PPAR-α–Null Mice Are Protected From High-Fat Diet–Induced Insulin Resistance

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Peroxisome proliferator–activated receptor (PPAR)-α controls the expression of genes involved in lipid metabolism. PPAR-α furthermore participates to maintain blood glucose during acute metabolic stress, as shown in PPAR-α–null mice, which develop severe hypoglycemia when fasted. Here, we assessed a potential role for PPAR-α in glucose homeostasis in response to long-term high-fat feeding. When subjected to this nutritional challenge, PPAR-α–null mice remained normoglycemic and normoinsulinemic, whereas wild-type mice became hyperinsulinemic (190%; $P < 0.05$) and slightly hyperglycemic (120%; NS). Insulin tolerance tests (ITTs) and glucose tolerance tests (GTTs) were performed to evaluate insulin resistance (IR). Under standard diet, the response to both tests was similar in wild-type and PPAR-α–null mice. Under high-fat diet, however, the efficiency of insulin in ITT was reduced and the amount of hyperglycemia in GTT was increased only in wild-type and not in PPAR-α–null mice. The IR index, calculated as the product of the areas under glucose and insulin curves in GTT, increased fourfold in high-fat–fed wild-type mice, whereas it remained unchanged in PPAR-α–null mice. In contrast, PPAR-α deficiency allowed the twofold rise in adiposity and blood leptin levels elicited by the diet. Thus, the absence of PPAR-α dissociates IR from high-fat diet–induced increase in adiposity. The effects of PPAR-α deficiency on glucose homeostasis seem not to occur via the pancreas, because glucose-stimulated insulin secretion of islets was not influenced by the PPAR-α genotype. These data suggest that PPAR-α plays a role for the development of IR in response to a Western-type high-fat diet. *Diabetes* 50: 2809–2814, 2001

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AUC, area under the curve; G6Pase, glucose-6-phosphatase; GK, glucokinase; GTT, glucose tolerance test; 11β-HSD-1, 11β-hydroxysteroid dehydrogenase type 1; IR, insulin resistance; ITT, insulin tolerance test; PPAR, peroxisome proliferator–activated receptor.

Mitochondrial β-oxidation is the major metabolic process by which fatty acids are utilized intracellularly, thus providing energy primarily for the heart and skeletal muscles. In the liver, β-oxidation also provides the substrates required for the synthesis of ketone bodies and supplies ATP and reducing equivalents to sustain gluconeogenesis. The physiological impact of enzymatic defects in these pathways is evidenced by the phenotype of patients with inherited β-oxidation deficiency. The clinical presentation includes cardiomyopathy, liver and muscle dysfunction, and episodes of nonketotic hypoglycemia (rev. in Eaton et al. [1]). Recently, a mouse model of β-oxidation deficiency was produced by targeted disruption of long-chain acyl-CoA dehydrogenase, an enzyme that catalyzes the initial step of this pathway. These mice display several features that resemble those of patients with β-oxidation defects, including reduced tolerance to fasting as a result of hypoglycemia and hepatic and cardiac disturbances (2).

The peroxisome proliferator–activated receptor (PPAR)-α plays a central role in the control of mitochondrial β-oxidation of fatty acids. PPAR-α–null mice (3) exhibit a reduced capacity to metabolize long-chain fatty acids (4,5), which likely contributes to dyslipidemia (6) and larger adipose stores observed in these mice with aging (7). When fasted, PPAR-α–null mice develop a severe and prolonged hypoglycemia (8,9). Blood glucose levels are tightly controlled through a coordinated interplay among the liver, the pancreatic β-cells, and peripheral insulin-sensitive tissues. Because PPAR-α is expressed in these tissues, with the highest levels in the liver (10,11), this factor could function in glucose homeostasis. In the present study, this hypothesis was tested by using PPAR-α–null mice and a nutritional challenge known to induce insulin resistance (IR) in rodents. We show that in the absence of PPAR-α, mice develop increased adiposity in response to a high-fat diet but are protected from the development of IR. Although beneficial during fasting, the function of PPAR-α may prove to be deleterious under long-term metabolic stress induced by a high-fat Western-type diet.

RESEARCH DESIGN AND METHODS

Animals. This study was conducted according to the *Guidelines for the Care and Use of Experimental Animals.* Male PPAR-α–null mice on either pure Sv/129 (3) or 10 generations–backcrossed C57BL/6N (12) genetic background were used. Mice were maintained under a constant light-dark cycle (light from 7:00 A.M. to 7:00 P.M.) and received either a standard diet (AO4; UAR, Epinay, France) or a high-fat diet containing hydrogenated coconut oil (29% wt/wt) according to Surwit et al. (13). Body weight and food intake, monitored by

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weighing special gridded metal food containers at regular intervals, were recorded throughout the feeding period. Two independent series of experiments were conducted, one with C57BL/6N mice for 16 weeks and one with Sv/129 mice for 22 weeks of high-fat feeding. Mice were 2 months old at the beginning of the feeding period. Except when glucose tolerance tests (GTTs) or insulin tolerance tests (ITTs) were performed, food was removed 4 h before the mice were killed, between 10:00 and 12:00 A.M. Blood was obtained from the orbital sinus. Serum was separated and stored at −20°C. Tissues were dissected, weighed, frozen in liquid nitrogen, and stored at −80°C until further use.

GTT. Food was removed for 2 h before the mice were lightly anesthetized with halothane between 9:00 and 10:00 A.M. Glucose (1 g/kg) was administered by intraperitoneal injection, and blood samples were collected from the orbital sinus at various times after the glucose load, as indicated. Plasma glucose was immediately determined on a glucose Analyzer II (Beckman Instruments, Fullerton, CA). The areas under the curves (AUCs) were determined with GraphPad Prism software. The product of glucose and insulin AUCs was taken as an IR index, as described by Mukherjee et al. (14). Mice on the Sv/129 genetic background, fed either the standard or high-fat diet as described above, were used. Wild-type and PPAR-α–null mice were submitted to concurrent GTT.

ITT. Food was removed for 2 h before the mice were lightly anesthetized with halothane, between 9:00 and 10:00 A.M. Insulin (Endopancrine, 0.01 units/mouse) was administered by intraperitoneal injection, and blood samples were collected from the orbital sinus at the time points indicated. Plasma glucose was immediately determined as above. Mice on the Sv/129 genetic background, fed either the standard or high-fat diet as described above, were used. Wild-type and PPAR-α–null mice were submitted to concurrent ITT.

Islet isolation and static incubations. Pancreas was obtained from C57/BL6N mice fed either the standard or high-fat diet as described above. After pancreas digestion by intraductal injection of collagenase, isolated islets were purified by double-hand picking, as described previously (15). After two washes of 15 min at 37°C in Krebs buffer containing 2.8 mmol/l glucose, islets were incubated for 60 min in the presence of 2.8 or 16.7 mmol/l glucose. Each incubation tube contained five islets, and each condition was run at least in triplicate. Preliminary experiments showed that the genetic background (Sv/129 versus C57BL/6N) did not influence the rate of insulin secretion in isolated islets. Islets from wild-type and PPAR-α–null mice were studied in concurrent incubations.

Serum assays. Except for GTT and ITT, glucose concentrations were measured using the glucose oxidase method. Insulin concentrations were determined with a radioimmunoassay kit (SB INS 15; CIS Bio International, Gif sur Yvette, France) with rat insulin standards. Leptin was measured using the rat Linco radioimmunoassay kit (St. Charles, MO), as described by the manufacturer.

Statistical analysis. Data are shown as means ± SE. Comparison between groups was performed by Student's t test. Two-way analysis of variance was performed to compare glucose curves in ITT. A difference at $P < 0.05$ was considered statistically significant.

RESULTS

PPAR-α–null mice are protected from high-fat diet–induced hyperinsulinemia. To evaluate a potential role for PPAR-α in modulating diet-induced IR, Sv/129 wild-type and PPAR-α–null mice were fed a diet enriched with hydrogenated coconut oil for several weeks. Plasma glucose and insulin levels were measured at the end of the feeding period. As expected, a twofold increase in insulin levels, accompanied by a moderate increase in plasma glucose, was induced in response to this nutritional challenge in wild-type mice (Fig. 1). Surprisingly, PPAR-α–null mice remained normoinsulinemic. Moreover, their plasma glucose was not increased but rather tended to be lower than in mice that were fed a standard diet. These observations were confirmed in a second series of experiments, in which wild-type and PPAR-α–null mice on the C57BL/6N genetic background were submitted to high-fat feeding. A similar pattern of diet-induced changes occurred, demonstrating increased insulin levels in wild-type but not in PPAR-α–null mice, and no major alteration of plasma glucose in either genotype (Table 1). Moreover,

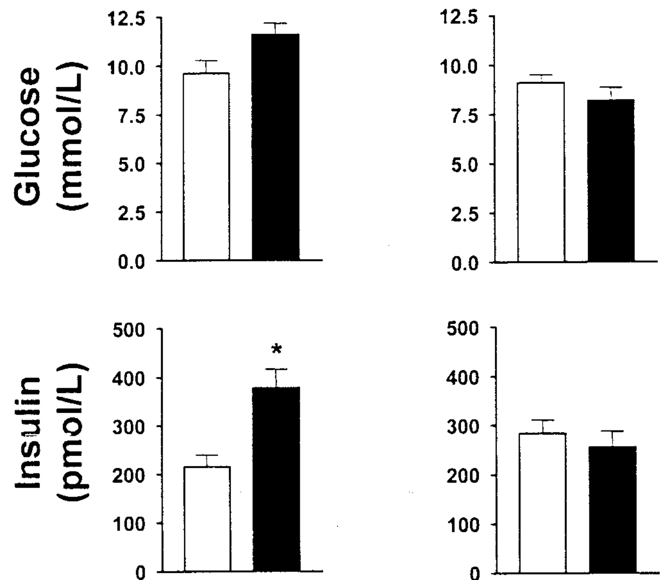

FIG. 1. Effect of high-fat feeding on plasma glucose and insulin. Plasma glucose and insulin concentrations were measured in blood obtained from the orbital sinus of mice that had been food-deprived for 4 h. Wild-type and PPAR-α–null Sv/129 mice were fed either standard diet (□; n = 8 per genotype) or high-fat diet (■; n = 8 per genotype) for 22 weeks. Data represent mean ± SE. *P < 0.01 versus standard diet.

preliminary data indicate that this feature also occurs in C57BL/6N female mice (data not shown). Thus, the absence of functional PPAR-α seems to protect the mice from high-fat diet–induced hyperinsulinemia.

PPAR-α deficiency did not preclude the marked enhancement of adipose tissue mass (Table 1) and circulating leptin (Fig. 2), which occurred in both strains in response to increased dietary fat. In mice on the Sv/129 genetic background, liver weight also increased, and this effect was more pronounced in PPAR-α–null mice (Table 1). Body weight was slightly increased in both strains, reaching statistical significance in wild-type mice only. In fitting with the known effect of high-fat feeding on food intake in rodents, these changes occurred despite similar caloric intake, independent of the PPAR-α genotype.

PPAR-α–null mice are protected from high-fat diet–induced IR. Diet-induced hyperinsulinemia is usually considered as a compensatory response to the development of IR. To evaluate more directly whether IR developed, intraperitoneal ITT and GTT were performed in Sv/129 wild-type and PPAR-α–null mice. Glucose and insulin response curves were not statistically different between PPAR-α–null and wild-type mice that were fed a standard diet (Figs. 3 and 4). By contrast, wild-type mice exhibited a reduced hypoglycemic response to insulin (Fig. 3) and increased amounts of hyperglycemia and insulin release in response to glucose load (Fig. 4) by the end of the high-fat feeding period. None of these effects was apparent in PPAR-α–null mice. Calculating the product of the areas under glucose and insulin curves obtained in GTT (IR index) allowed demonstration that high-fat feeding induced a fourfold increase in the IR index of

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TABLE 1
Effect of long-term high-fat feeding in wild-type and PPAR-α–null mice on two genetic backgrounds

	C57BL/6N mice				Sv/129 mice			
	Wild-type		PPAR-α–null		Wild-type		PPAR-α–null	
	Standard diet (n = 6)	High-fat diet (n = 6)	Standard diet (n = 6)	High-fat diet (n = 6)	Standard diet (n = 8)	High-fat diet (n = 8)	Standard diet (n = 8)	High-fat diet (n = 8)
Food intake (kcal · kg^{-1} · day^{-1})	465 ± 28.5	511 ± 15.2	506 ± 21.0	560 ± 28.9	383 ± 7.35	414 ± 13.3	369 ± 11.6	386 ± 22.4
Body weight (g)	32.7 ± 0.95	38.0 ± 1.37†	30.8 ± 0.94	32.4 ± 0.78	27.1 ± 0.80	34.5 ± 1.28†	29.6 ± 0.70	33.1 ± 0.90
Liver weight (g)	1.46 ± 0.12	1.44 ± 0.10	1.50 ± 0.07	1.48 ± 0.11	1.00 ± 0.03	1.26 ± 0.05†	1.26 ± 0.08	1.95 ± 0.10†
Adiposity index* (%)	1.60 ± 0.33	3.58 ± 0.34†	1.71 ± 0.25	3.74 ± 0.40†	2.94 ± 0.23	6.34 ± 0.78†	3.42 ± 0.36	5.93 ± 0.52†
Plasma glucose (mmol/l)	9.8 ± 0.67	11.5 ± 0.75	10.6 ± 0.72	9.3 ± 0.42	9.6 ± 0.56	11.6 ± 0.58	9.1 ± 0.41	8.2 ± 0.65
Plasma insulin (pmol/l)	209 ± 21.3	398 ± 50.1†	189 ± 31.8	225 ± 30.2	215 ± 23.9	378 ± 38.8†	284 ± 26.9	256 ± 32.3

Data are means ± SE. Mice on the C 57BL/6N genetic background were fed the high-fat diet for 16 weeks. Mice on the Sv/129 genetic background were fed the high-fat diet for 22 weeks. *Adiposity index was the ratio of epididymal adipose tissue to body weight for C57BL/6N mice and the ratio of epididymal + inguinal adipose tissue to body weight for Sv/129 mice. †$P < 0.05$ versus standard diet.

wild-type mice, whereas no change was observed in PPAR-α–null mice (Table 2). Moreover, individual IR index increased with the degree of adiposity in wild-type (r^2 = 0.63; $P < 0.05$) but not PPAR-α–null mice (data not shown). Thus, in the absence of PPAR-α, high-fat feeding does not seem to induce IR.

Islets from PPAR-α–null mice exhibit normal insulin secretion. We next tested whether the absence of PPAR-α could alter pancreatic β-cell function, by assessing insulin release in static incubations of islets isolated from C57BL/6N wild-type or PPAR-α–null mice. In mice that were fed a standard diet, equal amounts of insulin were secreted at low glucose concentration in both groups, and a similar increase in response to stimulatory glucose occurred, regardless of the PPAR-α genotype (Fig. 5). Moreover, glucose-stimulated insulin secretion potentiated by palmitate, 3-isobutyl 1-methylxanthine, or carbachol was not altered in the absence of PPAR-α (data not shown). In response to high-fat feeding, insulin release from wild-type islets tended to increase at low glucose and remained normal at high glucose concentration. In high-fat–fed PPAR-α–null mice, neither basal nor glucose-stimulated insulin secretion was affected as compared with mice that were fed a standard diet.

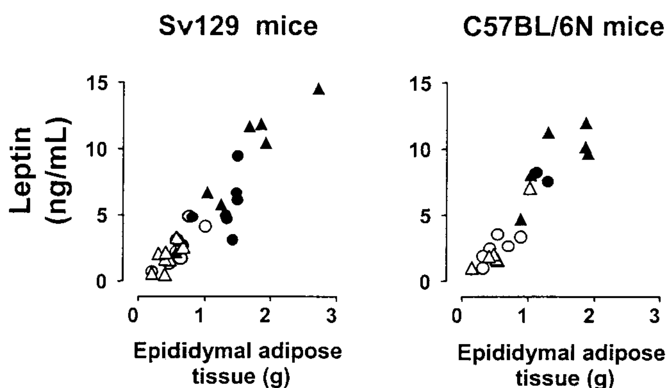

FIG. 2. Relationship between leptin and adipose tissue weight. Plasma leptin was plotted against the epididymal adipose tissue weight for wild-type (triangles) and PPAR-α–null (circles) Sv/129 and C57BL/6 mice, fed either standard diet (open symbols) or high-fat diet (closed symbols).

DISCUSSION

The present study, coupled with previous observations (8,9), reveals an unexpected role of PPAR-α in glucose homeostasis. Indeed, PPAR-α–null mice display abnormal systemic glucose metabolism in response to both short-term (8,9) and long-term (this study) nutritional challenges. In fasted conditions, PPAR-α–null mice develop severe hypoglycemia, suggesting that PPAR-α function is crucial to maintain blood glucose during fasting. Our data suggest that PPAR-α is involved in the alterations of glucose metabolism induced by high-fat feeding, which lead eventually to hyperglycemia and hyperinsulinemia. Thus, although this receptor can have a beneficial effect during fasting, PPAR-α seems to provoke potentially deleterious changes in glucose homeostasis after long-term high-fat feeding.

PPAR-α–deficiency did not alter systemic glucose and insulin levels or insulin sensitivity in response to standard diet feeding, as assessed by ITT and GTT. Consistent with these observations, normal glucose clearance in response to intraperitoneal glucose load has been reported in PPAR-α–

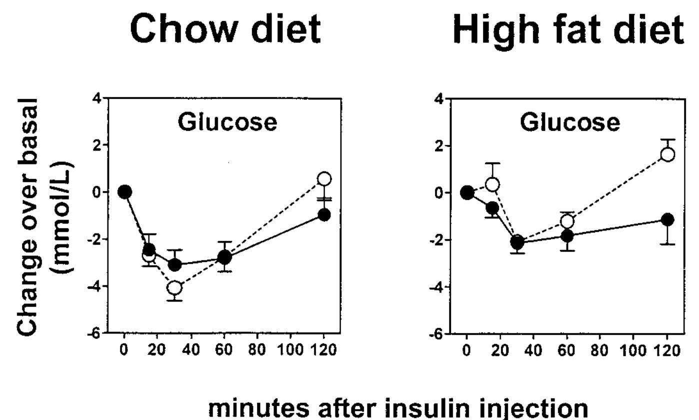

FIG. 3. Effect of high-fat feeding on ITT. Intraperitoneal ITTs were performed in Sv/129 wild-type (○) and PPAR-α–null (●) mice fed either standard or high-fat diet for 22 weeks. Data are expressed relative to time 0 and represent the means ± SE of seven independent tests. Response curves were compared by two-way analysis of variance. The diet effect was statistically significant ($P < 0.01$) in wild-type mice. The genotype effect was statistically significant ($P < 0.05$) in high-fat–fed mice.

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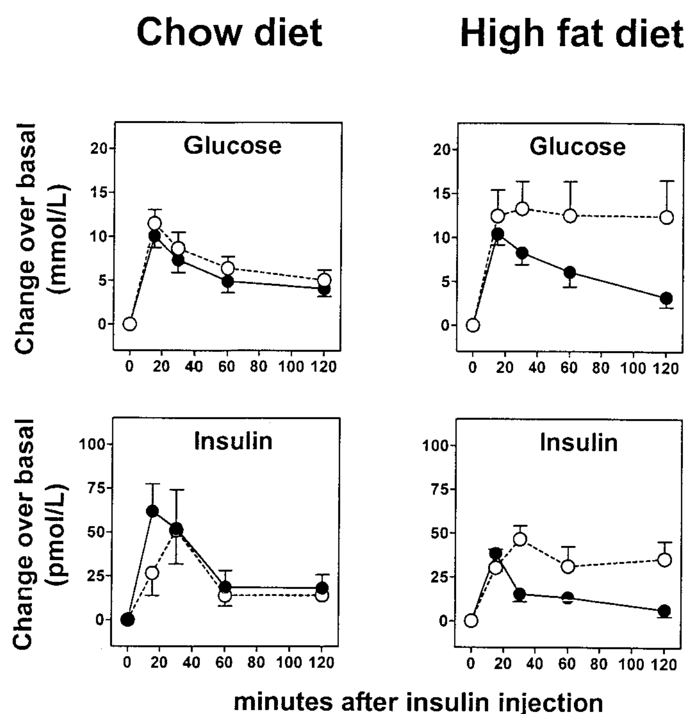

FIG. 4. Effect of high-fat feeding on GTT. Intraperitoneal GTTs were performed in Sv/129 wild-type (○) and PPAR-α–null (●) mice fed either standard or high-fat diet for 22 weeks. Data are expressed as changes relative to time 0 and represent the means ± SE of five independent tests.

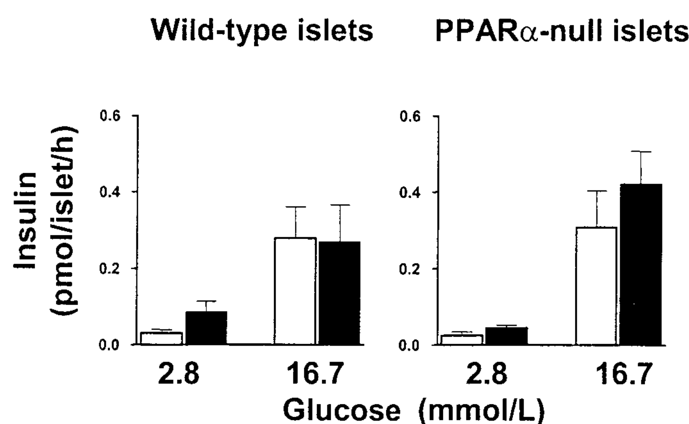

FIG. 5. Insulin secretion in isolated islets. Insulin secretion was measured in static incubations of isolated islets from wild-type and PPAR-α–null mice on the C57BL/6N genetic background fed standard diet (□) or high-fat diet (■) for 16 weeks. Data are the means ± SE of three (high-fat diet) and five (standard diet) separate incubations.

null mice (8). However, a PPAR-α–dependent alteration of glucose homeostasis was observed in response to the high-fat diet. Indeed, in agreement with changes in plasma glucose and insulin levels, ITT and GTT suggested a phenotype of IR in wild-type mice, which was absent in PPAR-α–null mice. These observations suggest a role for PPAR-α in the control of peripheral glucose utilization in response to dietary fat. This nuclear receptor is expressed in muscles and brown adipose tissue, which are major sites of insulin-sensitive glucose utilization. According to Randle et al. (16), increased fatty acid oxidation inhibits insulin-stimulated glucose uptake. The absence of PPAR-α, by reducing the rate of fatty acid oxidation, would favor glucose utilization in these tissues, despite lower glucose availability as in high-fat–fed mice. Shulman (17) recently suggested that intracellular fatty acid metabolite(s) could alter insulin signaling. If such a mechanism is involved in diet-induced IR, then our observations suggest that the potential mediator(s) derives from PPAR-α–dependent pathways. The PPAR-α–null mice could help to elucidate the molecular link between fatty acid metabolism and insulin signaling.

In line with our observations, a recent study reported that PPAR-α expression is increased in the liver of high-fat–fed rats, potentially as an adaptive response to attenuate liver steatosis (18). Our present data suggest that this might also result in the activation of metabolic pathways leading to IR. For example, increased glucose production from enhanced gluconeogenesis that is stimulated by increased fatty acid oxidation (19) could be dependent on a functional PPAR-α. It is interesting that when this article was under review, Tordjman et al. (20) reported that insulin was more efficient to suppress endogenous hepatic glucose production in the absence of PPAR-α in Western diet–fed apolipoprotein E–null mice. Although not studied here, it is likely that a similar mechanism is operative under the experimental conditions of our study.

The present study did not reveal major alterations of insulin secretion in islets isolated from PPAR-α–null mice, regardless of the diet. These observations are surprising because decreased fatty acid oxidation (as a result of the absence of PPAR-α) and, in turn, fat overload are likely to occur in these islets. On a long-term basis, increased lipid content results in high basal secretion and decreased insulin response to glucose. This feature is prominent in islets of obese prediabetic *fa/fa* ZDF rats (11) and in INS-1 β-cells that are exposed long-term to high glucose (21). Moreover, both conditions are associated with a marked downregulation of PPAR-α expression. It is interesting that the PPAR-γ ligand troglitazone reverses the secretory

TABLE 2
Effect of high-fat feeding on IR index

	Wild-type		PPAR-α–null	
	Standard diet (n = 5)	High-fat diet (n = 5)	Standard diet (n = 6)	High-fat diet (n = 5)
AUC glucose (mg/dl · 2 h)	115 ± 15	296 ± 78*	106 ± 16	128 ± 27
AUC insulin (ng/dl · 2 h)	17.3 ± 3.1	27.6 ± 2.7*	23.2 ± 6.3	12.8 ± 2.5
IR Index (AUC glucose · AUC insulin)	1,970 ± 413	8,400 ± 2,790*	2,490 ± 730	1,680 ± 548

Data are means ± SE. Areas under the insulin and glucose curves were calculated from the data shown in Fig. 4. The IR index is the product of glucose and insulin AUCs. *$P < 0.05$ versus standard diet.

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and lipid alterations of the β-cells in ZDF rats (22). This raises the hypothesis of a compensatory increase in PPAR-γ in PPAR-α–null islets, which might lower fat content and reduce the deleterious effects of PPAR-α deficiency on insulin release. In support of this idea, PPAR-γ mRNA is increased in the liver of PPAR-α–null mice (7,23). Nevertheless, the efficiency of PPAR-γ to compensate for the absence of PPAR-α is likely to depend on several factors, including level of expression and cellular environment in a given tissue.

Increasing dietary fat in rodents enhances adiposity and results in elevated plasma leptin levels (13,18,24,25). PPAR-α–null mice exhibit the expected phenotype irrespective of the Sv/129 or C57BL/6 genetic background. This indicates that *lep* gene regulation is not influenced by PPAR-α signaling, in agreement with previous observations (26,27). Thus, PPAR-α–null mice define a model for diet-induced obesity uncoupled from IR. Resistin is a newly discovered adipose protein that could represent a molecular link between increased adipose tissue mass and IR (28). PPAR-α–null mice may prove to be highly suitable to test whether altered release and/or action of resistin accounts for the lack of IR despite increased adipose tissue mass. Because resistin is primarily, if not exclusively, expressed in white adipose tissue, a direct transcriptional effect of PPAR-α is unlikely.

Similar phenotypes, in which IR dissociates from increased adiposity, have been described in some models of transgenic mice, including mice deficient in 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) (29). This enzyme is involved in the conversion of glucocorticoids from inactive to active form, notably in the liver. As a result of intrahepatic glucocorticoid deficiency, 11β-HSD-1–null mice fail to activate gluconeogenic enzymes under starvation. Although not demonstrated directly, it is likely that the gluconeogenic flux remains low in response to high-fat feeding. In turn, this would reduce hepatic glucose output and attenuate the diet-induced rise in glycemia. To test whether this feature might occur in PPAR-α–null mice, we measured 11β-HSD-1 mRNA in the livers of Sv/129 PPAR-α–null and wild-type mice. No change in 11β-HSD-1 gene expression was detected with any PPAR-α genotype or diet (data not shown). Nevertheless, a low rate of gluconeogenesis, as a result of fatty acid β-oxidation deficiency in PPAR-α–null mice, might account for similarities with the phenotype of mice deficient in 11β-HSD-1. Recently, mice expressing one extra copy of the glucokinase (GK) gene were also reported to be resistant to the development of diet-induced type 2 diabetes (30). In this model, supplementation of GK expression maintained a high GK–to–glucose-6-phosphatase (G6Pase) ratio in liver. By favoring glucose uptake, this could reduce the net hepatic glucose output, thereby reducing hyperglycemia. To our knowledge, whether PPAR-α exerts a direct or indirect transcriptional activation of GK is unknown. Data presented in an abstract form indicate that hepatic expression of sterol regulatory element binding protein 1 is very low in PPAR-α–null mice (31). Because this factor is known to control GK expression (32), increased GK activity in these mice is unlikely. However, Aoyama et al. (4) previously showed that G6Pase activity is not altered by PPAR-α deficiency. Even if the GK/G6Pase ratio is not increased, this does not exclude the possibility of reduced net hepatic glucose output in PPAR-α–null mice as a result of a low rate of gluconeogenesis. Altogether, these observations raise the hypothesis that the lack of diet-induced IR in these three models relies on reduced liver glucose output, although through different mechanisms.

The targeted mutation of the adipose fatty acid binding protein aP2 results in uncoupling obesity from IR in the context of both high-fat feeding (33) and extreme obesity (34). Although not expressed in the same tissues, aP2 and PPAR-α participate in a similar metabolic pathway of intracellular fatty acid metabolism. This suggests that the integrity of this pathway is critical for the development of IR in obesity, although the mechanisms involved might differ depending on the tissue.

A fourth model of transgenic mice, heterozygous PPAR-γ mice, display refractoriness to high-fat diet–induced IR (35). However, in contrast to the models described above, these mice exhibit a reduction in food intake and do not develop obesity. This has been attributed to a partial release of the suppressive effect of PPAR-γ on leptin gene expression, resulting in higher plasma leptin levels, despite lower adipose tissue mass. In this model, the more insulin-sensitive phenotype of heterozygous PPAR-γ compared with wild-type mice has been ascribed to a lack of adipocyte hypertrophy under high-fat diet, a feature that does not occur in the PPAR-α–deficient mice and that might directly rely on low resistin release.

In conclusion, our observations suggest that PPAR-α participates in glucose homeostasis. Such a role of PPAR-α might be physiologically relevant to avoid hypoglycemia under states of acute metabolic stress, such as fasting and exercise. However, under long-term metabolic stress, such as high-fat feeding, this function might become health-threatening by allowing the development of IR.

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