Differential Influences of Peroxisome Proliferator–Activated Receptors γ and -α on Food Intake and Energy Homeostasis

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Chronic treatment with compounds activating peroxisome proliferator–activated receptor (PPAR)γ and -α influences body energy stores, but the underlying mechanisms are only partially known. In a chronic-dosing study, equiefficacious antihyperglycemic doses of the PPARγ agonist pioglitazone and PPARα/γ dual activator ragaglitazar were administered to obesity-prone male rats. The PPARα agonist fenofibrate had no effect on insulin sensitivity. Pioglitazone transiently increased and fenofibrate transiently decreased food intake, whereas ragaglitazar had no impact on feeding. As a result, body adiposity increased in pioglitazone-treated rats and decreased in fenofibrate-treated rats. PPARγ compounds markedly increased feed efficiency, whereas PPARα agonist treatment decreased feed efficiency. In fenofibrate-treated rats, plasma acetoacetate was significantly elevated. Plasma levels of this potentially anorectic ketone body were unaffected in pioglitazone- and ragaglitazar-treated rats. High-fat feeding markedly increased visceral fat pads, and this was prevented by pioglitazone and ragaglitazar treatment. Pioglitazone treatment enlarged subcutaneous adiposity in high-fat–fed rats. In conclusion, PPARγ activation increases both food intake and feed efficiency, resulting in net accumulation of subcutaneous body fat. The impact of PPARγ activation on feeding and feed efficiency appears to be partially independent because the PPARα component of ragaglitazar completely counteracts the orexigenic actions of PPARγ activation without marked impact on feed efficiency. *Diabetes* 52:2249–2259, 2003

The nuclear receptors, peroxisome proliferator–activated receptors (PPARs), constitute a family of three genes, PPARα, -γ, and -β(δ), all of which are involved in control of energy homeostasis (1,2). Unequivocal evidence of endogenous ligands for PPARα and -γ is lacking, but a number of synthetic PPAR activating ligands exist, of which hypolipidemic fibric acids are typical examples of PPARα activators while hypoglycemic thiazolidinediones are typical examples of PPARγ activators.

The antidiabetic effects of PPARγ agonists are partly mediated via increased insulin sensitivity of adipose tissue and skeletal muscle. From clinical experience, PPARγ agonists are associated with weight gain, whereas PPARα agonists appear body weight neutral (3). Part of the body weight increase may be caused by their oedema-inducing class effect, but activators of PPARγ also induce adipogenesis (4,5). They act preferentially on subcutaneous adipocytes, which in comparison to intrabdominal adipocytes express higher levels of PPARγ (6). The long-term metabolic consequences of the increased fat accumulation accompanying treatment with PPARγ agonists are not fully elucidated. Intrabdominal body fat accumulation is one of several hallmarks typifying the metabolic syndrome and, as such, an independent risk factor of type 2 diabetes (7–9). Many clinical trials of oral antidiabetic agents (including PPARγ agonists) are conspicuous by their absence of obese test subjects. Thus, it may be that the apparent lack of effect by PPARγ activation on intra-abdominal adipocytes is specific to normal-weight subjects, leaving an enhanced intra-abdominal fatty acid accumulation in PPARγ agonist–treated obese type 2 diabetic patients an ill-fated possibility.

Also, PPARγ activation is associated with decreased plasma levels of leptin despite increased body adiposity (10,11). The reason for this utter paradox is uncertain, but lowered leptin levels may increase food consumption. However, the reported effects of PPARγ agonists on feeding are ambiguous. In lean rodents, short-term (1-week) PPARγ activation increases feeding with no overt effect on body weight (11), whereas leptin signaling–deficient mice and rats markedly increase food intake and body adiposity when treated with PPARγ agonists (12,13). In contrast, PPARα agonists decrease body weight in both leptin receptor–deficient *fa/fa* rats and high-fat–fed rodents (14,15). In a recent study, we have observed that the dual PPARα and -γ activator, ragaglitazar (16), appears much less adipogenic compared with the known PPARγ activators rosiglitazone and pioglitazone (17). To further study the metabolic consequences of combined PPARα and -γ activation, food intake, body composition, and metabolic surrogate markers were analyzed in low-fat– and high-fat–fed obesity-prone rats treated for 32 days with either the dual PPARα/γ activator ragaglitazar or the reference PPARγ and -α activators pioglitazone and feno-

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Received for publication 5 May 2003 and accepted in revised form 10 June 2003.

LPL, lipoprotein lipase; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator–activated receptor; UCP-1, uncoupling protein-1.

TABLE 1
Body weight change during drug treatment (days 16–44)

	Vehicle	Pioglitazone	Fenofibrate	Ragaglitazar
Low fat fed				
Body weight change (g)	54.8 ± 5	102.9 ± 9*	46.9 ± 9†‡	77.8 ± 9*†
Body weight change (%)	12 ± 1	22 ± 2*	10 ± 2†‡	17 ± 2*†
High fat fed				
Body weight change (g)	65.9 ± 7	104.0 ± 7*	27.3 ± 6*†‡	99.3 ± 5*†
Body weight change (%)	14 ± 1	21 ± 2*	6 ± 1*†‡	21 ± 1*

Data are means ± SD. *$P < 0.05$ vs. vehicle on same diet; †$P < 0.05$ vs. pioglitazone, same diet; ‡$P < 0.05$ vs. ragaglitazar, same diet (n = 8 in all groups, one-factor ANOVA followed by Fisher projected least significant differences post hoc analysis).

fibrate, respectively. We have chosen this animal model because it clearly differentiates itself from monogenic or inbred rodent models of obesity by resembling the upper body weight segment of a normally distributed human population characterized by metabolic syndrome.

RESEARCH DESIGN AND METHODS

Animals. All animal experiments were conducted according to the rules given by the local institutional ethics committee and a personal license from the Danish Justice Department (to P.J.L.). Sixty-four selectively bred male rats, 8–10 weeks of age, displaying enhanced likelihood of developing diet-induced obesity (18), were used throughout the study. The diet-induced obese rats were chosen because they are likely to reflect the segment of the human population that develops diet-induced obesity and, subsequently, type 2 diabetes.

Upon arrival to the animal unit, rats were housed individually at controlled temperature conditions and in a 12-h light/dark cycle. After 1 week of acclimatization (day 0), animals were randomized into stratified groups to ensure equal body weight means. At day 0, animals were randomized (n = 32 in each group) to eat either a high-fat diet (4.41 kcal/g [carbohydrate 51.4% kcal, fat 31.8%, protein 16.8%], diet no. 12266B; Research Diets, New Brunswick, NJ) or a low-fat diet (4.41 kcal/g [carbohydrate 72.6% kcal, fat 10.6%, protein 16.8%], diet no. D12489; Research Diets). Measurements of food and water intake were initialized and continued every weekday throughout the study. Body weights were measured three times weekly and on the day of the oral glucose tolerance test (OGTT).

The experimental diets were administered in a schedule-fed regimen such that food was removed for a 7-h period during the light phase (from 0800 to 1500), ensuring that animals had not eaten 4–6 h before drug administration. From previous experience with feeding experiments on rats, we know that an acclimatization period of no less than 10 days should be allowed if novel food is introduced. At day 15, each diet group was further subdivided into four treatment groups (n = 8 in each group): vehicle (0.2% CMC + 0.4% Tween-80 [wt/vol] in NaCl), pioglitazone (30 mg · kg^{-1} · day^{-1}), fenofibrate (100 mg · kg^{-1} · day^{-1}), and ragaglitazar (3 mg · kg^{-1} · day^{-1}). Drugs were dosed twice daily for a total of 32 days (days 16–46). On day 41, animals were subjected to an OGTT. Six days later, on day 47, animals were killed in a semistarved state (80% of ad libitum food intake). In the morning period, animals were anesthetized by CO_2 inhalation and orbital blood samples were collected before decapitation and tissue dissection. Blood and tissue sampling were carried out in a room adjacent to the permanent stable to ensure the lowest possible level of stress. Fat depot analyses were carried out by removing mesenterial, retroperitoneal, epididymal, and subcutaneous inguinal fat.

OGTT. This test was carried out at 1400 h in the afternoon on day 41. Animals were mildly fasting (without food since 0700 h). Blood samples were taken as tail vein droplets and blood glucose measured on a conventional Glucometer at time points 0, 15, 30, 60, and 120 min after oral administration of 1 g/kg glucose (using 1 g/ml dH_2O). The oral glucose load was given as gavage.

Blood sampling and plasma measurements. On the day animals were killed, orbital blood was collected in three tubes: Vacutainer-EDTA, Vacutainer–EDTA + 1% NaF, and Vacutainer–EDTA + Aprotinin (750 KIU). Blood samples were subjected to analyses for HbA_{1c}, total cholesterol, and glycerol. These variables were all measured using standard enzyme assay kits on a fully automated analyzer (Hitachi). Plasma triacyl glycerol levels were measured by the GPO-Trinder triglyceride kit (Sigma). Samples taken in Vacuatiner–EDTA + 1% NaF were used for analyses of plasma nonesterified free fatty acids using a acyl-CoA oxidase–based colorimetric kit (NEFA-C; Wako, Osaka, Japan).

Plasma leptin, insulin, C-peptide, GLP-1(7-37), adiponectin, and glucagon were assayed using immunologically based assay kits (ELISA or RIA; Linco Research Immunoassay, St. Charles, MO).

Hepatic and adipose tissue gene expression. Small samples of liver and inguinal fat were submerged in RNAlater at room temperature and stored in RNAlater at −20°C until use. Total RNA was extracted in TRIzol (Life Technologies, Gaithersburg, MD). Subsequent cDNA synthesis was carried out using standard procedures based on Gibco's reverse transcriptase Superscript II and random primers. The PCR step used two or more primer sets per reaction and incorporated hot nucleotides during the PCR. The PCR was run between 16 and 25 cycles and analyzed on a 6% sequencing gel. Bands were quantified using a Phosphor Imager and the results expressed as a ratio of the gene in question to the internal standard.

The internal standards used depended on the gene being analyzed and the number of amplification cycles. In order of increasing cycles, the following internal standards were used: Tubulin, 36B4 (acidic ribosomal phosphoprotein), EF1-a, G6PDH, and TBP (up to 18, 18, 22, 24, and 25 cycles, respectively).

To test for drug-specific actions in target tissues, the following rat transcripts were analyzed: *1*) liver: PEPCK and fatty acyl-CoA oxidase; *2*) adipose tissues: aP2, leptin, adiponectin, lipoprotein lipase (LPL), and uncoupling protein-1 (UCP-1); compounds: ragaglitazar (also known as NNC 61–0029, DRF(−)2725) and pioglitazone (AD-4833, Actos) were both synthesized at Novo Nordisk (Måløv, Denmark). Fenofibrate was purchased from Sigma.

Statistical evaluation. All results are presented as means ± SE, unless otherwise stated. Data were analyzed for main effects by a two-factor ANOVA (diet times drug treatment). When a significant main effect was observed, data within each diet group were analyzed by a one-factor ANOVA followed by Fisher or Bonferroni post hoc analyses, as appropriate (n = 8 per group). All data were digitized using Excel 5.0 as database software, and statistical analysis were performed using Statview or Graph Pad software.

RESULTS

Body weight gain and tissue weights. At day 0, rats were stratified by weight and, consequently, mean body weights were similar in both groups (low fat 418.8 ± 5.65 g, high fat 418.8 ± 5.4 g). Thirteen days after starting the diet, the body weight increase of high-fat–fed animals was clearly different from that of low-fat–fed animals (51.8 ± 2.2 vs. 36.1 ± 2.1 g [means ± SE], n = 32; $P < 0.0001$). At day 14, high-fat– and low-fat–fed animals were further stratified into four different treatment groups. Analyses of body weight were carried out on temporal weight changes (Table 1). Two-factor ANOVA did not reveal a main effect of the diet on body weight ($F_{1,54}$ = 0.453; P = 0.504), but a main effect of drug treatment ($F_{3,54}$ = 40.191; $P < 0.0001$) was observed together with a clear diet-drug interaction ($F_{3,54}$ = 58.001; P = 0.013). In both low-fat– and high-fat–fed rats, pioglitazone and ragaglitazar significantly increased body weight compared with vehicle treatment. In high-fat–fed animals, fenofibrate-treated rats gained significantly less weight than vehicle-treated rats (Table 1).

Tissue weights were measured at day 47 and expressed as weight/100 g body wt (Table 2). Two-factor ANOVA showed no main effect of diet on liver weight ($F_{1,54}$ = 1.833; P = 0.181), but there was a significant main effect of drug treatment ($F_{3,54}$ = 5.101; P = 0.004). Livers of

TABLE 2
Tissue weights at day 47 (g/100 g body wt)

	Vehicle	Pioglitazone	Fenofibrate	Ragaglitazar
Low fat fed				
Liver	3.77 ± 0.06	2.93 ± 0.04*	5.64 ± 0.07*†‡	3.29 ± 0.04*†
Muscle	0.56 ± 0.02	0.52 ± 0.01	0.54 ± 0.02	0.55 ± 0.01
Inguinal fat	0.43 ± 0.03	0.57 ± 0.07*	0.49 ± 0.04	0.52 ± 0.05
Epididymal fat	1.50 ± 0.07	1.42 ± 0.06	1.22 ± 0.06*	1.33 ± 0.06
Perirenal fat	1.69 ± 0.11	1.58 ± 0.07	1.49 ± 0.14	1.54 ± 0.09
Mesenterial fat	1.84 ± 0.13	1.96 ± 0.08	1.73 ± 0.13	1.73 ± 0.09
Total fat pad	5.9 ± 0.3	6.08 ± 0.2	5.34 ± 0.4	5.3 ± 0.1
High fat fed				
Liver	3.34 ± 0.10§	2.81 ± 0.08*	5.93 ± 0.17*†‡§	3.18 ± 0.07†
Muscle	0.53 ± 0.01	0.49 ± 0.01*	0.56 ± 0.02†‡	0.51 ± 0.01
Inguinal fat	0.49 ± 0.06	0.69 ± 0.04*	0.56 ± 0.02	0.45 ± 0.05†
Epididymal fat	1.61 ± 0.12	1.53 ± 0.06	1.32 ± 0.10*†	1.41 ± 0.06
Perirenal fat	2.08 ± 0.12§	2.24 ± 0.09§	1.63 ± 0.06*†	2.03 ± 0.13§
Mesenterial fat	2.54 ± 0.24§	2.12 ± 0.17*	1.99 ± 0.10*	1.85 ± 0.11*
Total fat pad	7.2 ± 0.5§	7.2 ± 0.3§	6.05 ± 0.2*†	6.3 ± 0.4†

Data are means ± SD. *$P < 0.05$ vs. vehicle on same diet; †$P < 0.05$ vs. pioglitazone, same diet; ‡$P < 0.05$ vs. ragaglitazar; same diet; §$P < 0.05$ vs. low fat, same treatment ($n = 8$ in all groups, one-factor ANOVA followed by Fisher protected least significant difference post hoc analysis).

fenofibrate-treated animals were markedly enlarged, whereas pioglitazone significantly reduced liver weight. Treatment with ragaglitazar had no impact on liver weight (Table 2). Using two-factor ANOVA, small but statistically significant main effects of both diet ($F_{1,54} = 5.017$; $P = 0.029$) and drug treatment ($F_{3,54} = 3.632$; $P = 0.018$) were seen on muscle weight without interaction between the diet and drug treatment (Table 2).

Feeding rats a high-fat diet exerted main effects on all visceral fat depots: perirenal ($F_{1,54} = 35.173$; $P < 0.0001$), mesenterial ($F_{1,54} = 9.915$; $P = 0.003$), and epididymal ($F_{1,54} = 4.464$; $P = 0.039$). However, no effects of diet were seen on inguinal fat accumulation ($F_{1,54} = 3.086$; $P = 0.09$). In contrast, drug treatment influenced fat accumulation in all regional fat depots: perirenal ($F_{3,54} = 4.748$; $P = 0.005$), mesenterial ($F_{3,54} = 9.915$; $P = 0.003$), epididymal ($F_{3,54} = 5.642$; $P = 0.002$), and inguinal ($F_{3,54} = 5.560$; $P = 0.002$). An interaction between diet and drug treatment on resulting weights of fat depots was not observed. The high-fat–induced increase in regional adiposity was most pronounced for the perirenal (23%) and mesenterial (38%) depots (Table 2). Relative weights of the epididymal and inguinal adipose tissue depots remained unaffected by high-fat feeding. All compounds eliminated high-fat diet–induced weight gain of mesenterial adipose tissue depots. In high-fat–fed animals, perirenal adipose tissues remained enlarged in pioglitazone- and ragaglitazar-treated animals, whereas fenofibrate treatment abolished the effect of high-fat feeding on the size of the perirenal adipose tissue depot (Table 2). Similar results were seen for pooled adipose tissue samples, i.e., isolated main effects of both diet and drug treatment without interaction between the two variables (Table 2).

Food intake. To assess the underlying cause of diet- and pharmacologically induced alterations of body weight, food intake was assessed daily. Diets were equicaloric, such that the daily gravimetrically determined consumption of food accurately reflected the caloric consumption.

Cumulated food intake from day 16 to 44 is shown in Fig. 2*A*. Two-factor ANOVA revealed that drug treatment ($F_{3,54} = 9.110$; $P < 0.0001$) but not diet ($F_{1,54} = 3.893$; $P = 0.06$) exerted main effects on cumulated food intake of the entire treatment period. Interaction between drug treatment and diet was not observed for cumulated food intake between days 16 and 44. Cumulated food intake over the first 2 weeks of drug treatment was markedly elevated in pioglitazone-treated rats (Fig. 2*A*). Cumulated food intake for high-fat–fed fenofibrate-treated rats was significantly lower than that for vehicle-treated rats eating the same diet, as well as for low-fat–fed fenofibrate-treated rats, clearly indicating that the anorectic action of fenofibrate is diet dependent (Fig. 3).

Vehicle-treated animals displayed stable daily food intake throughout the dosing period. The temporal pattern of food consumption displayed marked elevation of food intake in pioglitazone-treated rats during the initial 2 weeks of drug treatment (Fig. 1 and Table 3). Because of this graphically evident difference in daily food consumption, analyses of cumulated food intake were arbitrarily divided into the first 2 weeks (days 16–30) and the last 2 weeks (days 31–44) of treatment (Table 3).

Feed efficiency (body weight change in grams [days 16–44] × 100/cumulated food intake [days 16–44]) was calculated for all treatments and diets (Fig. 2*B*). It was obvious that fenofibrate treatment reduced feed efficiency significantly in both low-fat– and high-fat–fed animals, whereas both pioglitazone and ragaglitazar treatments significantly increased feed efficiency when compared with diet-matched vehicle-treated groups.

As most experimental rat diets are rather dry and require moistening to allow proper mastication, virtually all orexigenic treatments are accompanied by moderate increases of fluid intake. We therefore assessed daily water intake in all groups. Water intake was affected to a lesser degree than food intake (Table 3). However, when assessing cumulated water intake from day 16 to 44, significant main effects of both drug and diet were clearly observed (drug $F_{3,45} = 4.201$, $P = 0.011$; diet $F_{1,45} = 8.043$, $P = 0.007$; two-factor ANOVA) but without interaction ($F_{3,45} = 0.073$; $P = 0.97$). Thus, high-fat–fed rats drank

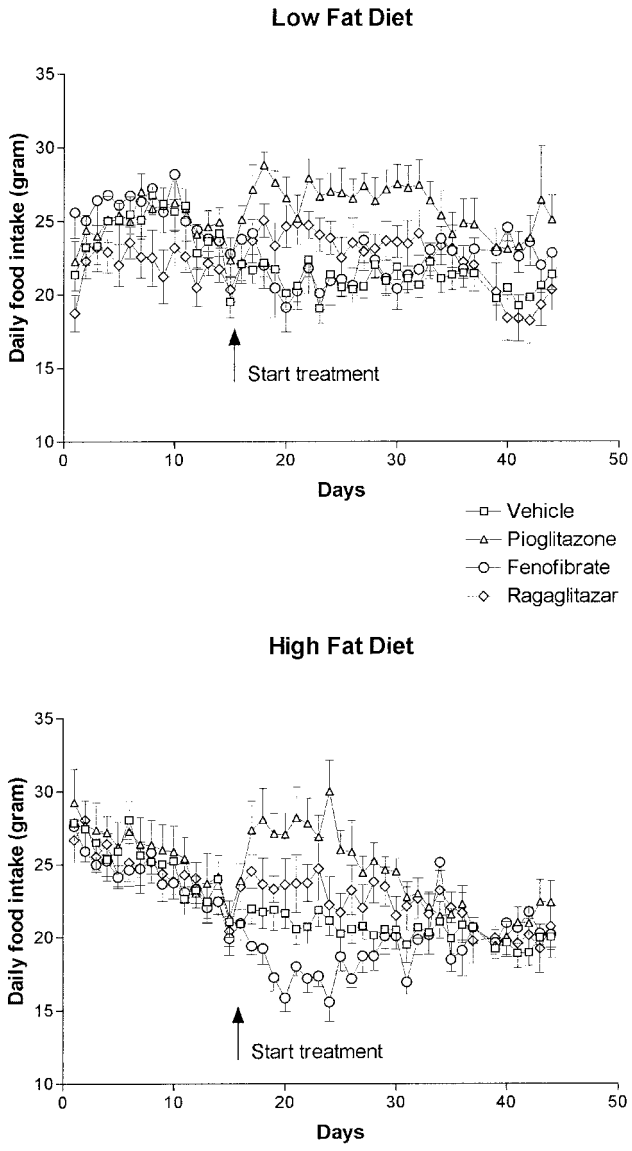

FIG. 1. Feeding behavior in groups of individually housed adult male rats fed either low- or high-fat diets for 44 days. During the last 32 days, animals were orally dosed with vehicle, pioglitazone, fenofibrate, or ragaglitazar. Food intake was registered daily throughout the study.

slightly more than low-fat–fed animals, and the orexigenic pioglitazone treatment resulted in elevated water intake in both low-fat–fed (694 ± 26 vs. 599 ± 22 ml, $n = 6–8$; $P = 0.02$) and high-fat–fed (752 ± 46 vs. 654 ± 31 ml, $n = 6–8$; $P = 0.02$) rats. None of the other drug treatments gave rise to elevated water intake.

OGTT. On day 41, all animals were subjected to an OGTT. Two-factor ANOVA revealed that both diet ($F_{1,54} = 7.637$; $P = 0.008$) and drug treatment ($F_{3,54} = 16.185$; $P < 0.0001$) exerted significant main effects on the OGTT without interaction between diet and drug treatment ($F_{3,54} = 0.823$; $P = 0.5$). The diet-dependent effect was seen, with high-fat–fed animals displaying poorer OGTT scores (Table 4). Both pioglitazone and ragaglitazar significantly reduced the area under the curve in both low-fat– and high-fat–fed animals, thereby eliminating the diet-dependent effect on glucose tolerance (Table 4). Fenofibrate treatment had no impact on OGTT scores.

Blood biochemistry (glucose, HbA_{1c}, and lipids). Fasting plasma glucose levels were neither affected by diet nor treatment (Table 5), and HbA_{1c} levels were also unaffected by diet (4.39 ± 0.03 vs. 4.51 ± 0.06%; $P = 0.11$). However, in contrast to fasting plasma glucose levels, HbA_{1c} levels were reduced by treatment with all PPAR-activating compounds in both low-fat– and high-fat–fed rats (Table 5).

As expected, blood lipid profiles were markedly affected by drug treatment, whereas high-fat feeding gave rise to a paradoxical small drop in plasma triglycerides and a marked increase in HDL cholesterol. Both PPARα compounds induced significant decreases in total plasma cholesterol. Although a lowering effect of pioglitazone on total cholesterol was absent, treatment with this compound for 44 days markedly increased plasma HDL cholesterol levels (Table 5). All of the compounds used induced a diet-independent reduction of lipolysis, as evidenced by decreased circulating levels of both glycerol and free fatty acids.

In the rat, PPARα agonists are potent stimulators of hepatic β-oxidation, but the extent to which PPARγ and dual activators stimulate such catabolism of nonesterified fatty acids is unknown. Plasma concentrations of ketone bodies were used as an indirect measure of hepatic β-oxidation (Table 6). The main effects of drug treatment and diet on plasma total ketone bodies were analyzed using two-factor ANOVA, revealing a significant effect of drug treatment ($F_{3,54} = 3.857$; $P = 0.014$) but not of diet ($F_{1,54} = 1.444$; $P = 0.24$), and there was no drug times diet interaction ($F_{3,54} = 0.526$; $P = 0.67$). Interestingly, plasma levels of 3-hydroxybutyrate were neither affected by drug treatment ($F_{3,54} = 2.654$; $P = 0.06$) nor by diet ($F_{1,54} = 1.485$; $P = 0.23$), whereas plasma levels of acetoacetate were markedly affected by drug treatment ($F_{3,54} = 20.130$; $P < 0.0001$). Diet had no impact on plasma concentrations of acetoacetate ($F_{1,54} = 0.888$; $P = 0.35$), and no interaction between drug and diet was observed ($F_{3,54} = 0.647$; $P = 0.59$).

Endocrine responses to 44 days of treatment with PPAR-activating compounds. A number of hormones associated with glucose homeostasis were measured (Table 7). Diet had no impact on plasma insulin levels, but all of the PPARγ-activating compounds induced a marked reduction of circulating insulin levels, irrespective of the diet.

Plasma levels of glucagon and of the incretin hormone, GLP-1(7-36NH_2) were completely unaffected by diet and pharmacological treatment. Two-factor ANOVA revealed that diet had no main effect on plasma levels of corticosterone ($F_{1,53} = 0.74$; $P = 0.39$), while drug treatment exerted a significant main effect on plasma corticosterone levels ($F_{3,53} = 4.37$; $P = 0.008$). Significant interaction between diet and drug treatment was also observed for plasma corticosterone ($F_{1,53} = 4.71$; $P = 0.006$). Thus high-fat feeding abolished fenofibrate- and ragaglitazar-induced elevation of plasma corticosterone (Table 7). As in most models of adiposity, high-fat feeding also elevated plasma corticosterone in vehicle-treated animals (Table 7).

Two-factor ANOVA revealed the main effects of both diet ($F_{1,55} = 12.059$; $P = 0.001$) and drug treatment ($F_{3,55} = 8.005$; $P = 0.0002$) on plasma leptin levels. No interaction

TABLE 3
24-h food and water intake on selected days and cumulated food intake over the first 2 weeks of drug treatment and over the 2 weeks of drug treatment (food: grams/day; water: milliliters/day)

	Food intake day 13	Food intake day 16	Food intake day 30	Food intake day 44	Cumulated food intake (days 16–30)	Cumulated food intake (days 31–44)
Low-fat diet						
Vehicle	2723.6 ± 1.2	22.1 ± 1.1	21.9 ± 0.9	21.4 ± 1.0	317 ± 12	268 ± 11
Pioglitazone	24.6 ± 1.1	25.1 ± 0.9	27.5 ± 1.3*	25.1 ± 1.7	404 ± 16*	325 ± 16*
Fenofibrate	23.8 ± 1.3	23.7 ± 1.7	20.4 ± 1.4†	22.9 ± 1.7	321 ± 18†	296 ± 12
Ragaglitazar	22.1 ± 0.9	22.1 ± 1.3	23.6 ± 1.1†	20.3 ± 1.4‡	356 ± 16†	275 ± 17†
High-fat diet						
Vehicle	22.4 ± 1.1	20.9 ± 1.1	20.5 ± 1.4	20.0 ± 0.9	315 ± 14	260 ± 11
Pioglitazone	23.6 ± 2.0	23.9 ± 1.2	24.5 ± 0.9*	22.4 ± 1.5	397 ± 19*	280 ± 8
Fenofibrate	22.0 ± 1.3	20.9 ± 1.2	20.1 ± 0.9†	20.1 ± 1.6	274 ± 7*†‡	263 ± 11
Ragaglitazar	22.3 ± 1.4	23.5 ± 1.6	21.5 ± 1.3	20.7 ± 1.4	348 ± 21†	272 ± 14
	Water intake day 13	Water intake day 16	Water intake day 30	Water intake day 44	Cumulated water intake (days 16–30)	Cumulated water intake (days 31–44)
Low-fat diet						
Vehicle	19.2 ± 0.9	19.8 ± 0.9	23.3 ± 2.7	18.5 ± 1.0	294 ± 9	307 ± 11
Pioglitazone	21.3 ± 1.5	25.1 ± 0.7*	21.3 ± 1.0	19.9 ± 2.8	342 ± 11*	351 ± 17*
Fenofibrate	20.9 ± 1.0	22.6 ± 1.2	20.6 ± 3.2	17.8 ± 1.3	303 ± 11†	320 ± 9
Ragaglitazar	17.2 ± 0.7†	21.6 ± 1.0	22.8 ± 1.4	19.9 ± 1.2	318 ± 10	318 ± 19
High-fat diet						
Vehicle	20.7 ± 1.3	24.7 ± 2.4	25.8 ± 2.0	21.7 ± 1.6	321 ± 13	337 ± 15
Pioglitazone	22.5 ± 1.5	24.8 ± 1.3	23.9 ± 0.7	24.1 ± 1.9	385 ± 24*	363 ± 16
Fenofibrate	22.9 ± 2.3	23.3 ± 1.3	27.0 ± 2.1†	21.6 ± 1.8	313 ± 18†	351 ± 15
Ragaglitazar	21.2 ± 1.3	24.5 ± 1.3	24.8 ± 1.0	21.4 ± 1.5	349 ± 10	346 ± 8

Data are means ± SE. Two-factor ANOVA of all data points revealed significant main effects of drug treatment on food intake on days 30 and 44, as well as on cumulated food intake in the period days 16–30 and 31–44. No main effects of diet and no interaction between drug and diet were seen on any of the days and periods. Two-factor ANOVA of all data points revealed main effects of diet on days 13, 16, 30, and 44 but no main effects of drug and no interaction between drug and diet were seen on any of the days. Two-factor ANOVA revealed significant effects of both drug and diet for cumulated water intake in the periods days 16–30 and 31–44 but with no interaction. Significantly different values, as revealed by subsequent one-factor ANOVA (Bonferroni correction for repeated measures) are as follows: *$P < 0.05$ vs. vehicle on same diet; †$P < 0.05$ vs. pioglitazone, same diet; ‡$P < 0.05$ vs. ragaglitazar, same diet.

between diet and drug treatment was observed ($F_{3,55}$ = 1.970; P = 0.13). In low-fat–fed rats, fenofibrate treatment gave rise to significantly lower levels of leptin. Interestingly, the dose of pioglitazone used elevated leptin to levels significantly higher than those seen in any other high-fat–fed group (Table 7).

Finally, plasma levels of adiponectin were measured. Main effects of both diet ($F_{1,56}$ = 13.483; P = 0.0005) and drug treatment ($F_{3,56}$ = 66.1919; $P < 0.0001$) were observed without interaction between the two variables. In vehicle-treated rats, high-fat feeding had no impact on plasma adiponectin concentrations, but in both pioglitazone- and ragaglitazar-treated groups, high-fat–fed rats displayed lower adiponectin levels compared with low-fat–fed groups. Also, it was quite clear that treatment with either of the PPARγ activators pioglitazone or ragaglitazar significantly increased adiponectin levels compared with vehicle-treated animals fed the same diet. Fenofibrate treatment had no impact on plasma adiponectin levels in any of the diet groups.

In vehicle-treated rats, plasma adiponectin levels were not correlated to plasma levels of leptin (regression line: Y = 2.8 + 0.05X; R^2 = 0.046), irrespective of diet (Fig. 3). Interestingly, pioglitazone treatment induced a marked change such that plasma adiponectin levels became negatively correlated to plasma leptin levels (regression line: Y = 14.6–0.46X; R^2 = 0.615). None of the other drug treatments introduced such correlations, indicating that a specific PPARγ action was absent in the concomitant presence of PPARα stimulation.

Hepatic and adipose tissue gene expression. To validate drug actions in the liver, we assessed hepatic expression of fatty acyl-CoA oxidase and PEPCK as markers of PPARα activation and insulin sensitivity, respectively (Table 8). In addition, drug actions were assessed in inguinal adipose tissue by measurements of aP2 expression and by measurement of interscapular brown adipose tissue UCP-1 expression (Table 8).

In the liver, compounds with PPARα-activating potential, fenofibrate and ragaglitazar, both enhanced acyl-CoA oxidase expression independent of diet. Hepatic PEPCK expression was markedly elevated in high-fat–fed rats, and this diet-induced expression was abolished by all compounds (pioglitazone, fenofibrate, and ragaglitazar). In low-fat–fed rats, both of the PPARγ activators enhanced brown adipose tissue uncoupling protein-1 expression (Table 7).

Similarly, both PPARγ activators stimulated white adipose tissue expression of the adipocyte marker aP2 (Table 8). Expression of other adipocyte-specific genes was also examined in epididymal fat sampled on day 47. Thus, two-factor ANOVA revealed main effects of both diet ($F_{1,55}$ = 17.811; $P < 0.0001$) and drug treatment ($F_{3,55}$ = 10.043; $P < 0.0001$) on leptin expression, which was significantly lower in low-fat–fed animals. Treatment with pioglitazone significantly lowered leptin expression in both low-fat–

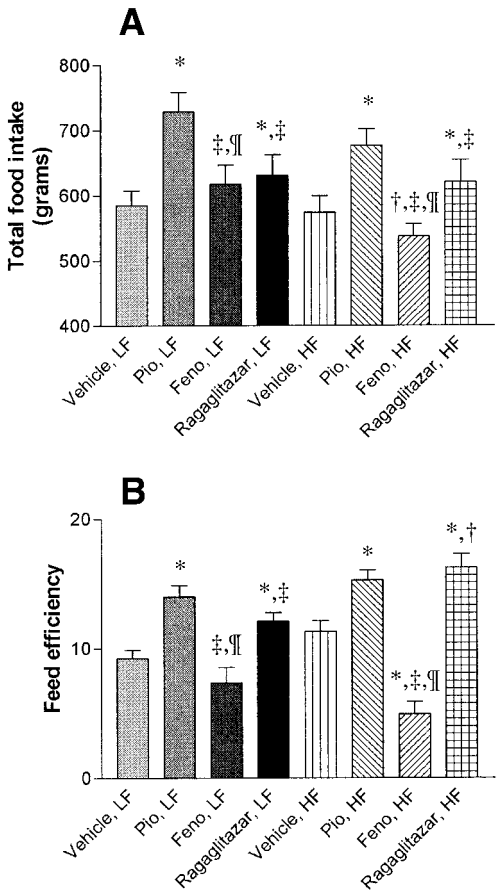

FIG. 2. *A*: Cumulated food intake (days 16–44) for individually housed adult male rats fed either low- or high-fat diets for 47 days. During the last 32 days, animals were orally dosed with vehicle, pioglitazone (Pio), fenofibrate (Feno), or ragaglitazar. *$P < 0.05$ vs. vehicle on same diet; †$P < 0.05$ vs. low fat, same treatment; ‡$P < 0.05$ vs. pioglitazone, same diet; ¶$P < 0.05$ vs. ragaglitazar, same diet ($n = 8$ in all groups, ANOVA followed by Fisher projected least significant difference post hoc analysis). *B*: Feed efficiency based on data from individually housed adult male rats fed either low- or high-fat diets for 47 days. Feed efficiency is expressed as body weight change in grams (days 16–44) × 100/cumulated food intake (days 16–44). During the last 32 days, animals were orally dosed with vehicle, pioglitazone (Pio), fenofibrate (Feno), or ragaglitazar. *$P < 0.05$ vs. vehicle on same diet; †$P < 0.05$ vs. low fat, same treatment; ‡$P < 0.05$ vs. pioglitazone, same diet; ¶$P < 0.05$ vs. ragaglitazar, same diet ($n = 8$ in all groups, ANOVA followed by Fisher projected least significant difference post hoc analysis).

and high-fat–fed animals (Table 8). Expression of adiponectin was also analyzed by two-factor ANOVA, and the main effects of both diet ($F_{1,52} = 9.609$; $P = 0.005$) and drug treatment ($F_{3,52} = 7.042$; $P = 0.0005$) were observed. A significant interaction between the two treatment modalities was also observed ($F_{3,52} = 9.018$; $P = 0.0002$), and it was reflected by a high-fat feeding–dependent increase of pioglitazone-stimulated adiponectin mRNA expression (Table 7). In low-fat–fed rats, drug treatment had no impact on adiponectin expression. The main effect of drug treatment on adipocyte expression of LPL mRNA was observed with the two-factor ANOVA ($F_{3,54} = 4.186$; $P = 0.009$). Diet had no main impact on LPL expression ($F_{1,54} = 0.132$; $P = 0.72$), and no interaction between the two treatment modalities was seen. Ragaglitazar markedly increased LPL expression in both low-fat– and high-fat–fed rats, while pioglitazone had no impact on the expression of LPL.

DISCUSSION

In line with earlier reports, the present series of experiments demonstrate that activators of PPARα and -γ exert pharmacologically differential effects on body energy homeostasis. However, this is the first study to directly compare the pharmacological effects of PPARα, -γ, and a dual PPARα/γ activator on feeding behavior and body energy homeostasis. For this purpose, we have used a unique animal model that excellently mimics human metabolic syndrome (18). Thus, once diet-induced obese rats are exposed to a high-fat diet, they develop visceral adiposity, glucose intolerance, and dyslipidemia. The diet-induced accumulation of visceral fat and its association with impaired glucose tolerance becomes clearly apparent when plotting the two variables as a regression line (Fig. 4). It is worthy of mention that the diet used to trigger this condition is not extremely energy dense and that dietary fat contributes relatively modestly to the total energy content (31.8%). Thus, a total dietary energy of 4.47 kcal/g is sufficient to trigger a syndrome with many similarities to human metabolic syndrome. Most other animal models used both in basic physiological experiments that scrutinize pathophysiological consequences of adiposity and in the pharmacological assessment of various drug treatments are based on nonphysiological diets with fat contributing to as much as 60%, or even more, of the total energy. Invariably, such diets give rise to altered consummatory behavior, as both texture and taste are far from anything normal. Another clear advantage of the presently employed animal model is its apparent presence of all major endocrine and neural regulators of feeding and energy expenditure. The diet-induced obese rats may become prematurely resistant to leptin (B.E. Levin, personal communication); however, in contrast feeding studies (19–22) performed on diabetic animal models, the rats used in this study still have an intact leptin signaling system.

In addition to recognized stimulatory effects of PPARγ activation on insulin sensitivity and peripheral adipogenesis (23,24), our present study clearly demonstrates that

TABLE 4
Areas under the curve (AUCs) from 0 to 120 min of OGTTs

	Vehicle	Pioglitazone	Fenofibrate	Ragaglitazar
Low fat				
AUC ($mmol \cdot l^{-1} \cdot min^{-1}$)	775 ± 7	711 ± 15*	838 ± 34†‡	682 ± 13*
High fat				
AUC ($mmol \cdot l^{-1} \cdot min^{-1}$)	847 ± 25§	751 ± 17*	843 ± 33†‡	735 ± 12*

Data are means ± SE. *$P < 0.05$ vs. vehicle on same diet; †$P < 0.05$ vs. pioglitazone, same diet; ‡$P < 0.05$ vs. ragaglitazar, same diet; §$P < 0.05$ vs. low fat, same treatment.

TABLE 5
Blood biochemistry (day 44)

	Vehicle	Pioglitazone	Fenofibrate	Ragaglitazar
Low Fat				
Glucose (mmol/l)	6.9 ± 0.2	6.5 ± 0.1	6.8 ± 0.3	6.9 ± 0.1
Hgb (g/dl)	21.3 ± 0.8	18.7 ± 0.7*	18.1 ± 0.7*	19.7 ± 0.5
HbA_{1c} (%)	4.51 ± 0.06	4.26 ± 0.10*	4.11 ± 0.05*	4.25 ± 0.07*
Free fatty acids (μmol/l)	202.6 ± 32	172.9 ± 35*†	221.4 ± 16†	92.50 ± 23*‡
Glycerol (μmol/l)	0.16 ± 0.01	0.12 ± 0.01	0.13 ± 0.02	0.12 ± 0.01
Triglycerides	6.6 ± 0.3	1.8 ± 0.1*	2.5 ± 0.1*†	1.2 ± 0.9*
Total cholesterol (mmol/l)	2.3 ± 0.14	1.9 ± 0.08	1.8 ± 0.14*	1.4 ± 0.05*
HDL cholesterol (mmol/l)	0.86 ± 0.08	1.33 ± 0.01*	0.83 ± 0.18‡	1.04 ± 0.03‡
High Fat				
Glucose (mmol/l)	7.4 ± 0.1	7.0 ± 0.1	6.9 ± 0.6	7.2 ± 0.2
Hgb (g/dl)	21.8 ± 0.5	18.9 ± 0.6*	19.1 ± 0.5*	19.2 ± 0.3*
HbA_{1c} (%)	4.39 ± 0.03	4.19 ± 0.05*	4.14 ± 0.05*	4.20 ± 0.04*
Free fatty acids (μmol/l)	232.1 ± 16	166.3 ± 31	163.9 ± 11†§	122.4 ± 14*
Glycerol (μmol/l)	0.25 ± 0.05‖	0.17 ± 0.01*	0.16 ± 0.01*	0.14 ± 0.01*
Triglycerides	5.2 ± 0.3‖	2.2 ± 0.2*	3.0 ± 0.2*†	1.8 ± 0.3*
Total cholesterol (mmol/l)	2.4 ± 0.10	2.1 ± 0.06	1.6 ± 0.13*	1.5 ± 0.10*‡
HDL cholesterol (mmol/l)	1.28 ± 0.10‖	1.54 ± 0.06	0.78 ± 0.07*†‡	1.12 ± 0.07‡

Data are means ± SE. *$P < 0.05$ vs. vehicle on same diet; †$P < 0.05$ vs. ragaglitazar, same diet; ‡$P < 0.05$ vs. pioglitazone, same diet; §$P = 0.06$ vs. vehicle on same diet; ‖$P < 0.05$ vs. low fat, same treatment (ANOVA followed by Fisher protected least significant difference post hoc analysis).

orexigenic actions of PPARγ-preferring ligands contribute markedly to positive energy balance. Pioglitazone and other PPARγ agonists increase fat mass by increasing the number of newly formed small-sized adipocytes and by enhancing intracellular triacylglycerol formation (13). PPARγ-induced intracellular storage of metabolically inert triacylglycerol is mediated by marked upregulation of glycerol kinase, providing excess glycerol as substrate for triglyceride formation (25).

The currently observed actions of pioglitazone emphasize that increased adipogenesis is not simply a result of increased storing capacity of triacylglycerol, but that it also arises from an increased urge to ingest energy. A previous study using the PPARγ agonists BRL49653 similarly showed increased feeding over 1 week of drug administration but with no apparent effect on total body weight (11). A number of reports have shown that PPARγ induced food intake in rodents with deficient leptin receptors, but there was a lack of effect on feeding in normal rats (13,14,19–21). Obviously, neither *fa/fa* rats nor *db/db* mice constitute optimal animal models for the study of feeding behavior because data are bound to be skewed by absence of key components of normal energy homeostasis. Also, in some of the earlier studies, PPARγ agonists were administered as part of the food, potentially affecting texture and palatability and resulting in altered feeding patterns.

The orexigenic action of pioglitazone was independent of diet, as cumulated food intakes in both low-fat– and high-fat–fed rats were markedly elevated. Pioglitazone-treated rats were both heavier and fatter than other drug-treated groups, but both plasma leptin and leptin mRNA levels were comparable to those observed in ragaglitazar-treated rats displaying normal food intake throughout the treatment period. Agonists of PPARγ have been shown to inhibit adipocyte leptin synthesis (11), suggesting that pioglitazone treatment actually increases sensitivity to leptin. The apparent altered sensitivity to plasma leptin concentrations of PPARγ-treated rats emphasizes why animals with deficient leptin signaling should be avoided in experimental analysis of the potential impact of these compounds on energy homeostasis.

In *fa/fa* Zucker rats, pioglitazone has no impact on whole-body energy expenditure (22), but these measures may not have been properly adjusted for the expected increase of thermogenesis associated with excess energy intake. Thus, it is likely that adjustment for food intake yield lowers measures of energy expenditure per consumed calorie. Both pioglitazone and ragaglitazar markedly improved feed efficiency, with or without a concomitant increase of caloric intake, emphasizing that positive energy balance associated with PPARγ activation is not solely ascribed to the orexigenic actions of these compounds. Thus, PPARγ may favor positive energy balance by a combined action of enhanced food intake and decreased energy expenditure.

Treatment with fibrates is associated with decreased body weight gain and reduced adiposity in animal models

TABLE 6
Plasma levels of ketone bodies, day 44

	Vehicle	Pioglitazone	Fenofibrate	Ragaglitazar
Total ketone bodies (μmol/l)	83.0 ± 16	163.7 ± 31*	190.7 ± 17*	134.0 ± 25
3-Hydroxybutyrate (μmol/l)	53.2 ± 11	125.4 ± 26	107.7 ± 13	96.4 ± 21
Acetoacetate (μmol/l)	29.9 ± 6	38.3 ± 6	82.9 ± 6*†‡	34.6 ± 4

Data are means ± SE. *$P < 0.05$ vs. vehicle; †$P < 0.05$ vs. pioglitazone; ‡$P < 0.05$ vs. ragaglitazar (ANOVA followed by Fisher protected least significant difference post hoc analysis).

TABLE 7
Plasma levels of glucose homeostasis related hormones, day 44

	Vehicle	Pioglitazone	Fenofibrate	Ragaglitazar
Low fat				
Insulin (pmol/l)	536.8 ± 68	247.4 ± 29*	410.6 ± 70†‡	208.5 ± 16*
Glucagon (pg/ml)	62.3 ± 20	47.5 ± 3	44.8 ± 10	56.5 ± 13
Leptin (ng/ml)	13.09 ± 1.4	10.36 ± 0.5	6.99 ± 1.0*†‡	10.70 ± 1.6
Corticosterone (nmol/l)	32.01 ± 3	103.7 ± 56	184.8 ± 48*	178.6 ± 46*
GLP-1(7-36NH_2) (pmol/l)	10.34 ± 3.3	6.27 ± 0.3	7.60 ± 0.5	6.21 ± 0.3
Adiponectin (μg/ml)	4.1 ± 0.5	10.1 ± 0.7*	3.9 ± 0.5†‡	12.0 ± 1.2*†
High fat				
Insulin (pmol/l)	498.6 ± 94	232.8 ± 25*	385.5 ± 35†	250.3 ± 41*
Glucagon (pg/ml)	56.4 ± 3	49.8 ± 2	71.1 ± 21	81.8 ± 22
Leptin (ng/ml)	13.74 ± 0.9	16.50 ± 1.1§	9.69 ± 1.4*†	12.81 ± 1.2†
Corticosterone (nmol/l)	103.6 ± 19§	82.49 ± 12	145.4 ± 21	97.03 ± 14
GLP-1(7-36NH_2) (pmol/l)	7.62 ± 0.4	9.18 ± 1.2	8.56 ± 0.5	10.63 ± 1.7
Adiponectin (μg/ml)	3.3 ± 0.3	6.8 ± 0.3*§	3.1 ± 0.7†‡	10.2 ± 0.8*†

Data are means ± SE. *$P < 0.05$ vs. vehicle on same diet; †$P < 0.05$ vs. pioglitazone, same diet; ‡$P < 0.05$ vs. ragaglitazar, same diet; §$P < 0.05$ vs. low fat, same treatment (ANOVA followed by Fisher protected least significant difference post hoc analysis).

of diet-induced obesity and in *fa/fa* Zucker rats (14,15,26). A few clinical studies have shown decreased body weight, fat mass, and waist circumference in fibrate-treated humans but no apparent effect on insulin sensitivity, which is in line with our current observations (27). Fenofibrate-mediated negative impact on energy homeostasis is most pronounced in high-fat–fed animals (15) and was reflected by a marked reduction of food intake in high-fat–fed animals in the present study. Data on cumulated caloric intake have not been published for PPARα$^{-/-}$ mice, which display a nonsignificant lowering of daily food intake that reflects a higher feed efficiency (28). This is in line with the current observations of markedly reduced feed efficiency in high-fat–fed fenofibrate-treated rats, which is likely to be the result of the combined actions of reduced feeding and increased resting energy expenditure. In a recent study, Mancini et al. (15) demonstrated that fenofibrate markedly enhances hepatic mitochondrial and peroxisomal palmiotyl–dependent oxygen consumption, leading to a slight increase in resting metabolic rate. Thus, amelioration of the metabolic syndrome markers associated with fibrate treatment may actually be accounted for by the anorectic effects of these compounds.

Increased hepatic fatty acid oxidation is significantly elevated in fenofibrate-treated animals, as evidenced by marked upregulation of hepatic acyl CoA oxidase and carnitine palmiotyl transferase expression (14). The currently observed hepatomegali and upregulated acyl-CoA oxidase expression supports a profound fenofibrate-induced peroxisome proliferation. Despite a moderate

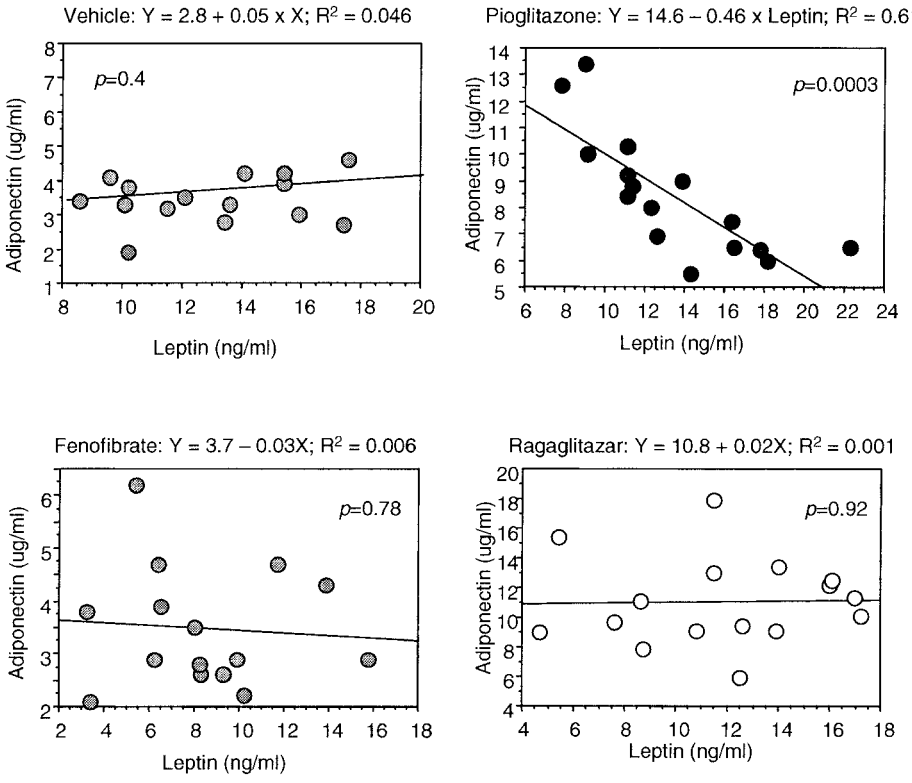

FIG. 3. Effect of pharmacological treatment on adiponectin/leptin correlation. Regression analysis of plasma adiponectin dependency on plasma leptin was based on data from identically drug-treated low-fat– and high-fat–fed rats.

FIG. 4. Regression plot displaying association of glucose tolerance as examined by a 2-h OGTT (area under the curve [AUC]) and visceral adiposity as expressed by weight of mesenterial fat depot in obesity-prone rats fed either a low- or high-fat diet. Regression line: AUC ($mmol \cdot l^{-1} \cdot min^{-1}$) = 631.5 + 81.9 × mesenterial fat (g/100 g body wt); R^2 = 0.7.

induction of hepatic acyl-CoA oxidase expression, treatment with the dual activator ragaglitazar had no impact on liver size. Fenofibrate treatment activated formation of anorectic signals markedly enhanced by intake of dietary fats, but neither free fatty acids nor circulating triacylglycerol levels were directly accountable for this anorectic action, as these parameters were similarly decreased in both pioglitazone- and ragaglitazar-treated rats. However, it is tempting to speculate that fibrate-induced intracellular accumulation of long-chain fatty acids within hepatocytes may constitute a nutritive signal curtailing further intake of nutrients. Hepatic fatty acid oxidation constitutes an important metabolic variable in the control of feeding, and much experimental evidence points toward hepatic ketone body formation comprising at least some of the measured variables (29). PPARα activation constitutes a major stimulus to hepatic expression of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mHMG-CoAS), a key enzyme in ketogenesis (30). Furthermore, the anorectic actions of systemically administered 3-hydroxybutyrate are abolished by selective hepatic vagotomy (31), but the brain may also detect circulating ketone bodies because direct intracerebroventricular injections of 3-hydroxybutyrate induce hypophagia (32). However, our observations suggest that acetoacetate rather than 3-hydroxybutyrate serves an anorectic role, as this ketone body was selectively elevated in fenofibrate-treated rats. The underlying mechanism mediating information about hepatocyte fatty acid oxidation to afferent terminals of hepatic vagus nerve is unknown. Lipoprivic feeding induced by intraportal infusion of mercaptoacetate depolarizes hepatocytes and enhances the discharge rates of the hepatic vagus branch (33,34). Thus, it is possible that the enhanced lipid oxidation induced by fibrates and other activators of PPARα mediate anorexia via their stimulatory action of the hepatic vagus branch.

The apparent lack of pharmacological effects on feeding seen in both diet groups treated with the dual PPARα/γ activator ragaglitazar may be explained in several ways. Based on the results of the OGTT and measures of plasma insulin concentrations, it was evident that equipotent

TABLE 8
Hepatic and inguinal adipose tissue expression of selected genes

	Low-fat diet				High-fat diet			
	Vehicle	Pioglitazone	Fenofibrate	Ragaglitazar	Vehicle	Pioglitazone	Fenofibrate	Ragaglitazar
Hepatic acyl-CoA oxidose	0.11 ± 0.0	0.09 ± 0.01	1.0 ± 0.06*†‡	0.36 ± 0.03*†	0.09 ± 0.0	0.10 ± 0.01	1.1 ± 0.05*†‡	0.33 ± 0.03*†
Hepatic PEPCK	3.5 ± 0.5	2.2 ± 0.3*	1.2 ± 0.2*‡	1.8 ± 0.3*	5.3 ± 0.3§	2.7 ± 0.2*	1.0 ± 0.1*†‡	3.0 ± 0.4*§
Brown adipose tissue UCP-1	1.5 ± 0.2	4.0 ± 0.4*	0.8 ± 0.1*†‡	3.4 ± 0.2*	2.7 ± 0.2§	2.9 ± 0.2§	0.7 ± 0.1*†‡	3.8 ± 0.4*†
White adipose tissue aP2	1.4 ± 0.1	2.1 ± 0.1*	1.5 ± 0.2†‡	1.8 ± 0.1‖	1.4 ± 0.2	1.8 ± 0.0¶	1.2 ± 0.1†‡	2.0 ± 0.2*
White adipose tissue leptin	2.1 ± 0.2	1.3 ± 0.1*	1.6 ± 0.3‡	1.0 ± 0.1*	2.7 ± 0.2§	1.5 ± 0.1*	2.2 ± 0.2†	2.0 ± 0.3*
White adipose tissue adiponectin	3.0 ± 0.5	2.1 ± 0.4	1.9 ± 0.3	2.5 ± 0.4	3.2 ± 0.5	5.3 ± 0.5*	2.3 ± 0.3†	2.1 ± 0.2†
White adipose tissue LPL	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.1‡	1.1 ± 0.1*	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.0 ± 0.2*

Data are means ± SE. Levels of mRNA are expressed in arbitrary units based on calculation expression level relative to an internal standard. See RESEARCH DESIGN AND METHODS for details. *$P < 0.05$ vs. vehicle on same diet; †$P < 0.05$ vs. pioglitazone, same diet; ‡$P < 0.05$ vs. ragaglitazar, same diet; §$P < 0.05$ vs. low fat, same treatment; ‖$P = 0.09$ vs. low-fat–fed vehicle; ¶$P = 0.07$ vs. high-fat–fed vehicle (ANOVA followed by Fisher protected least significant difference post hoc analysis).

insulin-sensitizing doses of pioglitazone and ragaglitazar were used. Also, the comparable induction of brown adipose tissue UCP-1 and white adipose tissue aP2 provides evidence that equiefficacious PPARγ-activating doses were used. It is therefore tempting to speculate that the PPARα component of ragaglitazar counterbalances PPARγ-induced feeding. However, the absence of marked activation of hepatic peroxisomes and lipid oxidation suggests that lack of orexigenic actions may not simply be due to PPARα activation. Interestingly, plasma concentrations of adiponectin were significantly higher in ragaglitazar-treated rats than in pioglitazone-treated rats (Table 8). Adiponectin is an anti-inflammatory protein, synthesized and released from adipocytes upon PPARγ stimulation and in response to weight reduction (35–37). In mice, the globular head domain of adiponectin decreases weight gain via enhanced muscular lipid oxidation (38).

In conclusion, we have shown that selective PPARγ activation is associated with weight gain due to both increased food intake and improved feed efficiency, whereas selective PPARα activation leads to opposite effects. This observation calls for increased awareness of possible disadvantageous metabolic consequences for type 2 diabetic patients subject to long-term clinical use of selective PPARγ agonists. The actions were independent of dietary fat content; therefore, caloric restriction of type 2 diabetic patients treated with selective PPARγ agonists is of utmost importance. Dual activators of both PPARα and -γ appear less devastating for metabolic control, as they are devoid of orexigenic actions. Thus, it seems likely that this class of compounds may constitute therapeutically more interesting drugs, as they induce favorable metabolic control of glucose homeostasis accompanied by a lower degree of body fat accumulation.

ACKNOWLEDGMENTS

Financial support for this study was obtained from the Danish Medical Research Council, Danish Diabetes Association, the Novo Nordisk Foundation, and Fonden til Lægevidenskabens Fremme (to P.J.L. and N.V.).

Part of this study has appeared in abstract form [Larsen PJ, Jensen PB, Larsen LK, Vrang N, Sørensen RV, Wassermann K: Ragaglitazar prevents intra-abdominal fat accumulation and is devoid of orexigenic and adipogenic effects in diet-induced obese rats (Abstract). *Diabetes* 51 (Suppl. 2):A140, 2002].

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