Tetradecylthioacetic acid prevents high fat diet induced adiposity and insulin resistance

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Abstract Tetradecylthioacetic acid (TTA) is a non-β-oxidizable fatty acid analog, which potently regulates lipid homeostasis. Here we evaluate the ability of TTA to prevent diet-induced and genetically determined adiposity and insulin resistance. In Wistar rats fed a high fat diet, TTA administration completely prevented diet-induced insulin resistance and adiposity. In genetically obese Zucker (fa/fa) rats TTA treatment reduced the epididymal adipose tissue mass and improved insulin sensitivity. All three rodent peroxisome proliferator-activated receptor (PPAR) subtypes were activated by TTA in the ranking order PPARα > PPARδ > PPARγ. Expression of PPARγ target genes in adipose tissue was unaffected by TTA treatment, whereas the hepatic expression of PPARα-responsive genes encoding enzymes involved in fatty acid uptake, transport, and oxidation was induced. This was accompanied by increased hepatic mitochondrial β-oxidation and a decreased fatty acid/ketone body ratio in plasma. These findings indicate that PPARα-dependent mechanisms play a pivotal role, but additionally, the involvement of PPARα-independent pathways is conceivable. Taken together, our results suggest that a TTA-induced increase in hepatic fatty acid oxidation and ketogenesis drains fatty acids from blood and extrahepatic tissues and that this contributes significantly to the beneficial effects of TTA on fat mass accumulation and peripheral insulin sensitivity.—Madsen, L., M. Guerre-Millo, E. N. Flindt, K. Berge, K. J. Tronstad, E. Bergene, E. Sebokova, A. C. Rustan, J. Jensen, S. Mandrup, K. Kristiansen, I. Klimes, B. Staels, and R. K. Berge. **Tetradecylthioacetic acid prevents high fat diet induced adiposity and insulin resistance.** ***J. Lipid Res.*** **2002.** 43: **742–750.**

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Insulin resistance with ensuing hyperinsulinemia and dyslipidemia characterizes the metabolic syndrome, which eventually may develop into type II diabetes. Since most type II diabetic patients are obese, and obesity is virtually always associated with insulin resistance, a causal relationship has been suggested. Although it is generally assumed that high fat diets promote obesity, (1–3), it still remains uncertain how obesity may induce insulin resistance. In this respect, it is of interest that saturated and ω-6 unsaturated fatty acids in the diet may lead to insulin resistance in experimental animals, whereas ω-3 fatty acids prevents the development of insulin resistance (4–6). Particularly, polyunsaturated fatty n-3 acids have been shown to ameliorate metabolic dysfunctions, including improvement of insulin sensitivity (4, 7) and lowering of plasma triacylglycerol levels (8).

Results from both in vivo and in vitro experiments indicate that reduced triacylglycerol synthesis in and secretion from the liver due to increased fatty acid oxidation contribute to the hypolipidemic effect of n-3 fatty acids (9–12). Similarly, feeding the 3-thia substituted fatty acid {tetradecylthioacetic acid (TTA) [CH_3-$(CH_2)_{13}$-S-CH_2-COOH]} to rats causes a significant reduction of plasma triacylglycerol accompanied by increased mitochondrial and peroxisomal β-oxidation in the liver (13, 14). TTA is unable to undergo β-oxidation due to the sulfur substitution, but TTA is otherwise handled as a normal fatty acid and incorporated into triacylglycerols and phospholipids. Prolonged feeding of TTA to rats also changes the fatty acid composition in liver, heart, kidney, and adipose tissue (15–17).

These observations prompted us to investigate whether TTA could prevent high fat diet induced insulin resis-

Abbreviations: CMC, carboxymethyl cellulose; CPT, carnitine palmitoyltransferase; FATP, fatty acid transport protein; L-FABP, liver fatty acid binding protein; PPAR, peroxisome proliferator-activated receptor; TTA, tetradecylthioacetic acid.

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tance in Wistar rats and reduce insulin resistance in obese Zucker (fa/fa) rats. In this report we demonstrate that TTA completely prevented high fat diet induced insulin resistance and adiposity. In obese Zucker (fa/fa) rats TTA also reduced adiposity and hyperglycemia, and markedly improved insulin sensitivity as determined by the intravenous glucose tolerance test. The exact mechanisms mediating these effects still remain to be deciphered. We show that TTA efficiently activates peroxisome proliferator-activated receptor (PPAR)α and PPARδ, whereas activation of PPARγ requires relatively high concentrations of TTA. In keeping with this we show that PPARα responsive genes, but not PPARγ responsive genes, are upregulated in TTA-treated rats, suggesting that TTA-dependent activation of PPARα is of importance.

MATERIALS AND METHODS

Animals

All animal studies were conducted according to the Guidelines for the Care and Use of Experimental Animals, and the Local Animal Care Committees approved the protocols in the individual research centers.

Obese Zucker (fa/fa) rats. The young obese Zucker (fa/fa) rats (5 weeks old) used in this study were bred at the U465 INSERM animal facility from pairs originally provided by the Harriet G. Bird Laboratory (Stow, MA) and the old obese Zucker (fa/fa) rats (4 month old) were from IFFA-CREDO (France). The animals were maintained under a constant light-dark cycle (light from 7 AM to 7 PM) at 21 ± 1C° and were given free access to food and water. Three rats were housed per cage. Weight gain was recorded daily. In a first experiment, young male rats (5 weeks old) receiving a standard diet (UAR, Epinay/Orge, France) were given either 300 mg/kg/day TTA in 0.5% carboxymethyl cellulose (CMC) (n = 6) by oral gavage in the morning or an equal amount of CMC only (n = 6). After 11 days of treatment, rats were killed by cervical dislocation between 9 AM and 10 AM. Blood was collected. Liver and epididymal adipose tissue were dissected out and weighed. In a second series of experiments, the rats were given either a standard diet (n = 6) or a standard diet enriched with 0.15% TTA (n = 6) for 15 days (corresponding to approximately 150 mg/kg/day). Intravenous glucose tolerance tests were performed on these rats. In a third experiment, 4 months old obese Zucker rats were given a standard chow either with (n = 5) or without 0.15% wt/wt TTA in chow (n = 6) for 21 days.

Wistar rats. Male Wistar rats weighing 280–358 g were purchased from AnLab Ltd. (Prague, Czech Republic) and housed in wire-mesh cages at 22 ± 1°C with light from 7 AM to 7 PM. They were given free access to chow and water. Three rats were housed per cage. Weight gain and food intake were recorded daily. One group of animals was fed a standard pellet diet (ST1, Velaz Prague, Czech Republic) containing 10 cal% of fat, and is referred to as the control group. The second group received a high fat (HF) diet, containing 70 cal% fat prepared according to Storlien et al. (18). Fatty acid composition of the standard chow and the HF diets were as published earlier (19). The third group received the HF diet with 0.4% wt/wt TTA in chow (corresponding to 400 mg/kg/day). Following 3 weeks of ad libitum feeding, rats were subjected to vascular surgery in preparation for in vivo measurements of insulin sensitivity (see below).

In a second series of experiment, rats fed the same experimental diets for 3 weeks were used for the collection of blood and tissues.

Physiological techniques

Intravenous glucose tolerance tests. Male Zucker (fa/fa) rats (5 weeks old) were fasted for 5 h and subsequently anesthetized at 2 PM by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The rats were injected with glucose (0.55 g/kg) in the saphenous vein and blood samples were collected from the tail vein in heparinized tubes at time 0, 5, 10, 15, 20, and 30 min after the glucose injection. Samples were kept on ice, centrifuged, and plasma was stored at −20 C° until analysis was performed.

Hyperinsulinemic euglycemic clamp. After 21 days on their respective diets (see above), the rats were anesthetized by injection of xylazine hydrochloride (Rometar SPOFA, Prague, Czech Republic; 10 mg/ml) and ketamine hydrochloride (Narkamon SPOFA, Prague, Czech republic; 75 mg/ml), and fitted with chronic carotid artery and jugular vein cannulas as described by Koopmans et al. (20). The cannulated rats were allowed to recover for 2 days after surgery before the clamping studies, which were carried out according to Kraegen et al. (21). On the third day after surgery, unrestrained conscious rats were given a continuous infusion of porcine insulin (Actrapid, Novo Nordisk, Denmark) at a dose of 6.4 mU/kg/min to achieve plasma insulin levels in the upper physiological range. The arterial blood glucose concentration was clamped at the basal fasting level, by variable infusion of a 30% w/v glucose solution (Leciva, Prague, Czech Republic). Blood samples for determination of plasma glucose and insulin concentrations were collected every 15 min from the start of the glucose infusion. After 90 min, the rats were disconnected from the infusions and immediately decapitated. Blood was collected for plasma separation, and liver and epididymal adipose tissues were dissected out and weighed.

Measurement of plasma parameters. In Zucker (fa/fa) rats glucose (GLU, Boehringer Mannheim, Germany), free fatty acids (NEFA C ACS-ACOD kit; Wako Chemicals, Dalton, USA) and β-hydroxybutyrate (310-A kit; Sigma Diagnostics Inc., St. Louis) concentrations were measured using enzymatic methods, and insulin concentrations were determined with radioimmunoassay (CIS bio International, Gif sur Yvette, France) using rat insulin as standard. In the Wistar rats, plasma glucose concentrations were measured using a Beckman Glucose Analyzer (Fullerton, CA, USA). Plasma insulin levels were measured using a radioimmunoassay kit from Linco Research Inc. (St. Charles, MO, USA). Plasma triacylglycerol levels were measured using the Monotest triacylglycerol kit (Boehringer Mannheim, Germany)

Preparation of post-nuclear and mitochondrial fractions and measurement of enzyme activities. Freshly isolated livers from individual old Zucker rats were homogenized in ice-cold sucrose buffer [0.25 M sucrose, 10 mM HEPES (pH 7.4) and 2 mM EDTA]. Post-nuclear and mitochondrial fractions were prepared using preparative differential centrifugation according to DeDuve et al. (22). Acid soluble products were measured in post-nuclear and mitochondrial enriched fractions, using [1−^{14}C]palmitoyl-CoA (Radiochemical Centre, Amersham, England) as substrates as described earlier (23). Carnitine palmitoyltransferase-I and -II activities were measured in the post-nuclear fractions essentially as described by Bremer (24) and 3-hydroxy-3-methylglutharyl-CoA synthase activity in the mitochondrial fractions was measured according to Clinkenbeard et al. (25).

RNA analysis. RNA extraction (26), and Northern blot and slot blot analysis were performed as earlier described (14). The following cDNA fragments were used as probes: carnitine palmitoyltransferase (CPT)-I (27) and CPT-II (28). The relative levels of CPT-I and CPT-II RNA were determined by normalizing to the level of hybridization to 28S rRNA. The expression of liver fatty acid binding protein (L-FABP) and fatty acid transport protein (FATP) was analyzed by multiplex RT-PCR essentially as described (29). Total RNA was reverse transcribed (M-MLV Reverse

Transcriptase kit, Life Technologies) and selected mRNAs were amplified by 25 cycles of hot PCR with Tata binding protein serving as internal standard. The following primers were used. TBP: 5′-ACCCTTCACCAATGACTCCTATG-3′ and 5′-ATGATGACTGCAGCAAATCGC-3′; L-FABP: 5′-GCAAGTACCAACTGCAGAGC-3′ and 5′-CCAATGTCATGGTATTGGTGAT-3′; FATP: 5′-CATTGTGGTGCACAGCAGG-3′ and 5′-CATATTTCACCGATGTAGTGCAC-3′. Quantification was performed by phosphorimaging (Molecular Dynamics).

Transfection analysis. NIH-3T3 cells passaged in DMEM supplemented with 8% calf serum were transiently transfected at 50–60% confluence by the DC-Chol method (30) with a total of 2.5 μg DNA per 9.6 cm^2 well. The Gal4-mPPAR hinge region/ligand binding domain fusions used are described earlier (31). One hundred twenty five nanograms of pcDNA1-Gal4-mPPAR, 500 ng 5xUAS-TK-luciferase reporter, and 50 ng pCMVβ (Clontech) were used per well. For pcDNA1-Gal4-mPPARδ, an equimolar amount of pCMX-mRXRα (kindly provided by R. M. Evans) (32) was cotransfected. Empty expression vector was used to equalize plasmid load. Following exposure to liposomes for 6 h, cells were incubated for 20 h in DMEM supplemented with 10% resin-charcoal stripped CS and ligand/vehicle as indicated. β-galactosidase and luciferase activities from cell lysates were determined by use of the MicroLumat LB 96 P luminometer (EG&G Berthold). Luciferase values were normalized to the β-galactosidase values.

RESULTS

TTA prevents high fat diet induced increase in adipose tissue mass

It is well established that high fat feeding induces obesity and may lead to the development of insulin resistance. Accordingly, the relative weight of both epididymal and retroperitoneal fat pads increased in Wistar rats fed a high fat diet (**Table 1**). Inclusion of TTA in the high fat diet prevented the relative increase in adipose tissue mass (Table 1) without a concomitant decrease in food consumption (high fat: 15.1 ± 1.1 vs. high fat + TTA: 14.8 ± 1.3 g/rat/day, n = 6). To further investigate the effect of TTA on fat accumulation, we treated obese Zucker (fa/fa) rats, a well-established genetic model for obesity and insulin resistance, with TTA. The body weight, as well as weight gain per day, was similar in control and TTA-treated rats during the 11 days of treatment (Table 1). However, the relative adipose tissue weight was lower in TTA-treated than in control rats (**Table 2**). The liver weight was, however, increased by TTA treatment in both animal models (Table 1 and 2), as observed in earlier studies (13).

TABLE 1. Influence of high fat diets with and without TTA supplement for 3 weeks on liver and adipose tissue weights in Wistar rats

Parameters	Standard Chow Diet	High Fat Diet −TTA	High Fat Diet +TTA
Epididymal Adipose Tissue/ Body Weight (%)	0.81 ± 0.03[a]	1.29 ± 0.06[b]	0.96 ± 0.07[a]
Retroperitoneal Adipose Tissue/Body Weight (%)	0.62 ± 0.08[a]	1.36 ± 0.08[b]	0.82 ± 0.05[a]
Liver Weight (g)	8.9 ± 0.6[a]	9.8 ± 1.0[a]	12.2 ± 1.4[b]
Hepatic CPT-I Activity (nmol/min/mg protein)	2.5 ± 0.2[a]	3.1 ± 0.2[a,b]	3.7 ± 0.3[b]
Hepatic CPT-II Activity (nmol/min/mg protein)	5.7 ± 0.6[a]	7.0 ± 0.9[a]	25.7 ± 2.5[b]
Plasma Triacylglycerol (mM)	0.66 ± 0.16[a]	0.70 ± 0.16[a]	0.40 ± 0.16[b]

Data are means ± SEM of six animals in each group. Wistar rats (280–360 g) were fed three different diets (see Materials and Methods) for 3 weeks ad libitum. Afterwards, they were killed by decapitation; liver, retroperitoneal, and epididymal adipose tissue pads were dissected out and weighed.

[a,b] Different letters within a row indicates statistical significance ($P < 0.05$).

TTA prevents high fat diet induced hyperinsulinemia

Development of insulin resistance is known to be associated with hyperinsulinemia. Wistar rats fed a high fat diet to induce insulin resistance exhibited increased plasma insulin levels, compared with controls fed the standard chow diet (**Fig. 1**), yet the levels of plasma glucose were unchanged (not shown). The development of diet-induced hyperinsulinemia was completely prevented by inclusion of TTA in the diet (Fig. 1).

The obese Zucker (fa/fa) rats develop hyperinsulinemia spontaneously early in life and this defect worsens with age. TTA treatment reduced blood insulin concentrations in both 5 weeks and 4 months old obese Zucker (fa/fa) rats (**Fig. 2**). As expected, TTA had a marginal effect on plasma glucose levels in young normoglycemic animals (Fig. 2). The plasma glucose levels were, however, significantly reduced in 4 months old hyperglycemic obese rats treated with TTA (Fig. 2). Thus, the reduction in the insulin concentration observed after TTA treatment is not solely the result of decreased plasma glucose levels.

An intravenous glucose tolerance test performed in young normoglycemic obese Zucker rats demonstrated that TTA treatment resulted in a significantly lower plasma insulin response to glucose (**Fig. 3A**), whereas the

TABLE 2. Influence of TTA on liver and adipose tissue weights, hepatic enzyme activities, and plasma parameters in young (A) and old (B) obese Zucker (fa/fa) rats

Parameters	Control	Treated
A.		
Epididymal Adipose Tissue/ Body Weight (%)	0.88 ± 0.02	0.78 ± 0.02[a]
Final Body Weight (g)	186 ± 7	177 ± 7
Weight Gain (g/day)	5.9 ± 0.4	6.2 ± 0.3
Liver Weight (g)	7.8 ± 0.3	10.6 ± 0.7[a]
B.		
FFA/Ketone Ratio	0.40 ± 0.10	0.17 ± 0.09[a]
HMG-CoA Synthase Activity (nmol/min/mg protein)	13 ± 4	27 ± 6[a]
Mitochondrial β-Oxidation (nmol/min/mg protein)	1.3 ± 0.7	4.6 ± 1.2[a]
CPT-I		
Activity (nmol/min/mg protein)	2.3 ± 0.5	4.5 ± 0.7[a]
mRNA (relative values)	1.0 ± 0.2	2.4 ± 0.8[a]
CPT-II		
Activity (nmol/min/mg protein)	14 ± 1	42 ± 13[a]
mRNA (relative values)	1.0 ± 0.1	4.6 ± 0.9[a]

A: Five-week-old male obese Zucker (fa/fa) rats were fed 300 mg TTA/kg/day suspended in 0.5% CMC for 11 days. B: Four-month-old Zucker (fa/fa) rats were given a standard chow diet either with or without 0.15% TTA for 21 days. At the end of the experiments rats were killed by cervical dislocation; liver and epididymal adipose tissue pads were dissected out and weighed. Data are means ± SEM of six animals in each group.

[a] $P < 0.05$ when compared to nontreated obese rats.

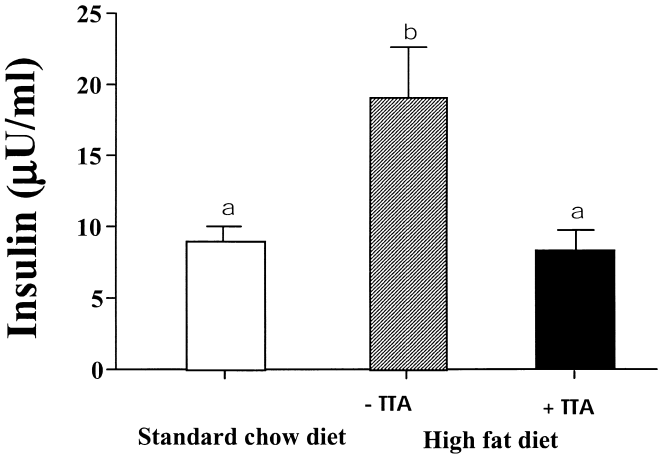

Fig. 1. Tetradecylthioacetic acid (TTA) treatment prevents high fat diet-induced hyperinsulinemia in Wistar rats. Wistar rats weighing 280–360 g were divided into three groups (n = 6) and fed with three different diets: standard chow, high fat diet (HF) and HF supplemented with TTA. After 21 days on the respective diets, blood was collected after an overnight fast from the tail vein. The data are presented as mean ± SEM. Results were analyzed by ANOVA and different letters denote statistical difference ($P < 0.05$).

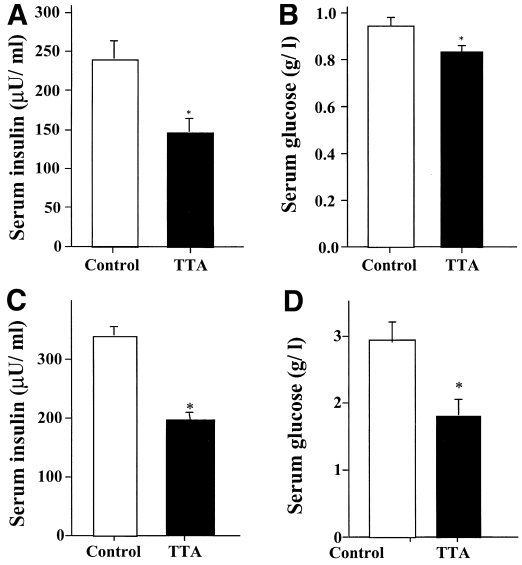

Fig. 2. TTA treatment reduces blood insulin and glucose concentrations in 5 weeks (A and B) and 4 months old (C and D) Zucker (fa/fa) rats. Five weeks old male obese Zucker (fa/fa) rats were fed 300 mg TTA kg/day suspended in 0.5% carboxymethyl cellulose (CMC) for 11 days. Control animals received CMC only. The 4 months old rats were given a standard chow either with (n = 5) or without (n = 6) 0.15% TTA for 21 days. At the end of both experiments blood was collected and the levels of insulin and glucose were measured. Data are means ± S.D. *Denotes statistical significant differences (Student's *t*-test) between control and TTA treated rats ($P < 0.05$).

kinetics of glucose clearance was similar in treated and untreated obese rats (Fig. 3B). This indicates that TTA improved insulin sensitivity.

TTA prevents high fat diet induced insulin resistance in vivo

To substantiate the insulin sensitizing effect of TTA, a 90 min euglycemic hyperinsulinemic clamp experiment was performed in Wistar rats fed a chow diet, a high fat diet, or a high fat diet supplemented with TTA. In keeping with the notion that high fat feeding leads to insulin resistance, the exogenous glucose infusion rate (GIR) required to maintain euglycemia in the high fat fed group was significantly reduced compared with that of the chow fed Wistar rats (**Fig. 4**). TTA completely prevented development of insulin resistance in these rats, as evidenced by a fully normal GIR in rats fed the high fat diet supplemented with TTA.

Elevated levels of plasma free fatty acids (FFA) characterize the fasted state of most obese subjects (33, 34) and have been associated with the development of insulin resistance (35–37). Interestingly, TTA prevented the increase in plasma FFA levels in fasted rats kept on a high fat diet (**Fig. 5**).

TTA decreases plasma triacylglycerol levels and activates PPARα-dependent pathways in vivo

It is suggested that elevated plasma triacylglycerol levels may inhibit peripheral glucose metabolism in humans (38). TTA reduced the plasma triacylglycerol levels by 40% in high fat fed Wistar rats (Table 1) and by 35% in old genetically hypertriacylglycerolemic obese Zucker (fa/fa) rats (not shown). As expected, TTA-treatment led to increased mitochondrial β-oxidation as well as increased activities of mitochondrial CPT-I and II in both Wistar rats fed a high fat diet (Table 1) and Zucker (fa/fa) rats (Table 2). In Zucker (fa/fa) rats CPT-I and -II mRNA levels were concomitantly increased by TTA (Table 2). Similarly, TTA treatment increased the mRNA levels of PPARα target genes, such as L-FABP and FATP above those observed in the high fat fed rats (**Fig. 6**), suggesting in vivo activation of PPARα by TTA.

Activation of PPAR subtypes by TTA

Simultaneous activation of PPARα and PPARγ has been shown to exert beneficial hypolipidemic and insulin sensitizing effects exceeding those observed with subtype-selective agonists (39–41). We have previously demonstrated that TTA is able to activate both human PPARα- and human PPARγ-mediated transactivation (42), and hence, we evaluated the transactivation profile of TTA on rodent PPARs using Gal4-mouse PPAR hinge region/ligand binding domain chimeras for transient transfection analyses. **Figure 7** shows that TTA in a dose-dependent manner activated all three subtypes. However, the TTA concentrations needed to activate the PPARs were clearly subtype-dependent. PPARα-dependent transactivation was significantly enhanced by 10 μM TTA to a degree similar to or exceeding that observed by 100 μM WY14643. PPARδ required 100 μM TTA to achieve a 6–7-fold induction comparable to that obtained with 1 μM of the PPARδ-selective ligand L165041. Finally, activation of PPARγ required 100 μM TTA, and the level of transactivation was only approximately 20% of that observed with 1 μM BRL 49653. Thus, these results indicate that PPARα is a main target of TTA in the treated rats. It is possible that also PPARδ is activated, whereas activation of PPARγ at most is very modest. Thus, the potency and efficacy of TTA are reminiscent of

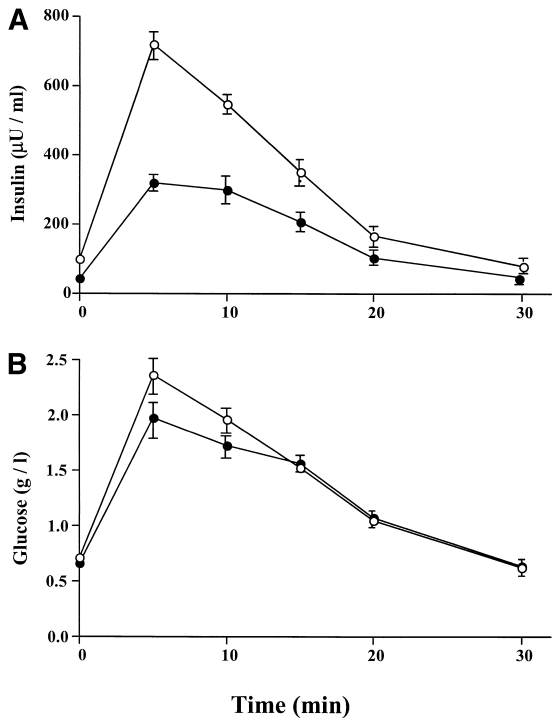

Fig. 3. TTA treatment decreases the plasma insulin response to glucose in 5 weeks old Zucker (fa/fa) rats. The rats were given either a standard diet (open circle) (n = 6) or a standard diet enriched with 0.15% TTA (closed circle) (n = 6) for 15 days and subsequently submitted to an intravenous glucose tolerance test as described in Materials and Methods. Results are presented as the mean ± SEM. Area under the curve (AUC) for insulin: $P < 0.01$, AUC for glucose, not statistically significant.

those of naturally occurring polyunsaturated fatty acids, which have been shown to activate PPARα and PPARδ in the 10–100 μM range, whereas activation of PPARγ is either not detected or low (43, 44).

In keeping with the results from the transfection experiments showing that a very high concentration of TTA was required to activate PPARγ, TTA treatment did not alter the expression of PPARγ target genes in adipose tissue. Northern blot analysis demonstrated that TTA did not affect the levels PEPCK mRNA (control; 100 ± 8 vs. TTA; treated 106 ± 12), leptin mRNA (control; 100 ± 9 vs. TTA treated; 97 ± 5) and lipoprotein lipase mRNA (control; 100 ± 9 vs. TTA treated; 108 ± 7) in epididymal adipose tissue of old obese Zucker rats.

DISCUSSION

Increased fat consumption is associated with a wide range of metabolic abnormalities, including hyperglycemia, dyslipidemia, and insulin resistance. In the present report, we demonstrate that inclusion of TTA in a high fat diet completely prevented the development of insulin resistance and adiposity. Moreover, we show that TTA markedly improved insulin sensitivity, as determined by intravenous glucose tolerance tests, and reduced adipose tissue mass in animal models of genetically determined and diet induced insulin resistance and obesity.

While a clear link between adiposity and development of insulin resistance has been established, details of the underlying molecular mechanisms remain elusive. High levels of plasma FFA, a characteristic of most obese subjects, are suggested to act directly or indirectly as messengers (33, 34). In obese subjects, an increased rate of lipolysis from the expanded fat cell mass (45, 46) would increase the plasma levels of FFA leading to inhibition of insulin action (35–37). Here we demonstrate that a high fat diet increased the plasma levels of FFA in the fasted state more than two fold, and notably, this increase was totally prevented by TTA. In a separate study, basal and β-adrenergic stimulated lipolysis in epididymal adipocytes from TTA-treated and untreated rats did not differ, and accordingly, no differences in the activity of hormone sensitive lipase were detected (A. Rustan and R. K. Berge, unpublished observations). Thus, we consider it unlikely that TTA reduced plasma FFA levels by decreasing lipolysis in fat. Increased fatty acid oxidation and ketone body formation accompanied by a decreased plasma FFA-ketone ratio rather suggest an increased flux of fatty acids to the liver. Increased fatty acid oxidation accompanied by reduced expression of apolipiprotein CIII (42) as well as increased FABP and FATP mRNA levels will diminish the availability of substrates for triacylglycerol synthesis (13), thereby reducing the rate of fat accumulation.

Increased triacylglycerol content in skeletal muscle is also related to insulin resistance and obesity (47). By lowering plasma triacylglycerols, TTA may diminish the delivery of triacylglycerol to skeletal muscle. We have recently observed that TTA treatment reduced the volume fraction of fat droplets concomitantly with an induction of mitochondrial proliferation in skeletal muscle (48). In agreement with this hypothesis, insulin stimulated glucose uptake was increased by 50% after TTA treatment in the epitrochlearis muscle in Zucker (fa/fa) rats (data not shown). Thus, the improved glucose homeostasis observed by TTA treatment might at least partly be explained by improved insulin action in skeletal muscle.

How TTA exerts the observed effects on adiposity and insulin resistance is not yet known in detail. However, several of our findings clearly indicate that molecular mechanisms governing the action of TTA differ from those of thiazolidinediones, which are high affinity ligands of PPARγ and known to exert their effect as insulin sensitizers by virtue of their ability to activate this PPAR subtype. Thus, treatment of rats with thiazolidinediones markedly influences gene expression in adipose tissue (49, 50), whereas treatment with TTA, which we show is a poor PPARγ ligand, did not alter the expression of PPARγ target genes in adipose tissue in the obese Zucker rats. Moreover, thiazolidinedione treatment increases food intake and adipose tissue mass (50–52), whereas TTA did not

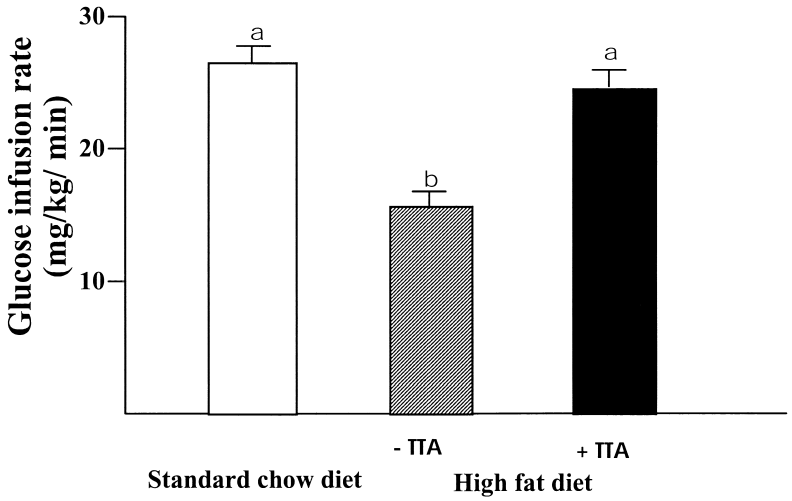

Fig. 4. TTA treatment prevents high fat diet-induced insulin resistance in Wistar rats. Rats weighing 330 ± 20 g were divided into three groups (n = 9) and fed with three different diets: standard rat chow, high fat diet (HF), and HF supplemented with TTA. After 21 days on the respective diets, a 90 min euglycemic hyperinsulinemic clamp was performed in unrestrained conscious animals as described in Materials and Methods. The glucose infusion rate (GIR) was determined after glucose levels had stabilized, i.e., between 45–90 min after initiation of the clamp. The data are presented as mean ± SEM. Results were analyzed by ANOVA and different letters denote statistical difference ($P < 0.05$).

change food intake and decreased adipose mass in obese Zucker rats.

TTA treatment resulted in an increased expression of PPARα target genes. We show that TTA is a potent activator of rodent PPARα, and hence, PPARα-dependent processes most probably contribute significantly to the lowering of plasma FFA and plasma triacylglycerol, and the improved insulin sensitivity. Although it was recently reported that PPARα null mice were protected from insulin resistance (53), our findings are in accordance with the reported beneficial effects on insulin sensitivity by administration of the PPARα activators of the fibrate class (54–57).

Although PPARα appears to play an important role in the effects of TTA on lipid and glucose metabolism, it cannot be excluded that activation of other transcription factors, such as PPARδ, are involved in the regulation of glucose metabolism and improvement of insulin sensitivity. However, available evidence obtained in feeding experiments using PPARδ-selective ligands would argue that this is unlikely (41). It is likely, however, that TTA may exert PPAR independent effects. PPAR-independent effects of TTA on the growth of human keratinocytes (58) and rat glioma cells (59) have been recently documented. The effects of TTA on lipid homeostasis are in some aspects comparable to those of ω-3 polyunsaturated fatty acids (13). TTA is known to affect the lipid profile of cells and TTA in itself is a substrate for the Δ^9 desaturase (17). Both TTA and its Δ^9 desaturated product are incorporated into hepatic triacylglycerols and phospholipids (17). Therefore it is likely that other transcription factors and cellular signaling pathways that are affected by fatty acids are also influenced by TTA. Potential targets include the sterol regulatory element binding protein 1c (SREBP-1c) the activity of which is regulated by polyunsaturated fatty acids at the level of transcription, mRNA stability, and protein processing. Furthermore, it was recently demonstrated that mono and poly-unsaturated fatty acids could antagonize ligand-dependent liver X-activated receptors-mediated transactivation (60). Finally, as increased oxidative stress contributes to poor insulin action (61–63), the antioxidant capacity of the sulfur atom (64) may contribute to the insulin sensitizing effect of TTA.

Taken together, our findings indicate that PPARα-dependent mechanisms play a pivotal role, but additionally, the involvement of PPARα-independent pathways is conceivable. Our results suggest that TTA-induced increase in hepatic fatty acid oxidation and ketogenesis will drain fatty acids from blood and extrahepatic tissues and that this

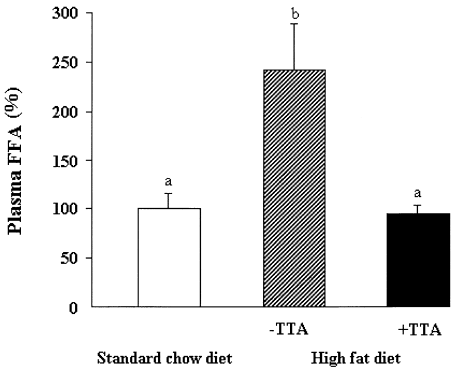

Fig. 5. TTA treatment reduces plasma levels of free fatty acids in Wistar rats fed a high fat diet. Rats weighing 330 ± 20 g were divided into three groups (n = 9) and fed with three different diets: standard rat chow, HF, and HF supplemented with TTA. The values are from decapitation after 16 h of fasting. The data are presented as % of control. Results were analyzed by ANOVA and different letters denote statistical difference ($P < 0.05$).

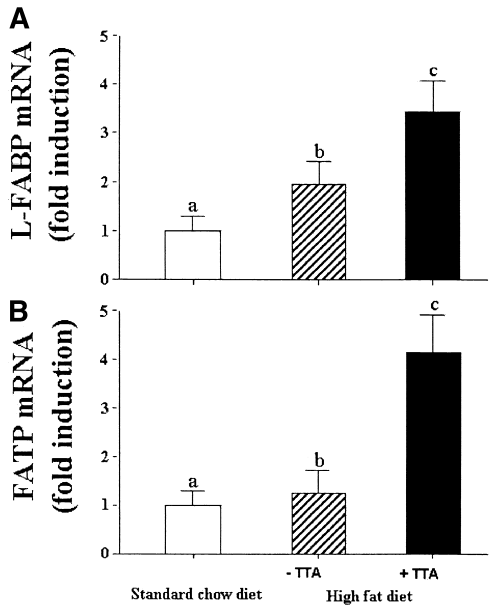

Fig. 6. TTA modulates gene expression of peroxisome proliferator-activated receptor (PPAR)α target genes in the liver of Wistar rats. Rats weighing 330 ± 20 g were divided into three groups (n = 9) and fed with three different diets: standard rat chow, high fat diet (HF) and HF supplemented with TTA. After 21 days on the respective diets, the livers were removed and RNA extracted. RNA-profiles for liver fatty acid binding protein (L-FABP) and fatty acid transport protein (FATP) were determined by phosphoimager analysis of multiple RT-PCR bands and normalized to TBP values. Determinations were repeated twice. Columns represent the average normalized values in the respective groups. Standard deviations are indicated. Results were analyzed by ANOVA and different letters denote statistical difference ($P < 0.05$).

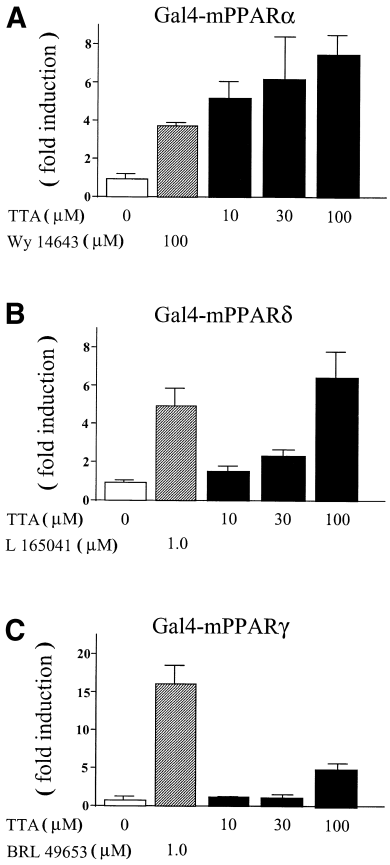

Fig. 7. Mouse PPARs are activated by TTA NIH-3T3 cells were transiently transfected with a 5xUAS-TK-luciferase reporter, β-galactosidase-control, and either Gal4-mPPARα, Gal4-mPPARδ, or Gal4-mPPARγ expressing the Gal4 DNA binding domain fused to the hinge region/ligand binding domains of PPARα, PPARδ, or PPARγ, respectively. The retinoid X receptor (RXR) expression vector pCMX-mRXRα was included in transfection with Gal4-mPPARδ. Empty expression vector was included to achieve for equal plasmid load. Cells were treated with the vehicle alone (DMSO), 10, 30, or 100 μM TTA, or 100 μM Wy14643 (A), 1 μM L165041 (B), or 1.0 μM BRL49653 (C) as indicated. All transfections were performed a minimum of two times in triplicate and normalized to the β-galactosidase values. Results from one representative experiment are shown. The DMSO control values for each subtype were set equal to 1.

contribute significantly to the beneficial effects of TTA on fat mass accumulation and peripheral insulin sensitivity. The draining of fatty acids by the liver may relieve the fatty acid pressure on adipose tissue and muscle, where according to Randle (65) glucose uptake and oxidation may be improved.

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