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Phenotype-based treatment of dietary obesity: differential effects of fenofibrate in obesity-prone and obesity-resistant rats☆

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Abstract

High-fat diets (HFDs) promote hyperphagia and adiposity in animals and human beings. To test the hypothesis that limitations on fat oxidation underlie this propensity for diet-induced obesity, rats were treated with fenofibrate, which enhances fat oxidation mainly in liver by inducing expression of enzymes and proliferation of organelles involved in fatty acid oxidation. Male Sprague-Dawley rats were fed a HFD (42% fat calorie) for 2 weeks. Rats ranked in the top and bottom thirds for weight gain during this feeding period were designated as obesity prone (OP) and obesity resistant (OR), respectively. Fenofibrate was added to the HFD (0.025% wt/wt) for half of the OP and OR rats. During the next 10 days, fenofibrate treatment significantly ($P < .05$) reduced food intake, weight gain, feed efficiency, and adiposity in OP rats to levels seen in control OR rats, but had no such effects in OR rats. Fenofibrate treatment increased whole-body fatty acid oxidation, and in liver, the expression of carnitine palmitoyl transferase I only in OP rats, but enhanced expression of acyl-CoA oxidase in both OP and OR rats. Restricting food intake of OP rats to levels seen in rats given fenofibrate similarly reduced weight gain but had little effect on weight of fat pads. Treatment with the daily dosage of fenofibrate given as a bolus did not produce a conditioned flavor aversion. These results suggest that enhancement of mitochondrial fatty acid oxidation in liver may be an effective phenotype-based treatment strategy for dietary obesity.

1. Introduction

The propensity to overeat and become obese when eating a high-fat diet (HFD) varies widely among individuals both across and within species or strains [1-4]. Genetic factors play a role in this variation, but the pathways responsible for these phenotypic differences are still largely unknown. It has been hypothesized that obesity-prone (OP) individuals do not oxidize fat fuels as readily as obesity-resistant (OR) ones [5-7]. As a consequence, slow fat oxidation leads to excessive accumulation of dietary fat in adipose tissue of OP individuals, which in turn leads to overeating in an attempt to compensate for the decreased availability of fat fuels for oxidation. This hypothesis has been supported mostly by indirect evidence (eg, Refs [8-13]). Recently, however, we demonstrated that rats that oxidize fat at a low rate and have high levels of circulating triglycerides after a fast are more hyperphagic and gain more weight during subsequent feeding of a HFD than do those with higher rates of fat oxidation and lower fasting triglyceride levels [14]. This provided direct evidence that the preexisting slow rate of fat oxidation is related to overeating and obesity in rats fed HFDs.

If slow oxidation of fat is one of the contributing factors for overeating and adiposity in dietary obesity, then promoting fat oxidation should reduce food intake, body weight, and body fat in those prone to obesity, but have lesser, if any, effects in those who already oxidize fat at higher rates and are thus resistant to dietary obesity. Fenofibrate is an agonist of peroxisome-proliferator receptor activator-α (PPARα). PPARα is a nuclear receptor that regulates expression of the enzymes and proliferation of the organelles involved in the oxidation of fatty acids primarily in liver and to a small extent in other fat-oxidizing tissues such as muscle, kidney, and brown adipose tissue [15,16]. PPARα agonists promote fatty acid oxidation and lower circulating lipids, and have been used as hypolipidemic drugs [17,18]. Recently, several studies have examined the effects of PPARα agonists on energy intake, body weight, and body fat in rodent models of obesity. In selectively bred OP rats, fenofibrate (100 mg/kg per day) reduced body weight gain, adiposity, food intake, and feed efficiency [19].

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The same dose induces a nonsignificant 10% reduction in weight gain of *db*/*db* mice and a significant reduction in weight gain of fatty Zucker rats [20]. In Wistar rats fed a HFD, fenofibrate (320 mg/kg per day) reduces weight gain and adiposity to levels seen in untreated control rats fed a standard diet [21]. When supplemented in diets, fenofibrate (0.05% wt/wt) prevents diet-induced obesity in C57BL/6 mice without affecting food intake, and ciprofibrate (0.005% wt/wt), another PPARα agonist, reduces weight gain and adiposity of young Zucker rats [22]. Dietary fenofibrate (100 mg/kg wt/wt) also reduces intake, body weight, and adiposity of obese Otsuka Long-Evans Tokushima Fatty (OLETF) rats [23].

Although these studies indicate that activation of PPARα reduces food intake, weight gain, and adiposity in rodent models of genetic or dietary obesity, they do not provide some critical information about the actions of fenofibrate and other PPARα agonists. In particular, it is unclear whether the effects of these agents are seen in all animals or only in obese ones. This is an important issue because if PPARα agonists only affect obese animals, it would imply that there are specific pathways underlying the obese phenotype and would offer a more phenotype-based treatment for obesity. Further, it has not been addressed whether reduction in adiposity is the result of intake reduction alone or if an increase in fat oxidation plays a role as well. It is also unknown whether PPARα agonists decrease food intake via a physiological mechanism or a nonspecific untoward action. To address these issues, we examined the effects of fenofibrate on energy intake, weight gain, adiposity, fatty acid oxidation, and expression of hepatic enzymes for fatty acid oxidation in Sprague-Dawley rats. Because about half of these rats overeat and develop obesity, and the rest remain lean when fed HFDs [2,24], this allows us to assess the interactions of fenofibrate with the phenotype. To test whether fenofibrate reduces food intake by causing malaise, we determined whether the drug produces a learned flavor aversion.

2. Materials and methods

Male Sprague-Dawley CD rats (150-175 g) from Charles River Laboratories (Wilmington, MA) were housed individually in hanging stainless-steel cages with free access to water and food except when fasted or match fed as specified below. The environmental temperature was set at 22°C and lighting followed a 12:12-hour day/night cycle. All protocols involving rats were in compliance of NIH guidelines for animal care and use and approved by the Institutional Animal Care and Use Committee of the Monell Center before the experiments.

2.1. Experiment 1: effects of fenofibrate in OP and OR rats

This experiment was designed to examine the effects of fenofibrate on food intake, body weight, and body fat in OP and OR rats. Thirty-six rats were given standard chow to eat for 1 day upon arrival in the laboratory, after which body weights were recorded and a semisynthetic HFD (42% fat, 41% carbohydrate, and 17% protein by energy [25]) was fed. Two weeks later, rats were weighed, and 12 rats with the largest and 12 rats with the smallest weight gains were designated as OP and OR, respectively. Fenofibrate (0.025% wt/wt) was mixed into the HFD and fed to half of the OP and OR rats; the other rats continued to have access to the plain HFD and served as controls. Body weights and food intakes were recorded daily for 10 days. On the 11th day, food was taken away at the onset of night period. Eighteen hours later, rats were anesthetized with an intramuscular injection of a mixture of Ketaset (100 mg/kg; Fort Dodge Animal Health, Fort Dodge, Iowa) and acepromazine maleate (1 mg/kg; Boehringer Ingelheim Vetmedica, Inc, St Joseph, Mo). Tail blood was collected into microcentrifuge tubes containing EDTA; whole liver and 3 major fat pads (epididymal, retroperitoneal, and mesenteric) were removed and weighed. Plasma samples were prepared from the blood and store at −80° until further analysis. Plasma concentrations of glucose and triglycerides were determined using diagnostic kits from Sigma, St Louis, MO. Free fatty acids were quantified using the NEFA-C kit from Wako USA Inc, Richmond, Va. Plasma concentration of ketone bodies was quantified enzymatically according to the method described by Ramirez [26].

2.2. Experiment 2: effects of fenofibrate on in vivo fatty acid oxidation

Eight OP and 8 OR rats were selected out of 24 rats screened as describefd above. Fenofibrate was then supplemented in the HFD (0.025% wt/wt) for half of the OP and OR rats. At the onset of night period on day 13, each rat was gavaged with 1 μCi [U-^{14}C]palmitic acid (Perkin Elmer Life Sciences, Boston, Mass) suspended in 0.5 mL of 0.5% methyl cellulose. Expired carbon dioxide was collected for 12 hours as described previously [14]. Percentage radioactivity recovered in carbon dioxide was calculated as a measure of fatty acid oxidation.

2.3. Experiment 3: effects of fenofibrate on expression of hepatic genes involved in fatty acid oxidation

This experiment was designed to determine whether fenofibrate affects the expression of the genes involved in fatty acid oxidation in liver. After a 2-day recovery, 16 rats used in experiment 2 were anesthetized with Ketaset and acepromazine maleate, and a piece of liver (median lobe) excised. The liver sample was immediately cut into small pieces and immersed in RNAlater (Ambion, Austin, Tex) overnight at 4°C and then stored at −20°C until RNA isolation. Rats were killed by cutting the diaphragm. Total RNA was extracted using TRIZOL reagent (Invitrogen, Carlsbad, Calif). Complementary DNA was synthesized using the SuperScript II Reverse Transcriptase (Invitrogen). To determine the expression of acyl-CoA oxidase (AOX)

and carnitine palmitoyl transferase I (CPT1A, gene encoding the liver isoform), relative quantitative reverse transcriptase polymerase chain reaction was performed on an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, Calif) using the Taqman Assay-on-Demand Gene Expression Products (Applied Biosystems) for rat AOX (assay ID: Rn00569216_m1) and CPT1A (assay ID: Rn00580702_m1) with Taqman Rodent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Control Reagents (product no. 4308313) as the endogenous control. Forty cycles of amplification was performed under the conditions recommended by the manufacture. Briefly, the reaction was activated at 50°C for 2 minutes followed by holding at 95°C for 10 minute. A cycle consisted of a 15-second denaturing at 95°C followed by a 1-minute annealing/extension at 60°C. No amplification was detected by these assays when genomic DNA was used as a substrate, which confirms that the assays measured only messenger RNA (mRNA). Contents of CPT1A and AOX mRNA were normalized against GAPDH mRNA content.

2.4. Experiment 4: role of reduced food intake on body weight and body fat in fenofibrate-treated OP rats

This experiment was designed to assess whether the reductions in body weight gain and adiposity seen in experiment 1 were due solely to the decrease in intake. In a third group of rats, 24 OP and 8 OR rats were identified as above. Obesity-prone rats were further divided into 3 groups of 8 matched for weight gain during the screening period. OR rats and one group of OP rats were fed the HFD ad libitum and served as controls, whereas a second group of OP rats was given the HFD containing 0.025% (wt/wt) fenofibrate and a third group of OP rats was fed the unadulterated diet in the amount matching those of fenofibrate-treated group. Body weights and food intakes were recorded daily for 12 days. After an overnight fast, rats were anesthetized and 3 major fat pads (epididymal, retroperitoneal, and mesenteric) were removed and weighed.

2.5. Experiment 5: role of malaise in food intake suppression during fenofibrate treatment

To assess whether the effect of fenofibrate on intake was due to malaise, we performed a learned flavor aversion experiment. Twenty rats were fed the HFD for 3 weeks and divided randomly into 2 groups of 10. During this feeding period, mock gavage (insertion of the feeding tube only) was performed several times to adapt rats to the gavage procedure. After an 18-hour water deprivation, rats were given a solution of 0.06% orange-flavored unsweetened Kool-Aid solution to drink for 30 minutes. One group was then gavaged with fenofibrate (15 mg/kg) suspended in 0.5% methyl cellulose, whereas the other group was gavaged with only methyl cellulose. This dose of fenofibrate was approximately equivalent to the daily intake of the drug when added to the diet as described above. Three days later, each rat was given a bottle of orange-flavored and a bottle of lemonade-flavored Kool-Aid solution to drink after an 18-hour water deprivation. Fluid intake from each bottle was recorded at 0.5, 1, 2, and 6 hours.

A week after the fenofibrate gavage, rats were again randomly divided into 2 groups. The experiment was repeated, this time with rats given 4.61 mL/kg of either 0.65 mol/L concentration of LiCl or NaCl paired with a grape-flavored Kool-Aid solution. Three days later, fluid intake was recorded when each rat was given both grape-flavored and lemonade-flavored Kool-Aid solutions after an 18-hour water deprivation. The preference for the paired flavor was calculated as percent total fluid intake.

Fenofibrate and all other chemical reagents were purchased from Sigma, unless specified in the text.

Results are presented as mean ± SEM. Data were analyzed using either *t* test, 1-way, or 2-way analysis of variance (ANOVA) with repeated measures as appropriate. Tukey's test was used for post hoc comparisons. Significance level was set at $P < .05$.

3. Results

3.1. Experiment 1: effects of fenofibrate in OP and OR rats

Adding fenofibrate to the diet decreased food intake, body weight, weight gain, feed efficiency, and adiposity in OP, but not OR, rats. As shown in Fig. 1 (top panel), daily food intake of control OP rats was significantly greater than that of control OR rats ($F_{19,1} = 55.6$, $P < .001$ by 2-way ANOVA). Fenofibrate treatment significantly reduced intake in OP rats but did not affect food intake in OR rats ($F_{19,1} = 33.5$, $P < .001$ by 2-way ANOVA); intake was

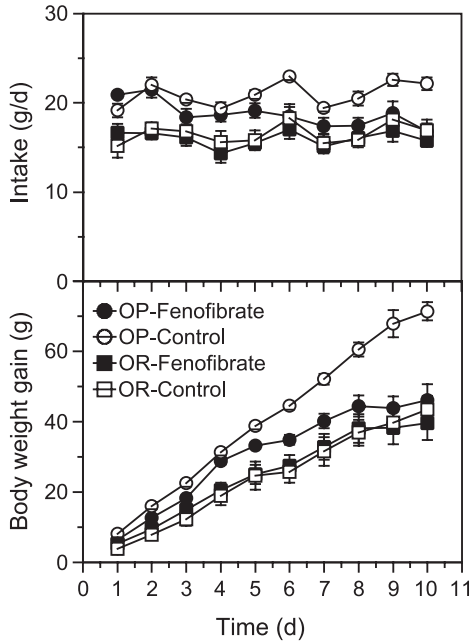

Fig. 1. Daily food intake (top panel) and body weight gain (bottom panel) of OP and OR rats given plain (control) or fenofibrate-supplemented HFD. Values are means ± SEM. N = 6.

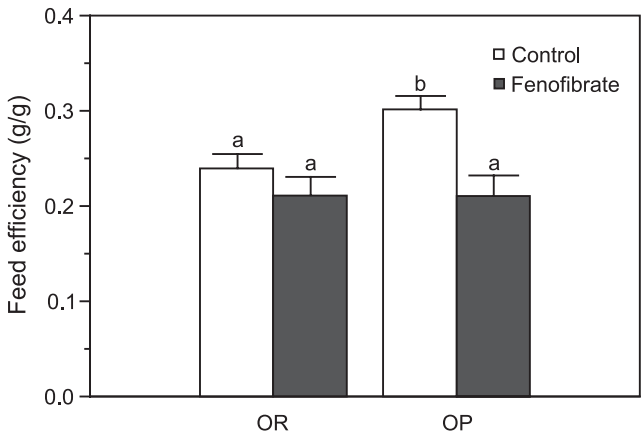

Fig. 2. Feed efficiency (weigh gain/food intake) over the 10-day treatment period of OP and OR rats given plain (control) or fenofibrate-supplemented HFD. Values are means ± SEM. N = 6. Values with same letters are not significantly different ($P > .05$).

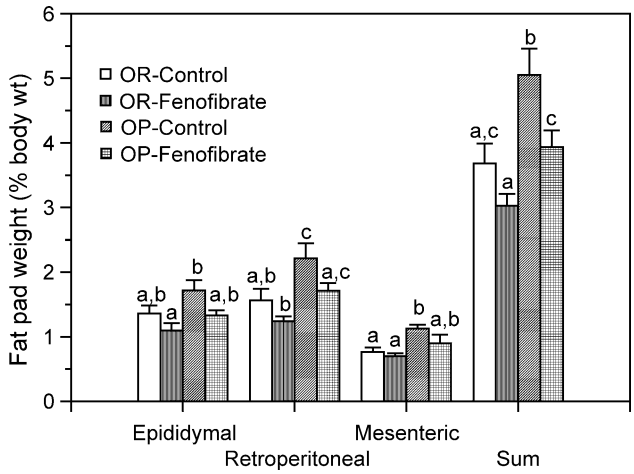

Fig. 3. Weights of epididymal, retroperitoneal, and mesenteric fat pads as percent body weight in OP and OR rats given plain (control) or fenofibrate-supplemented HFD. Values are means ± SEM. N = 6. Values in the same group with same letters are not significantly different ($P > .05$). Results presented as absolute weights of the fat pads yield an identical pattern of treatment effects.

significantly reduced by fenofibrate in OP rats on days 6 and 8 to 10 ($P < .05$). During days 6 to 10, intake of OP rats given fenofibrate was not significantly different from that of control OR rat ($P > .05$). Fenofibrate had no effect on total intake during the 10 days in OR rats (165.2 ± 8.9 vs 160.1 ± 7.2 g; control vs fenofibrate treated), but reduced it in OP rats from 209.5 ± 4.3 to 187.7 ± 8.4 g ($t_{10} = 2.3$, $P < .05$).

Obesity-prone rats weighed significantly more than did OR rats before treatment with fenofibrate (366.7 ± 3.4 vs 319.6 ± 4.6 g; N = 12; $t_{22} = 8.3$, $P < .001$). After fenofibrate treatment for 10 days, body weights of fenofibrate-treated OR rats did not differ significantly from those of control OR rats (366.7 ± 10.1 vs 355.0 ± 10.0 g, control vs treated), whereas body weights of treated OP rats were significantly lower than those of control OP rats (439.1 ± 3.6 vs 411.8 ± 9.4 g, control vs treated) ($t_{10} = 2.7$, $P = .02$). Fenofibrate treatment had no effect on weight gain of OR rats (Fig. 1, bottom panel), but significantly reduced it in OP rats ($F_{19,1} = 130$, $P < .001$ by 2-way ANOVA). Cumulative weight gain of treated OP rats was significantly less than that of control OP rats on days 2, 3, and 5 to 10 ($P < .05$). Cumulative weight gain of treated OP and control OR rats did not differ during the last 4 days of fenofibrate treatment ($P > .05$).

Feed efficiency (weight gain/intake) over the treatment period was significantly higher in OP rats than in OR rats ($F_{3,1} = 6.0$, $P = .02$ by 2-way ANOVA) (Fig. 2) and it was reduced in both OP and OR rats ($F_{3,1} = 11.3$, $P = .003$ by 2-way ANOVA). When compared in each phenotype, fenofibrate had no effect in OR rats, but significantly decreased feed efficiency in OP rats ($t_{10} = 4.4$, $P = .001$) to the level observed in OR rats.

After 10 days, weights of retroperitoneal and mesenteric fat pads and the total weight of all 3 individual pads (expressed as percentages of body weight) were higher in control OP rats compared with control OR rats (Fig. 3). Although fenofibrate treatment tended to reduce the weight of individual fat pads, a significant effect was observed only for total fat pad weights and then only in OP rats as compared with OR rats ($t_{10} = 2.33$, $P < .05$). Total fat pad weight in fenofibrate-treated OP rats was not different from that in control OR rats. When absolute weights of the fat pads were analyzed, an identical pattern of treatment effects was obtained.

Fenofibrate treatment caused hepatomegaly in both OP and OR rats to a similar degree (Fig. 4). At the end of treatment, fasting plasma glucose was significantly higher in both treated groups than it was in control rats. There were trends for fasting plasma fat fuels (triglycerides, free fatty acids, and ketones) to be lower in both treated groups compared with control groups, but these effects were not statistically significant (Table 1).

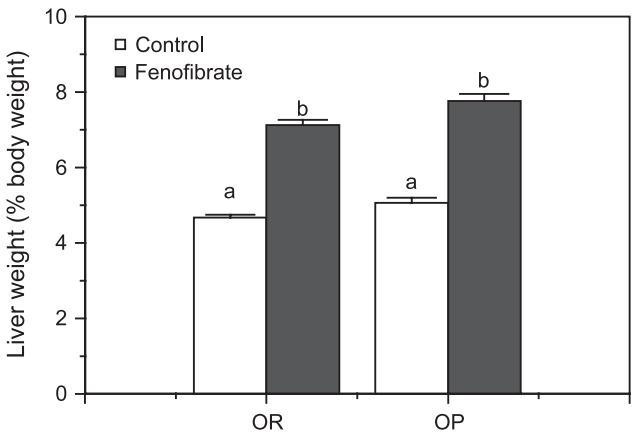

Fig. 4. Liver weight as percent body weight in OP and OR rats given plain (control) or fenofibrate-supplemented HFD. Values are means ± SEM. N = 6. Values with same letters are not significantly different ($P > .05$).

Table 1
Effect of fenofibrate on levels of fasting plasma fuels

Metabolic fuels (mmol/L)	OR rats (N = 6)		OP rats (N = 6)		Effect of fenofibrate by 2-way ANOVA (P)
	Control	Fenofibrate	Control	Fenofibrate	
Glucose	7.79 ± 0.51	9.06 ± 0.30	7.79 ± 0.34	9.09 ± 0.32	.003
Triglycerides	0.30 ± 0.05	0.27 ± 0.03	0.38 ± 0.05	0.31 ± 0.06	.28
Free fatty acids	0.92 ± 0.15	0.71 ± 0.07	0.98 ± 0.12	0.80 ± 0.06	.08
Ketones	3.01 ± 0.34	2.70 ± 0.62	3.95 ± 0.24	2.79 ± 0.44	.11

Blood samples were collected from control or fenofibrate-treated rats after 18 hours of fasting. Values are means ± SEM.

3.2. Experiment 2: effects of fenofibrate on in vivo fatty acid oxidation

To understand the physiological mechanism by which fenofibrate reduced food intake, body weight, and adiposity, we studied its effect on fat oxidation in vivo by measuring the oxidation rate of an exogenous fatty acid. Regardless of fenofibrate supplement, more radioactivity was recovered from OR rats than from OP rats during the 12 hours after the administration of [^{14}C]palmitic acid ($F_{3,1}$ = 28.3, P = .0002 by 2-way ANOVA) (Fig. 5). The effect of fenofibrate was not significant in OP and OR rats combined ($F_{3,1}$ = 2.3, P = .15 by 2-way ANOVA). However, when compared in each phenotype, fenofibrate increased recovery of radioactivity in OP rats by 21% (t_6 = 2.47, P < .05), whereas it had no effect in OR rats.

3.3. Experiment 3: effects of fenofibrate on the expression of hepatic genes involved in fatty acid oxidation

We examined hepatic mRNA levels of CPT-I and AOX, which are involved, respectively, in mitochondrial and peroxisomal fatty acid oxidation to elucidate the molecular mechanisms of fenofibrate actions in OP and OR rats. Fenofibrate significantly increased mitochondrial CPT1A mRNA levels in only OP rats (t_6 = 3.8, P < .01), but neither group of OP rats was significantly different from either control or treated OR rats (Fig. 6). Two-way ANOVA analysis showed that there was a significant effect of fenofibrate ($F_{3,1}$ = 5.6, P < .05), but not phenotype ($F_{3,1}$ = 0.10, P = .76). In contrast, fenofibrate increased peroxisomal AOX mRNA levels substantially and similarly in both OP and OR rats ($F_{3,1}$ = 33.9, P < .0001 for fenofibrate, and $F_{3,1}$ = 1.8, P = .21 for phenotype, by 2-way ANOVA).

3.4. Experiment 4: role of reduced food intake on body weight and body fat in fenofibrate-treated OP rats

In this experiment, a group of OP rats was match fed to a group of OP rats treated with fenofibrate. Intake of OP rats during the 12-day treatment period was again reduced by fenofibrate. As seen in experiment 1, fenofibrate reduced the rate of weight gain of OP rats to that of OR rats (Fig. 7). Match-feeding reduced weight gain to a similar extent, and by the end of 12 days, cumulative weight gains of OR, fenofibrate-treated OP, and match-fed OP groups did not differ but were all significantly lower compared with that of control OP group ($F_{3,28}$ = 6.1, P < .005). Although

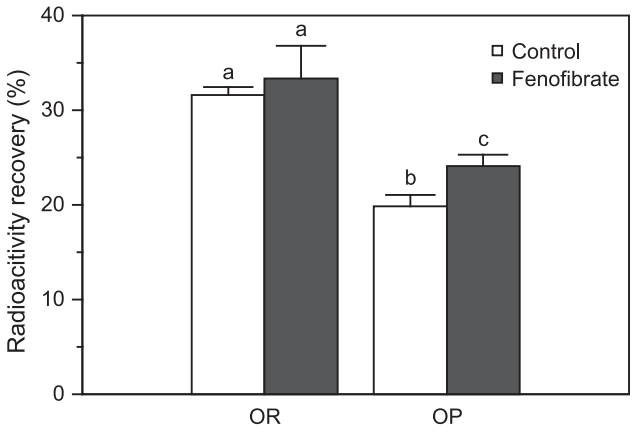

Fig. 5. Recovery of radioactivity in expired carbon dioxide during 12 hours after gavage of 1 μCi of [U-^{14}C]palmitate in OP and OR rats fed either plain (control) or fenofibrate-supplemented HFD. Values are means ± SEM. N = 4. Values with different letters are significantly different (P < .05).

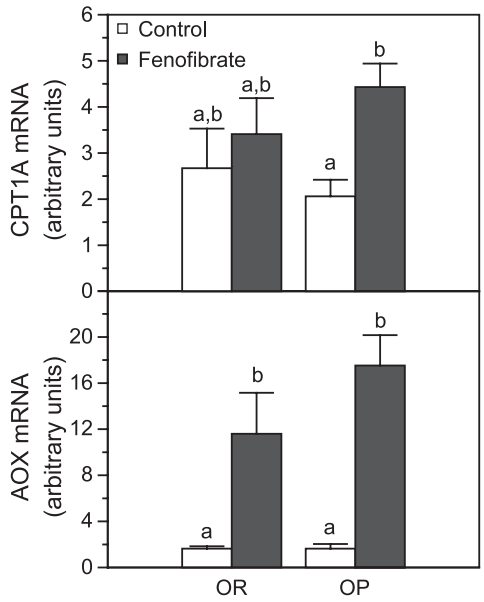

Fig. 6. Effects of fenofibrate on the expression of CPT1A and AOX in liver. Rats were fed either plain (control) or fenofibrate-supplemented HFD. Complementary DNA was synthesized from total RNA then analyzed by RT-PCR. Values shown (means ± SEM) are normalized against GAPDH mRNA. N = 4. Values with different letters are significantly different (P < .05).

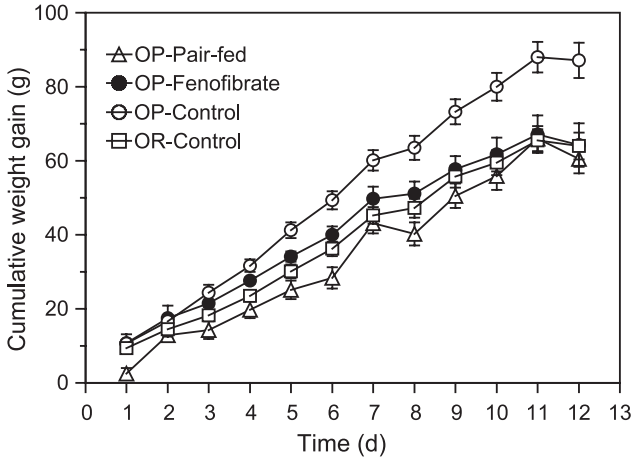

Fig. 7. Weight gain of OR rats fed the plain (control) diet and OP rats fed either plain or fenofibrate-supplemented diet or match-fed to fenofibrate-treated rats. Values are means ± SEM. N = 8.

match-feeding of OP rats reduced body weight gain to the level seen in fenofibrate-treated OP rats, fat pad weights (as percent of body weight) of match-fed OP rats were similar to those of control OP rats (Fig. 8). Fenofibrate reduced individual and total fat pad weights significantly in OP rats ($P < .05$). Again when absolute weights of the fat pads were analyzed, an identical pattern of treatment effects was obtained.

3.5. Experiment 5: role of malaise in food intake suppression during fenofibrate treatment

To assess whether fenofibrate treatment reduced intake by causing malaise, we performed a standard learned flavor aversion test. Rats showed no evidence that administration of fenofibrate at the dose used produced a learned flavor aversion. Rats given fenofibrate, like those given just the vehicle, showed no aversion to the flavor of Kool-Aid

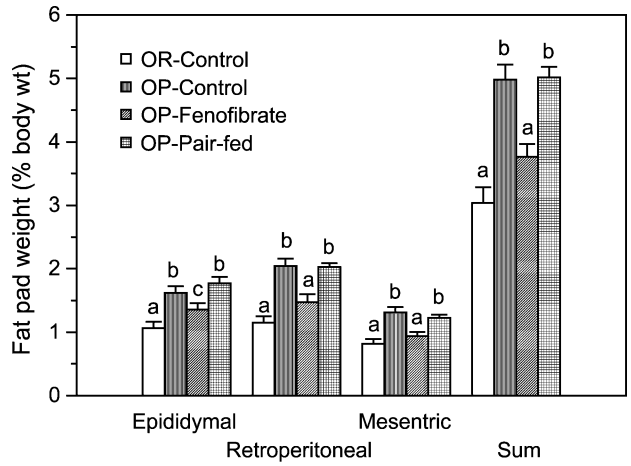

Fig. 8. Weight of epididymal, retroperitoneal, and mesenteric fat pads as percent body weight in OR rats fed plain (control) diet and OP rats fed either plain or fenofibrate-supplemented HFD or match-fed to fenofibrate-treated rats. Values are means ± SEM. N = 8. Values in the same group with same letters are not significantly different ($P > .05$). Results presented as absolute weights of the fat pads yield an identical pattern of treatment effects.

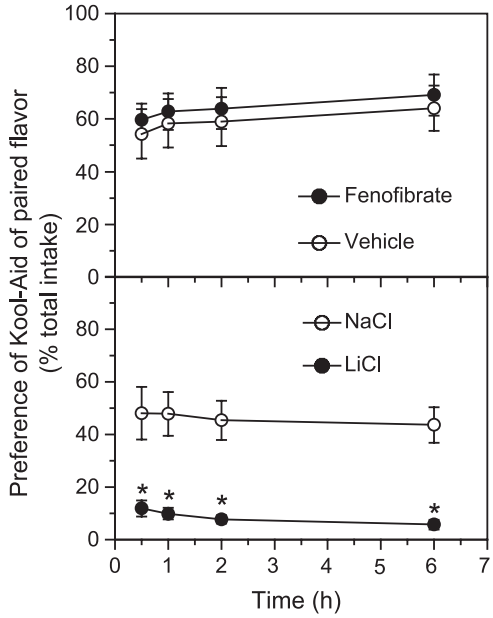

Fig. 9. Preference of the flavor of a Kool-Aid solution paired with treatment of (top panel) fenofibrate (15 mg/kg) or (bottom panel) LiCl (3 mmol/kg) over a novel flavor. Vehicle (0.5% methyl cellulose) or NaCl was used as a control. Values are means ± SEM. N = 10. The asterisk indicates significant difference from control ($P < .001$).

solution consumed with the drug treatment because intakes of the flavored Kool-Aid solutions paired with fenofibrate or vehicle treatment were similar (Fig. 9, top panel). When rats were assigned post hoc into OP and OR according to their weight gain during the high-fat feeding and the data reanalyzed, OP rats tended to prefer the flavor associated with fenofibrate slightly more (~15%) than did OR rats, although this difference was not statistically significant. In contrast, when rats were tested again for a conditioned aversion associated with LiCl solution, a strong avoidance to the flavor paired with LiCl, but not to that paired with NaCl, was observed (Fig. 9, bottom panel).

4. Discussion

Previous studies examined the effects of fenofibrate treatment only in obese animals, showing that the drug reduced body weight and adiposity [19-23]. By taking advantage of the variable propensity among outbred Sprague-Dawley rats to develop obesity while eating HFDs, the present study showed that at the dose used, only rats prone to obesity reduce energy intake and lose body fat when treated with fenofibrate. The findings reported here therefore demonstrate that fenofibrate is an effective and phenotype-specific treatment for dietary obesity in rats.

Enhancement of fatty acid oxidation is a major action of fenofibrate. Fenofibrate induces mRNA expression of the enzymes involved in fatty acid oxidation in a variety of species [21,23,27-33] and promotes fatty acid oxidation in vitro [34-37]. The experiments described here demonstrated for the first time that fenofibrate significantly promotes

hepatic CPT-I expression and whole-body fatty acid oxidation only in OP rats, but not in OR rats. It is possible that the overall lower rates of fatty acid oxidation observed in OP as opposed to OR rats reflected the fact that OP rats weighed more and had more body fat, which may have diluted the exogenous radioactive palmitic acid. However, this result replicates our previous observation [14] made in OP and OR rats before the development of obesity, indicating that the difference in fatty acid oxidation rates is not secondary to obesity. Thus, differential effects of fenofibrate as a function of obesity phenotype reported here are consistent with the hypothesis that a relatively low capacity for fat oxidation contributes to the development of obesity in OP rats fed a HFD [14].

Fenofibrate treatment decreased body fat (as measured by fat pad weights) in OP rats to the level seen in control OR animals. This effect cannot be attributed to the reduction in energy intake seen in fenofibrate-treated OP rats because restricting food intake in untreated OP rats to the level of treated ones did not reduce adiposity (as percent body weight) at all. Presumably, fenofibrate also increased energy expenditure to induce a loss of body fat. Whereas food-restricted (by about 10% on average) OP rats lost little body fat, they did lose body weight, suggesting that the decrease in body weight was due largely to the loss of fat-free mass. This implies that whereas a moderate energy restriction may reduce body weight, it may have little impact on body fat in the short term.

Fenofibrate supplement to the diet increased CPT1A expression significantly only in OP rats, whereas it greatly increased AOX expression in both OP and OR rats. The effect on AOX expression was consistent with results reported by others [19,21,38]; however, the effect on CPT1A was smaller than that seen in OLEFT rats [23]. This discrepancy might be due to the 7-week treatment for OLEFT rats as opposed to our 2-week treatment protocol. Although a 20- or 24-hour fast increases CPT-I expression, it seems unlikely that the small (10%) decrease in food intake during fenofibrate treatment would have a similar effect [39,40]. Because fenofibrate treatment also significantly increases liver carnitine content [34], the actual activity of CPT-I might in fact be increased more substantially than the increase in its mRNA levels suggests. These results suggest that mitochondrial fatty acid oxidation is more important for the effects of fenofibrate on body fat than is peroxisomal oxidation, even though peroxisomal genes may be more up-regulated. This is in line with the fact that peroxisomal β-oxidation only shortens long-chain fatty acids, and shortened fatty acids are eventually oxidized in mitochondria.

The results reported here, along with those of other studies, suggest that fenofibrate suppressed appetite by stimulating mitochondrial fatty acid oxidation. The match-feeding experiment (experiment 4) indicated that the loss of body fat during fenofibrate treatment was due directly to the drug's well-known effect of enhancing fatty acid oxidation and not secondary to a decline in food intake. Consistent with this conclusion is the finding that the effect of fenofibrate on weight gain starting on the second day of treatment preceded that on food intake, which was first seen on the sixth day. Given that suppression of fatty acid oxidation due to inhibition of CPT-I stimulates food intake in rats and human beings [11,13,41], it seems plausible that the suppression of food intake during fenofibrate treatment resulted from an increase in fatty oxidation produced by the drug. Because liver is the main target tissue of fenofibrate [23], it is also possible that fenofibrate reduces appetite by stimulating fatty acid oxidation and consequently hepatic energy production, which has been suggested as a signal for control of food intake [11,13,41].

It should be noted that the differential effects of fenofibrate reported here may depend on its dose and duration of administration. The dose (0.025% in diet) we used was much lower and the duration of treatment was much shorter than most other studies [19-23,42] examining the effects of PPARα agonists on obesity. We noticed that longer treatment eventually reduced body weight gain in OR rats as well (unpublished data). In fact, OR rats tended to have reduced weight gain and weight of fat pads at the end of our short-term experiments reported here, probably because of accumulation of the drug or its effects.

Reduced food intake in OP rats during fenofibrate treatment does not appear to be due to an untoward side effect of the drug because a bolus injection at a dose that was sufficient to reduce intake when consumed over a 24-hour period did not produce a learned flavor aversion. In addition, hepatomegaly, which is seen in rodents (but not in human beings) given fenofibrate and other peroxisome proliferators, does not appear to play a role in the suppression of food intake because liver enlargement was observed in both fenofibrate-treated OP and OR rats, but only OP rats reduced food consumption. Fu et al [42] showed a natural ligand of PPARα, oleylethanolamide, suppresses intake in mice and rats, and suggested down-regulation of nitric oxide signaling to be a possible mechanism. It is unknown whether this signaling mechanism plays a part in the effects observed in the present study. It is difficult to compare their results with the present study because of different drugs, duration of treatment, and species or strain.

After a fast, fenofibrate treated rats had higher glucose and lower lipid contents in blood than did control rats. The reductions in blood lipids did not reach a significant level ($P > .05$), possibly because of an increase in blood lipids as a result of fat mobilization during the fast (eg, Ref [14]), which might minimize the differences between treated and control groups. Lowered blood lipid content is in line with reduced body adiposity in these animals. High blood glucose in these rats with less body fat seems contradictory to many observations that high blood glucose is associated with high body fat content as a result of insulin resistance [43-49], but it could simply be the consequence of enhanced fatty acid oxidation, caused by fenofibrate, which promotes

gluconeogenesis in liver. Fenofibrate had no effect on blood insulin levels in either OP or OR rats measured after a 12-hour fast (unpublished observation), suggesting the animals did not develop insulin resistance, at least during short-term treatment. It is not known from our experiments, but will be interesting to examine, whether fenofibrate increases blood glucose under fed conditions.

PPARs have attracted much interest recently because of their role in fat metabolism and their potential as targets for treating hyperlipidemia, fatty liver, insulin resistance, obesity, and metabolic syndromes [50]. Agonists of either PPARα or γ reduce blood lipids and enhance insulin sensitivity; however, PPARα agonists reduce food intake and body fat, whereas PPARγ agonists promote energy intake, lipid uptake into adipose tissue, and adiposity [19,20,51,52]. So far, we are not aware of any human study demonstrating the effect of PPARα agonists alone on body weight or adiposity. It is interesting that peroxisome proliferators seem to be less toxic in human beings than they are in rodents because they do not induce hepatomegaly or other hepatocarcinogenic effects [53]. Thus, further research into PPARα and its activators is warranted and may yield new and effective strategies to treat obesity and related diseases. The results presented here further this effort by showing that fenofibrate is effective in treatment of dietary obesity by reducing weight gain, food intake, and adiposity. The underlying mechanisms include enhancement of fat oxidation through increasing expression of genes involved in fatty acid oxidation.

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