalytic activity requires dimerization after processing of N-linked oligosaccharides [29]. Therefore the reduction in LPL activity by fenofibrate could be caused by inhibition of LPL processing and reduced conversion of the inactive monomer to the active dimer. An inhibitory effect on LPL activity, with no change in specific activity, was demonstrated after incubating cultured cardiomyocytes with WY-14643, a PPARα ligand [30]. The authors emphasized that PPARα activators may act by a transcriptional mechanism, but one that inhibits LPL processing [30]. Bergö et al [31] proposed that the fasting-induced fall in adipose tissue LPL activity is due to a posttranslational mechanism, and this downregulation requires that a gene, separated from the lipase gene, is switched on. The results suggest that the weight-losing effect of fenofibrate is, at least partially, consequent to a decrease in triglyceride uptake, resulting from a reduction in LPL activity.

In the present study, treatment with fenofibrate lowered de novo lipogenesis in adipose tissue, one of the pathways providing fatty acyl-CoA for tissue triglyceride synthesis and storage in adipose tissue. Consequently, diminished carbon flux through acetyl-CoA carboxylase could result in diminished malonyl-CoA content in adipose tissue and enhanced carnitine palmitoyltransferase I and FA oxidation [32]. Although the present data provide no information regarding the cellular mechanism of action of fenofibrate, they demonstrate that a predicted consequence of inhibition of lipogenesis is a reduction in lipid accumulation in adipose tissue.

The data show that when the lipolytic rate is increased by ISO, fenofibrate becomes a lipolytic agent. In earlier reports indicating that activators of PPARα decrease plasma FFAs [18,21,33,34] despite the increased fat mobilization [18], glycerol release from adipocytes (an index of lipolysis) had not been measured. To determine the effect of fenofibrate on lipolysis, we incubated adipocytes in basal or ISO-stimulated conditions and also evaluated the insulin sensitivity of lipolysis. Our results demonstrate that insulin sensitivity of lipolysis is decreased in fenofibrate-treated rats which resulted in 30% higher rate of glycerol release when compared to the control group. Besides its effects on β-oxidation in liver, other studies have shown that increases in PPARα induced by leptin in normal adipocytes stimulate a novel form of lipolysis in which glycerol is released without a proportional release of FFA [18]. In those studies, PPARα mRNA was upregulated, together with its target enzymes of FA oxidation, acyl-CoA oxidase, carnitine palmitoyltransferase, and uncoupling proteins 1 and 2. Body fat disappeared without any increase in plasma FFAs or ketones, which led to the hypothesis that the fat was being oxidized within or very close to the adipocytes themselves, and that the energy was being dissipated as heat [18]. Recent study demonstrated that chronic activation of adipocyte β_3-adrenergic receptor agonist (CL-316,243) remodels white adipose tissue and upregulates PPARα [19]. This upregulation closely correlated with stimulation of induction of genes involved in FA oxidation in wild-type mice, whereas drug treatment was severely compromised in PPARα knockout animals [19].

In summary, our results have shown that the effect of fenofibrate treatment on body weight gain and adiposity is mediated by an important decrease in the processes of uptake and synthesis of FAs, associated with an increased lipid mobilization in adipose tissue. Whether these effects are mediated directly through interaction with PPARα receptors in adipose tissue or are secondary to alterations in other metabolic pathways is unknown. Further studies are required to determine the mechanisms involved in the effects of fenofibrate on adipose tissue metabolism.

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