Human Metabolic Syndrome Resulting From Dominant-Negative Mutations in the Nuclear Receptor Peroxisome Proliferator–Activated Receptor-γ

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We previously reported a syndrome of severe hyperinsulinemia and early-onset hypertension in three patients with dominant-negative mutations in the nuclear hormone receptor peroxisome proliferator–activated receptor (PPAR)-γ. We now report the results of further detailed pathophysiological evaluation of these subjects, the identification of affected prepubertal children within one of the original families, and the effects of thiazolidinedione therapy in two subjects. These studies *1*) definitively demonstrate the presence of severe peripheral and hepatic insulin resistance in the affected subjects; *2*) describe a stereotyped pattern of partial lipodystrophy associated with all the features of the metabolic syndrome and nonalcoholic steatohepatitis; *3*) document abnormalities in the in vivo function of remaining adipose tissue, including the inability of subcutaneous abdominal adipose tissue to trap and store free fatty acids postprandially and the presence of very low circulating levels of adiponectin; *4*) document the presence of severe hyperinsulinemia in prepubertal carriers of the proline-467-leucine (P467L) PPAR-γ mutation; *5*) provide the first direct evidence of cellular resistance to PPAR-γ agonists in mononuclear cells derived from the patients; and *6*) report on the metabolic response to thiazolidinedione therapy in two affected subjects. Although the condition is rare, the study of humans with dominant-negative mutations in PPAR-γ can provide important insight into the roles of this nuclear receptor in human metabolism. *Diabetes* 52:910–917, 2003

Peroxisome proliferator–activated receptor (PPAR)-γ is a member of the nuclear hormone receptor superfamily that is expressed at high levels in adipose tissue, monocytes/macrophages, and colon and at lower levels in multiple other tissues (1). It regulates the transcription of target genes in response to a variety of naturally occurring lipid-based molecules, but no single dominant natural ligand has been unequivocally identified (1). PPAR-γ plays a critical role in the differentiation of preadipocytes to mature fat cells (2) and has become the subject of intense biomedical interest because the thiazolidinedione group of drugs, now widely used as insulin-sensitizing agents in the treatment of type 2 diabetes, are high-affinity ligands for this receptor (3). Despite substantial scientific endeavor, the precise mechanism by which PPAR-γ agonists improve insulin sensitivity remains in doubt (4). Current hypotheses favor a primary role for PPAR-γ in adipose tissue, although the facts that skeletal muscle is responsible for the bulk of insulin-stimulated glucose disposal and expresses low levels of PPAR-γ have ensured that a direct effect of PPAR-γ agonists in skeletal muscle remains a plausible alternative (5).

Our recent description of three subjects with extreme hyperinsulinemia and hypertension, in whom dominant-negative mutations in PPAR-γ were found (6), provided the first genetic evidence to support a role for this receptor in the control of glucose metabolism and blood pressure in humans. We have now undertaken further detailed clinical and pathophysiological studies in these subjects in an effort to further the understanding of the physiological role of PPAR-γ in human physiology and to explore the mechanism of action of thiazolidinediones. These studies support a key role for PPAR-γ in the regulation of human adipose tissue mass, distribution, and function and have enabled us to establish the principle that a genetic defect in a single molecule can recapitulate all the salient features of the metabolic syndrome, including insulin resistance, dyslipidemia, and hypertension. We also identified two prepubertal children with dominant-negative PPAR-γ mutations and significantly elevated insulin levels, thereby highlighting the early metabolic impact of a disturbance in

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Acrp30, adipocyte complement-related protein of 30 kDa; DEXA, dual-energy X-ray absorptiometry; FABP4, fatty acid–binding protein 4; HSD-1, 11-β-hydroxysteroid dehydrogenase 1; IMTG, intramyocellular triglyceride; MRI, magnetic resonance imaging; NEFA, nonesterified fatty acid; PPAR, peroxisome proliferator–activated receptor.

TABLE 1
Body composition details

	56-year-old female, P467L (S1)	32-year-old male, P467L (S2)	21-year-old female, V290M (S3)	Healthy adult ranges
Height (m)	1.53	1.72	1.64	
Weight (kg)	57.1	73.8	75.8	
BMI (kg/m^2)	24.4	24.9	28.1	18.5–24.9 (44)
Predicted total body fat (%)*	29.0	22.0	34.5	
Measured total body fat (%)	17.6	10.6	25.5	S1: 23–34% S2: 8–20% S3: 33–39% (45)
Waist-to-hip ratio	1.1	0.91	0.99	Female <0.85 Male <1.0
Visceral fat–to–total abdominal fat ratio	0.28	0.44	0.39	Female 0.25 ± 0.14 Male 0.42 ± 0.11 (46)
Fatty liver†	+	+	+	
IMTGs expressed as IMTG-to-creatine ratio for soleus muscle	13.7	9.7	—	Mean ± SE in 76 control subjects = 13.6 ± 6.6 (E.L.T., J.D.B., unpublished data)

*Predicted body fat was calculated as follows (47): men % fat = (1.281 × BMI) − 10.13; women % fat = (1.48 × BMI) − 7.00. †The diagnosis of fatty liver (+) was made on the basis of an echo-bright ultrasound picture in the absence of a history of alcohol consumption in excess of 40 g per week. Steatosis and cirrhosis were confirmed histologically in S1.

the function of PPAR-γ. Finally, we present direct evidence for resistance to the action of PPAR-γ ligand in cells derived from such patients and describe the response of two subjects with different PPAR-γ mutations to thiazolidinedione therapy.

RESEARCH DESIGN AND METHODS

All studies were approved by the local research ethics committees, and informed consent was provided by each subject and control subject for all procedures undertaken.

Case reports. Subject 1 (S1), a 56-year-old woman, was heterozygous for a proline-467-leucine (P467L) mutation in PPAR-γ. She presented with oligomenorrhoea and hirsutism at the age of 19 years, and type 2 diabetes and hypertension were noted in her twenties. Two pregnancies were complicated by preeclampsia, with one infant dying at birth. Despite high-dose insulin therapy, her glycemic control was persistently poor, and she developed retinopathy and nephropathy. Her treatment included metformin, 280 units of insulin daily, and four antihypertensive agents for the control of blood pressure. She developed cirrhosis and a hepatoma on a background of nonalcoholic steatohepatitis. After transplantation, she suffered fatal acute liver failure due to hepatic arterial thrombosis.

Subject 2 (S2), a 32-year-old man (son of S1), also carried the P467L mutation. He was found to be diabetic and hypertensive at age 28 years. He was treated with metformin, gliclazide, acarbose, enalapril, and amlodipine. His children, aged 3 and 9 years, were both found to be heterozygous for the P467L mutation.

Subject 3 (S3), a 21-year-old woman, was heterozygous for a valine-290-methionine (V290M) PPAR-γ mutation and presented with primary amenorrhoea, hirsutism, acanthosis nigricans, and hypertension at age 15 years. She developed diabetes at age 17 years. Her treatment included metformin, gliclazide, dianette, and atenolol. Her mother was wild-type at the PPAR-γ locus; her father was deceased, and there were no other family members available for study.

Body composition and fat distribution. Total body fat was measured with the use of a four-compartment model (7), which incorporated measurements of body weight, volume (measured by air displacement), total body water (measured by deuterium dilution), and bone mineral (measured by dual-energy X-ray absorptiometry [DEXA] [Lunar DXA]). Changes in body fat induced by rosiglitazone therapy were measured using DEXA alone. Adipose tissue distribution was assessed by T1-weighted magnetic resonance imaging (MRI). Abdominal visceral–to–total fat ratios were calculated from a single cross-sectional image at the level of the umbilicus. Intramyocellular triglycerides (IMTGs) were measured as described previously (8).

Studies of insulin sensitivity. Medication was stopped 36 h before the studies, and subjects were fasted for 12 h before and throughout the clamps. Normoglycemia (5–7 mmol/l) was maintained overnight (2000–0800) with a variable rate insulin infusion. 6,6-[$^2H_2$]glucose infusion was commenced at 0500 and maintained until the end of the study. Insulin (Actrapid) was infused at 10 mU · kg^{-1} · min^{-1} for 2 h (0800–1000). Blood glucose measurements were carried out at 5-min intervals throughout the study period, and euglycemia (5 mmol/l) was maintained by variable infusion of a 20% dextrose infusion (enriched with 6,6-[$^2H_2$]glucose). Samples for stable isotope measurements were obtained at 15-min intervals, except during the steady-state period (0930–1000), when sampling occurred at 5-min intervals. Glucose enrichment was determined using the methoxamine-trimethylsilyl ether derivative by selected ion monitoring by gas chromatograph mass spectrometry (Hewlett Packard 5971A mass spectrometer detector). Rates of glucose appearance and glucose disposal were measured according to calculations originally derived by Steele and subsequently modified for stable isotopes (9).

Examination of in vivo adipose tissue function. In vivo measurements of triglyceride clearance, nonesterified fatty acid (NEFA) output, and glycerol release from subcutaneous abdominal adipose tissue were obtained as described previously (10). The principle of this technique is as follows: differences in the composition of blood samples from the arterial supply to the tissue and venous drainage from the tissue reflect the net metabolic activity of the tissue. The net uptake of any substrate is then given by the following: net uptake = arteriovenous difference × blood flow.

Blood flow is determined by washout of ^{133}Xe after subcutaneous injection in the anterior abdominal wall. "Arterial samples" are taken from a retrogradely inserted cannula in a vein draining a hand, which is placed in a hot-box (65°), and arterialization of the sample is confirmed by checking that oxygen saturation is >95%. Adipose tissue venous blood is drawn from a catheter, introduced over a guide wire into one of the superficial veins on the anterior abdominal wall, and threaded toward the groin so that its tip lies just superficial to the inguinal ligament. The subject was fasted overnight before the study. After insertion of cannulas, baseline measurements were made for 30 min before consumption of a standardized mixed meal. The subject then relaxed on a bed while further measurements were made for 6 h. The principle measurements included glycerol, NEFA, and triglyceride fluxes.

Ex vivo studies of mutant PPAR-γ function. Mononuclear cell isolation and culture were performed as described previously (11). Fatty acid–binding protein 4 (FABP4) mRNA levels in monocyte mRNA were measured by real-time PCR (Taqman) as described previously (11).

RESULTS

Body composition and fat distribution. In vitro and rodent data strongly suggest that PPAR-γ is a key regulator of adipogenesis (12). S1 and S2 (P467L carriers) had BMIs within the healthy range, whereas S3 (V290M) was overweight on the basis of BMI (Table 1). However, formal measurements revealed that all three subjects with domi-

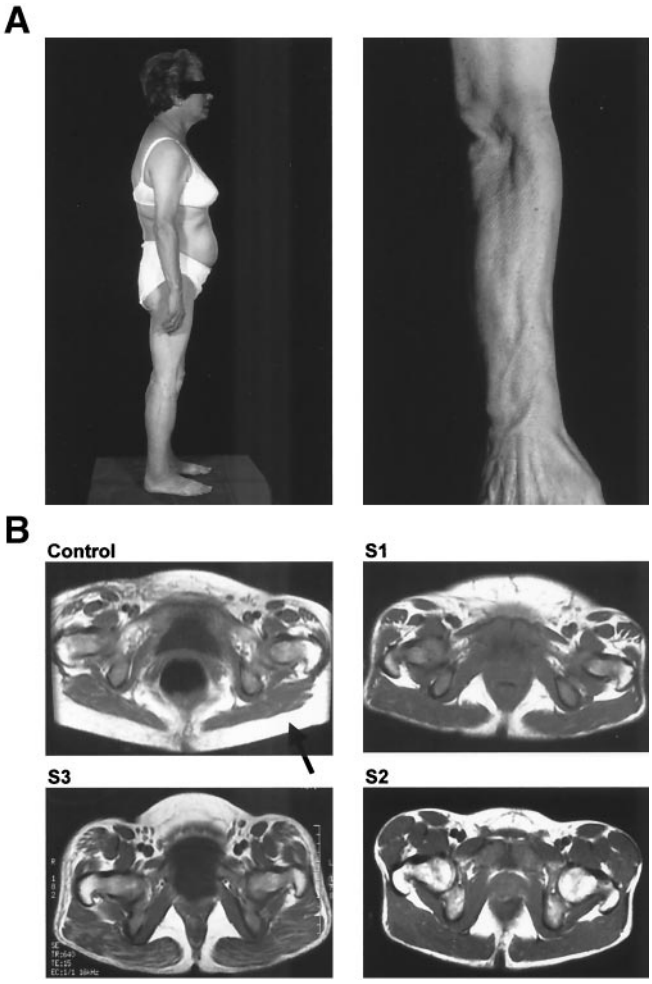

FIG. 1. Phenotypic features of individuals with dominant-negative PPAR-γ mutations. *A*: Photographs of a 56-year-old female P467L carrier (S1). Note the prominent forearm veins and musculature as well as the preservation of abdominal fat with loss of limb and gluteal fat depots. These images preceded her liver transplant and the subsequent use of immunosuppressive therapy. *B*: T1-weighted MRI images at the level of the gluteal fat pad indicate the striking loss of gluteal subcutaneous fat (arrow) in S1. Corresponding images from S2 and S3 demonstrate the consistency of these features. Control images were taken from a lean healthy female individual. None of the subjects were involved in either manual labor or regular physical exercise.

nant-negative PPAR-γ mutations had a total body fat content substantially lower than predicted from their BMI (Table 1). In addition, MRI of fat distribution revealed a consistent and striking paucity of subcutaneous limb and buttock fat (Fig. 1), whereas both visceral and subcutaneous abdominal fat were preserved. This is reflected by the increased waist-to-hip ratios in both female patients, where the loss of gluteal and femoral fat was particularly striking (Fig. 1 and Table 1). Although the detection of partial lipodystrophy in children is particularly difficult, both total and regional body fat measurements in the 9-year-old P467L carrier (total body fat 15%, individual regional fat range 12–16.3%) were similar to those predicted according to her height and weight (height 1.231 m, weight 25.2 kg, predicted total body fat 17.4% [13]). Lipodystrophy in rodents and humans is believed to result in "ectopic" lipid accumulation in liver and skeletal muscle, a phenomenon increasingly implicated in the pathogenesis of insulin resistance in these syndromes (14). On ultrasonographic study, all three subjects had hyperechoic livers, consistent with fatty infiltration. This radiological impression was confirmed histologically in S1 (data not shown), whose liver disease had in fact progressed to cirrhosis. Surprisingly, however, IMTG levels were within the normal range in two subjects (Table 1).

Metabolic status. The presence of acanthosis nigricans and fasting hyperinsulinemia suggested that all affected subjects were severely insulin resistant (6). To confirm this, S2 and S3 underwent hyperinsulinemic-euglycemic clamp studies. High-dose insulin infusion (10 mU · kg^{-1} · min^{-1}) failed to stimulate peripheral glucose disposal normally (rate of glucose disposal [mg · kg^{-1} · min^{-1}]: S2, 7.53; S3, 2.91; healthy adult range, 13–15 [15]) or to completely suppress hepatic glucose output (rate of glucose appearance [mg · kg^{-1} · min^{-1}]: S2, 1.07; S3, 0.31) in either subject (Table 2). Additional metabolic abnormalities found in the subjects include elevated serum triglycerides, low HDL cholesterol, hyperuricemia, and elevated serum transaminases (Table 3). Further evidence of the profound impact of dominant-negative mutations in PPAR-γ was provided by the three- to fourfold increases in fasting plasma insulin levels seen in the 3- and 9-year-old children of S2, both of whom carry the P467L mutation (Table 3). Besides hyperinsulinemia, and in contrast to the affected adults, neither child manifested features of the metabolic syndrome.

Adipose tissue function. The nature of PPAR-γ target genes in adipose tissue led to suggestions that PPAR-γ may be involved in the regulation of "trapping" NEFAs within adipocytes and that this may, at least in part, explain the insulin-sensitizing effects of the pharmacological PPAR-γ agonists (16). We hypothesized that dominant-negative mutations in PPAR-γ might impair this process, resulting in increased fatty acid flux to the liver and skeletal muscle, where the role of fatty acids in interfering with glucose metabolism is well described (17,18). We therefore undertook detailed in vivo functional studies of the abdominal subcutaneous adipose tissue depot of S2 by measuring arteriovenous differences of metabolites across this depot (10). The usual postprandial increase in plasma triglyceride clearance across the tissue was not seen; in fact, the clearance was very low both in the fasting state and postprandially (Fig. 2*A*). Interestingly, adipose tissue lipolysis, as assessed by glycerol output, was also low both in the fasted and postprandial states (data not shown). This apparent failure of adipose tissue to regulate lipolysis is reflected in Fig. 2*B*, where subcutaneous abdominal adipose tissue NEFA output remains low throughout the study in the P467L subject, whereas in both normal and obese subjects, it is suppressed postprandially (19). It is important to appreciate that, as was apparent in all the subjects of this study, dysregulation of postprandial fatty acid metabolism need not result in elevated fasting NEFA levels.

It has been suggested that PPAR-γ agonists increase the number of small insulin-sensitive adipocytes in adipose tissue. We therefore wondered if dominant-negative PPAR-γ mutations might alter adipose tissue morphology. The adipose mean cell diameter (81.56 ± 1.48 μm), area (5,734.67 ± 94.54 mm^2), and weight (0.33 ± 0.02 mg lipid/cell) in S2 were within normal limits (S.C., unpub-

TABLE 2
Response to PPAR-γ agonist therapy (4 mg rosiglitazone b.i.d.)

	P467L (S2)				V290M (S3)				Reference
Months of treatment	0	1	3	6	0	1	3	6	
Weight (kg)	73.8	—	—	76.8	75.8	—	—	76.6	
Fat mass (kg)	6.3	—	—	9.8	17.6	—	—	21.6	
Leptin (μg/l)	0.7	1.5	1.9	1.9	11.2	13.8	14.4	14.4	*
Acrp30 (units/ml)	0.25	—	—	0.5	0.17	—	—	0.24	2.23 ± 0.66 (32)
Blood pressure (mmHg)	140/97	147/84	153/85	156/99	136/84	114/82	135/88	140/85	
Glucose (mmol/l)	10	10.9	12	9.3	9.6	7.3	6.4	7.2	3.5–6.3
Insulin (pmol/l)	85	107	73	114	411	265	414	410	<80
HbA_{1c} (%)	8.7	8.8	8.3	5.6	7.3	6.9	6.7	7.1	4.9–6.3
Glucose disposal ($mg \cdot kg^{-1} \cdot min^{-1}$)	7.53	—	—	14.9	2.91	—	—	3.21	13–15 (14)
Hepatic glucose output ($mg \cdot kg^{-1} \cdot min^{-1}$)	1.07	—	—	0	0.31	—	—	0.52	0
Triglycerides (mmol/l)	5.7	6.6	7.6	6.3	4.5	7.1	3.5	6.9	<2.0
HDL cholesterol (mmol/l)	0.58	0.72	0.66	0.79	0.58	0.71	0.72	0.52	>0.9
NEFAs (mmol/l)	503	365	510	325	558	915	852	643	280–920
Alanine aminotranferase (units/l)	85	87	108	82	39	48	79	58	0–50
γ-Glutamyl transferase (units/l)	68	75	86	67	147	100	102	122	0–50

*Leptin adult reference values (unpublished data, mean, and 95% CIs): females: BMI 25–30 kg/m^2, $n = 348$ adults, 21.1 (8.6–38.9); males: BMI <25 kg/m^2, $n = 278$ adults, 3.3 (0.4–8.3).

lished data), and both light and electron microscopy showed typical white adipose tissue morphology (data not shown).

A growing body of evidence indicates that adipocytes secrete proteins ("adipokines") with paracrine and/or endocrine effects, several of which have the ability to influence insulin action, either positively or negatively (20). Plasma levels of leptin were within population reference ranges for sex- and BMI-matched individuals (Table 2), whereas both tumor necrosis factor-α and resistin mRNA were barely detectable in isolated adipocytes from S2 (data not shown and 11). The only striking abnormality in adipokine levels was the very low level of serum adipocyte complement-related protein of 30 kDa (Acrp30) seen in all adult subjects (12) (Table 2).

Ex vivo response of patient cells to PPAR-γ agonists. To establish whether cells obtained from the patients exhibited evidence of resistance to PPAR-γ ligands, we examined the induction of a known PPAR-γ target gene (1), namely FABP4 (human homologue of murine aP2), by rosiglitazone in cultured monocytes from S1 and S2. Monocytes from both subjects showed a right shift in responsiveness of FABP4 mRNA expression to rosiglitazone (Fig. 3), supporting the notion that these heterozygous mutations alter expression of PPAR-γ target genes in vivo.

Effects of rosiglitazone therapy. Because the mutant receptors present in these subjects retained some residual ability to respond to pharmacological agonists in vitro (6) and because their cultured monocytes also retained some ex vivo responsivity to a thiazolidinedione (Fig. 3), we administered a PPAR-γ agonist, rosiglitazone (4 mg twice daily), to S2 and S3 for a period of 6 months. Sizeable increases in total body fat were seen in both treated subjects (Table 2). In both cases, fat mass increased slightly more in the limb/gluteal depots than in the trunkal region. Consonant changes in plasma leptin and Acrp30 levels were also noted in both subjects. All of these changes were more marked in S2, and the metabolic impact of rosiglitazone was much more striking in S2, with insulin sensitivity and HbA_{1c} (Table 2) both being normalized, whereas S3 remained severely insulin resistant and showed little change in HbA_{1c} levels. Interestingly, the effects of rosiglitazone on fasting glucose and insulin levels were less impressive throughout the course of therapy. This may reflect the day-to-day variability of these measures and the fact that they may not be good surrogates for insulin sensitivity or total glycemic burden,

TABLE 3
Metabolic characteristics of S1, S2, and S3 and prepubertal P467L carriers

	56-year-old female, P467L*	32-year-old male, P467L	21-year-old female, V290M	9-year-old female, P467L	3-year-old male, P467L	Reference values
Glucose (mmol/l)	6.0	10	9.6	4.9	5.6	3.5–6.3
Insulin (pmol/l)	296	85	411	124†	85†	<80 (adults)
Triglycerides (mmol/l)	2.1	6.6	4.5	0.7	0.6	Desirable, <2.0
Total cholesterol (mmol/l)	3.4	4.7	3.4	4.1	4.8	Desirable, <5.2
HDL cholesterol (mmol/l)	0.9	0.72	0.6	0.84	1.05	Desirable, >1.0
NEFAs (μmol/l)	721	503	558	—	—	280–920
Uric acid (mmol/l)	0.29	0.35	0.37	—	—	0.15–0.35
Alanine aminotransferase (units/l)	70	85	39	29	33	0–50
γ-Glutamyl transferase (units/l)	454	68	147	18	20	0–50

*S1 is on hormone replacement therapy. †Reference values for fasting insulin in children: healthy 9-year-old subjects ($n = 50$, mean ± SE): 42.8 ± 5.7; healthy 3-year-old subjects ($n = 14$): 18.7 ± 1.6 (D.A. Dunger, K. Ong, personal communication).

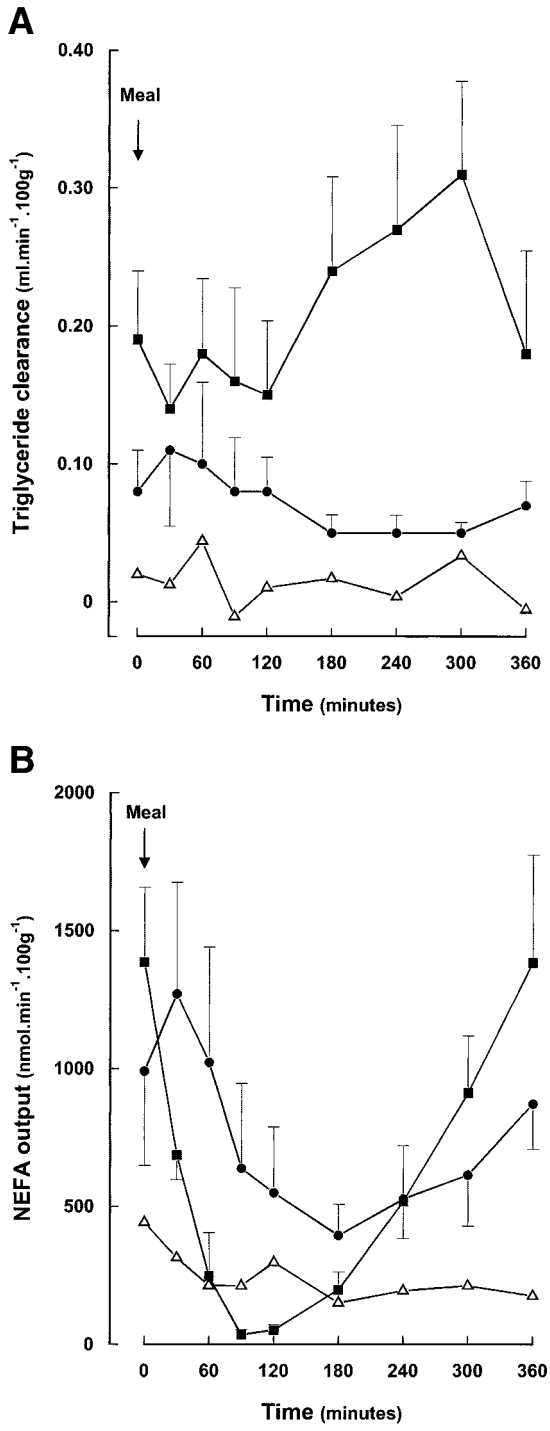

FIG. 2. Adipose tissue triglyceride and fatty acid metabolism in vivo. *A*: Adipose tissue clearance of plasma triglycerides in milliliters of plasma per minute^{-1} per (100 g adipose tissue)$^{-1}$ in S2 (P467L) (△), 10 normal subjects (■), and 8 obese subjects (●) (19). *B*: Adipose tissue NEFA output in nmol · min^{-1} · (100 g adipose tissue)$^{-1}$.

particularly in the setting of severe insulin resistance. Alternatively, it may be a feature of PPAR-γ agonist therapy per se, because Miyazaki et al. (21) noted little change in fasting glucose levels in type 2 diabetic subjects treated with thiazolidinediones, whereas glucose and insulin levels after an oral glucose challenge were significantly reduced. The notion that PPAR-γ activity is particularly important in the postprandial period is supported by the fact that, whereas fasting NEFA levels were normal in our subjects, postprandial fatty acid trapping by adipose tissue was significantly impaired.

How might the differential response to therapy in S2 and S3 be explained? In vitro studies of the function of the mutant receptors from these two subjects indicate that when cotransfected with a wild-type receptor, the V290M mutant receptor inhibited transcriptional activation of a reporter gene to a greater extent than did the P467L mutant (Fig. 4). Although the differences were small and extrapolating in vitro findings to the in vivo situation is always difficult, the differences were statistically significant and may, at least in part, explain the observed difference in clinical response to rosiglitazone. Lack of compliance in S3 is a formal possibility, but plasma rosiglitazone was detectable in this subject during the treatment period, and the small but consistent increases in fat mass, plasma leptin, and Acrp30 all suggest the presence of a PPAR-γ agonist effect, albeit insufficient to result in metabolic improvement.

DISCUSSION

Humans with dominant-negative mutations in PPAR-γ represent a novel subtype of inherited partial lipodystrophy. The paucity of limb and gluteal fat resembles that seen in familial partial lipodystrophy (Dunnigan-Kobberling syndrome) (22) and HIV-associated lipodystrophy (23), but differs from these syndromes in the preservation of normal facial and abdominal (subcutaneous and visceral) fat depots. The lack of excess facial fat clearly distinguishes people with dominant-negative PPAR-γ mutations from those with classic familial partial lipodystrophy but also renders recognition of the syndrome more difficult. In fact, the lipodystrophy was not initially recognized in our subjects (6). Consistent with our observations, Agarwal and Garg (24) have recently reported a heterozygous R425C mutation in PPAR-γ in a Caucasian female with limb and facial lipoatrophy. In contrast to other recently identified causes of inherited lipodystrophy, in which the genotype/phenotype relationships are as yet poorly understood (25,26), PPAR-γ is believed to be "the master regulator of adipogenesis" (2). The finding of lipodystrophy in association with dominant-negative germline mutations in human PPAR-γ is entirely consistent with a key role for this molecule in human adipogenesis. The loss of subcutaneous adipose tissue, particularly in the limb and gluteal depots, with preserved visceral adipose tissue is consistent with the selective increase in subcutaneous adipose tissue seen in patients treated with PPAR-γ agonists (27,28), but there is, to date, no clear explanation for the sparing of subcutaneous abdominal adipose tissue.

The presence of severe insulin resistance from early childhood in carriers of dominant-negative PPAR-γ mutations highlights the key role of this molecule in the control of insulin action. Our studies in these patients provide some possible insight into how interference with PPAR-γ signaling might result in insulin resistance. First, we have demonstrated that these subjects have a form of partial lipodystrophy with selective loss of gluteal and limb subcutaneous fat. Lipodystrophy, both of the partial and generalized types, is consistently associated with insulin resistance in animals and humans and thus is likely to

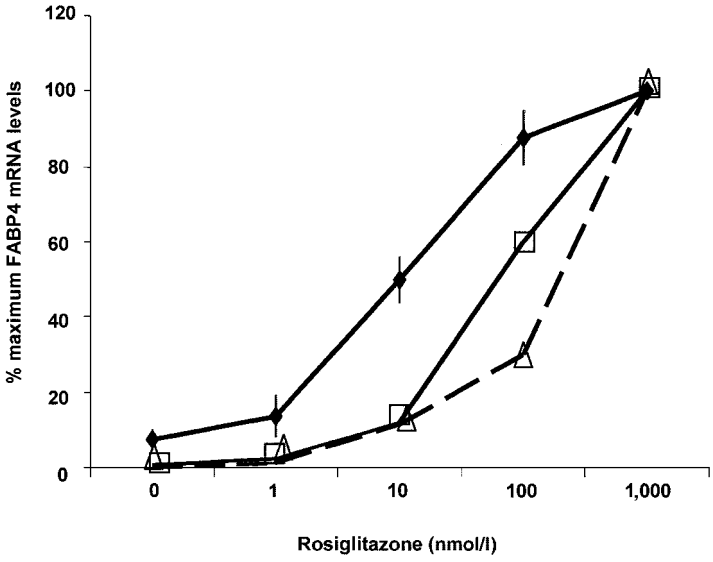

FIG. 3. FABP4 mRNA expression in freshly isolated peripheral blood monocytes from S1 (□) and S2 (△). Control data (♦) represents the mean ± SE of FABP4 expression in peripheral blood monocytes from four healthy individuals (BMI <28 kg/m², age 28–60 years). Results are expressed as a percentage of the maximum FABP4 mRNA levels obtained in each subject.

contribute to the insulin resistance seen in this disorder (29). In addition to diminished fat mass in the limbs and buttocks, we provide evidence for a major functional disturbance of the residual subcutaneous adipose tissue. In particular, the abdominal adipose tissue of S2 had both a reduced capacity to increase lipolysis in response to fasting and an inability to suppress fatty acid mobilization. In the postprandial state, this inability to "trap and store" NEFAs is likely to expose skeletal muscle and liver to NEFAs in an unregulated manner, a phenomenon that is known to impair insulin sensitivity in these tissues (18). The hepatic steatosis found in these subjects probably reflects this phenomenon, but we were surprised not to find a marked elevation of IMTGs. Although prior reports have suggested that IMTG levels are elevated in patients with total lipodystrophy, a recent report suggested that IMTGs were within the range seen in control subjects (30). The absence of elevated IMTGs does not preclude a pathogenic role for inappropriate NEFA delivery to skeletal muscle because it is increasingly acknowledged that long-chain acyl-CoAs, rather than triglycerides themselves, are likely to be directly involved in the induction of insulin resistance in muscle (31). Although we did not note any striking alterations in the protein or mRNA levels of adipokines such as leptin, resistin, or tumor necrosis factor-α in serum or adipocytes, serum Acrp30 levels were markedly reduced in all three subjects (32). This adipocyte-derived circulating protein is capable of potentiating insulin's actions in skeletal muscle and hepatocytes (33,34) and has been shown to be highly responsive to PPAR-γ agonists (35). Whether the reduced level seen in our patients is merely an in vivo marker of reduced PPAR-γ action or whether low levels of the protein actively contribute to the causation of insulin resistance is yet to be determined.

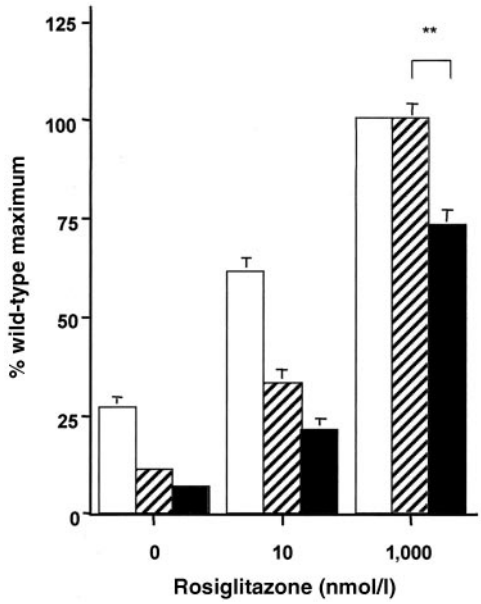

FIG. 4. Dominant-negative activity of mutant PPAR-γ. 293EBNA cells were transfected with 500 ng reporter construct, (PPARE)3TKLUC, 100 ng wild-type (WT) receptor plus an equal amount of either WT (□) or mutant (P467L [▨], V290M [■]) expression vectors, and 100 ng control plasmid Bos-β-gal. The transcriptional responses mediated by either 100 or 200 ng of WT receptor at each concentration of ligand did not differ significantly (data not shown). Results are expressed as a percentage of the WT maximum and represent the mean ± SE of eight independent experiments, each performed in triplicate. **$P < 0.001$.

In addition to insulin resistance, carriers of dominant-negative PPAR-γ mutations manifest early-onset hypertension, dyslipidemia, and hepatic steatosis. Although it is conceivable that these features may be secondary to severe insulin resistance per se (36), they have not been previously described as coexisting in patients with other forms of hereditary severe insulin resistance nor are they common in monogenic murine models of severe insulin resistance (37). Carriers of dominant-negative PPAR-γ mutations would thus appear to represent a unique human monogenic model of the metabolic syndrome. As reported previously (6), the consistent finding of early-onset hypertension was particularly striking in these subjects. The expression of several components of the renin-angiotensin system in adipose tissue (38) suggested the possibility that PPAR-γ mutations might lead to dysregulation of this system, thereby contributing to the development of hypertension. However, normal serum potassium levels coupled with normal plasma renin activity and aldosterone levels (data not shown) suggest that abnormal regulation of

mineralocorticoid metabolism may not be involved in the pathogenesis of hypertension in these subjects.

In conclusion, we believe that dominant-negative PPAR-γ mutations in humans represent a new member of a group of conditions involving an inherited impairment of nuclear receptor function. This group of conditions includes the androgen insensitivity syndrome, estrogen resistance, and hereditary vitamin D–resistant rickets. The closest analogy is with thyroid hormone resistance, where heterozygous dominant-negative mutations in thyroid hormone receptor-β are associated with pituitary and peripheral refractoriness to thyroid hormone action. Although the physiological ligand for PPAR-γ remains to be identified, we have demonstrated ex vivo resistance to a pharmacological agonist in mononuclear cells harboring the dominant-negative PPAR-γ mutation and therefore believe that PPAR-γ ligand resistance is almost certainly present in vivo. The fact that humans with dominant-negative mutations in PPAR-γ appear to represent a monogenic model of the metabolic syndrome in association with a stereotyped form of partial lipodystrophy is in keeping with the presence of lipodystrophy and insulin resistance noted by Yamauchi et al. (39) in heterozygous PPAR-γ knockout mice treated with retinoid X receptor antagonists. Although both PPAR-γ haplo-insufficient mice (40,41) and wild-type mice treated with either PPAR-γ or retinoid X receptor antagonists paradoxically demonstrated improved insulin sensitivity (39), we believe that the dominant-negative mutations identified in our human subjects induce a more severe impairment of PPAR-γ function than that seen in these states. The reduction in transcriptional activity of the PPAR-γ mutants is likely to be a consequence of aberrant corepressor recruitment (M.A., M.G., V.K.K.C., unpublished data). The ensuing combination of lipodystrophy of limb and gluteal subcutaneous fat with a major functional disturbance of the remaining adipose tissue is likely to be a major contributor to the observed metabolic dysfunction. This result may be compounded by the low circulating Acrp30 levels found in these patients. Details of the specific molecular abnormalities responsible for the adipose tissue dysregulation seen in carriers of dominant-negative PPAR-γ mutations remain to be identified, although several hypotheses exist. For example, Masuzaki et al. (42) recently described a murine model of syndrome X in mice selectively overexpressing 11-β-hydroxysteroid dehydrogenase 1 (HSD-1) in adipocytes. Because HSD-1 expression may be indirectly altered by PPAR-γ agonists (43), it is conceivable that elevated HSD-1 levels in the adipocytes of our patients could be contributing to the observed phenotype. Finally, reflecting the ex vivo ability of the mutant receptors to respond, at least partially, to pharmacological ligands, thiazolidinedione therapy can result in substantial reversal of pathophysiological disturbances together with clinically meaningful therapeutic benefits in some patients with this disease.

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