A Dominant Negative Peroxisome Proliferator-activated Receptor-γ Knock-in Mouse Exhibits Features of the Metabolic Syndrome*

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Peroxisome proliferator-activated receptor-γ (PPARγ), a member of the nuclear hormone receptor family, is a master regulator of adipogenesis. Humans with dominant negative PPARγ mutations have features of the metabolic syndrome (severe insulin resistance, dyslipidemia, and hypertension). We created a knock-in mouse model containing a potent dominant negative PPARγ L466A mutation, shown previously to inhibit wild-type PPARγ action in vitro. Homozygous PPARγ L466A knock-in mice die in utero. Heterozygous PPARγ L466A knock-in (PPARKI) mice exhibit hypoplastic adipocytes, hypoadiponectinemia, increased serum-free fatty acids, and hepatic steatosis. When subjected to high fat diet feeding, PPARKI mice gain significantly less weight than controls. Hyperinsulinemic-euglycemic clamp studies in PPARKI mice revealed insulin resistance and reduced glucose uptake into skeletal muscle. Female PPARKI mice exhibit hypertension independent of diet. The PPARKI mouse provides a novel model for studying the relationship between impaired PPARγ function and the metabolic syndrome.

The metabolic syndrome, or syndrome X, is characterized by a constellation of insulin resistance, dyslipidemia, obesity, and hypertension (1). The prevalence of the metabolic syndrome is increasing rapidly and is estimated to affect 24% of the United States population (2). Thiazolidinediones, a class of antidiabetic compounds that activate the peroxisome proliferator-activated receptor-γ (PPARγ),1 stimulate adipocyte differentiation, lower free fatty acids (FFAs), and enhance insulin sensitivity, thereby correcting several features of the metabolic syndrome (3). The insulin-sensitizing action of the thiazolidinediones suggests that PPARγ function may be central to the development and treatment of the metabolic syndrome.

PPARγ is a member of the nuclear hormone receptor superfamily and plays a pivotal role in adipogenesis (4–6). Although PPARγ is most highly expressed in adipose tissue, it is also present in colon, monocytes/macrophages, and at lower levels in many other tissues including skeletal muscle and liver (7, 8). Homozygous PPARγ null mice die in utero at 10.5–11.5 days post-coitum, likely because of placental abnormalities (9). Heterozygous PPARγ-deficient mice exhibit increased insulin sensitivity and are protected from high fat diet-induced obesity when compared with their wild-type littermates (10, 11). These observations may be partly explained by increased serum leptin, as heterozygous PPARγ knock-out mice have decreased food intake and increased energy expenditure (10). Cre-loxP strategies have been used to generate tissue-specific knockouts of PPARγ function (12–17). Muscle-specific PPARγ knock-out mice show progressive insulin resistance combined with increased adipose tissue mass (14, 16). Fat-specific PPARγ knock-out mice have lipodystrophy (hypocellularity and hyperplasia), elevated plasma FFAs and triglycerides (TGs) and decreased plasma leptin and adiponectin. These mice have insulin resistance in fat and liver but not in muscle (13).

There are several reports of heterozygous PPARγ mutations in humans (18–21). These patients exhibit partial lipoatrophy, severe insulin resistance, steatohepatitis, and hypertension (20, 21). These clinical manifestations include many features of the metabolic syndrome and are thought to result from a combination of loss of function and the dominant negative effects of the mutant PPARγ receptors. In this study, we created a knock-in mouse model containing a PPARγ L466A mutation, shown previously to inhibit wild-type PPARγ action in vitro (22). The potent dominant negative activity of the L466A mutation, located in the transactivation domain of PPARγ, is because of preserved DNA binding activity in combination with loss of coactivator binding but preserved corepressor recruitment (22). Homozygous PPARγ L466A knock-in mice die in utero, but heterozygous PPARγ L466A knock-in (PPARKI) mice are viable and manifest many of the features seen in patients with dominant negative PPARγ mutations.

MATERIALS AND METHODS

Generation of Mice—An exon 6 fragment of PPARγ was used to screen a 129SvJ mouse genomic library, resulting in the isolation of a 16-kb fragment of the PPARγ gene. Site-directed mutagenesis was used to mutate the leucine residue at codon 466 to alanine (L466A). Three nucleotides were modified (CCT to AGC) to generate a novel Eco47III enzyme restriction site. A targeting vector was constructed that contained most of the 16-kb clone, modified to include the L466A mutation, along with loxP-flanked neomycin and thymidine kinase cassettes in reverse orientation (Fig. 1A). The vector was linearized by KpnI digestion, electroporated into 129SvJ R1 ES cells (23), and subjected to selection with G418 and gancyclovir. PCR and Southern blot analysis were used to identify homologous recombination at the PPARγ locus. Two clones identified as carrying the L466A mutation were injected into murine blastocysts and implanted into pseudo-pregnant mice. Male chimeric mice were mated with female 129SvJ mice, and germ line transmission was confirmed by PCR screening and direct sequencing.

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1 The abbreviations used are: PPARγ, peroxisome proliferator-activated receptor-γ; FFA, free fatty acid; TG, triglyceride; PPARI, PPARα L466A knock-in; oWAT, epididymal white adipose tissue; WT, wild type; SREBP, sterol regulatory element-binding protein; HF, high fat; KI, knock-in; 2-DG, deoxyglucose; FKO, fat-specific PPARγ knock-out; TNFα, tumor necrosis factor α; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyltransferase-mediated UTP end labeling; RT, reverse transcription.
Mice were bred to CMV-Cre transgenic mice (129SvJ background) to excise the neomycin cassette and were maintained on a 129SvJ background. The 3-bp difference between the L466A mutated allele (CCT) and the wild-type PPARα allele (AGC) was used to confirm genotype (Fig. 1B).

**Phenotypic Evaluation of Mice**—Mice were housed in a pathogen-free barrier facility, maintained on a 12-h light/dark cycle, and ad libitum access to chow (Harlan Teklad Laboratory, Madison, WI) that contained 8 kcal % from fat unless otherwise specified. All animal protocols were approved by the Institutional Animal Care and Use Committee of the Northwestern University Feinberg School of Medicine or Vanderbilt Medical School in accordance with National Institutes of Health guidelines. In some experiments, mice were fed a high fat diet that contained 30 kcal % from fat (Harlan Teklad) or 45 kcal % from fat (Research Diets Inc., New Brunswick, New Jersey). In these experiments, other mice were fed standard control diets, 10 kcal % from fat, provided by the same suppliers. Animals were weighed on a weekly basis. When the animals were fasted for glucose and insulin tolerance tests, the fast lasted 6–8 h and was typically performed between 8 a.m. and 2 p.m.

**Blood Collection and Serum Measurements**—Blood samples were obtained by either the tail-cut method for small samples (<50 μl) or by retro-orbital bleeding under light anesthesia (halothane). Serum was stored at −80 °C. Fasting (6–8 h) and fed blood glucose levels were measured using a glucometer (FreeStyle20, TheraSense, Alameda, CA). Serum PFA measurements were performed using a diagnostic kit (Roche Diagnostics). Enzyme-linked immunosorbenent assay kits were used to measure serum insulin (Linco Research, St. Charles, MO), adiponectin (Linco), and TNFα (Chemicon International, Inc., Temecula, CA).

**Tissue Histology, Morphology, Immunohistochemistry, and Cell Counting**—Tissues were fixed in either Bouin’s buffer (white adipose tissue) or 10% neutral buffered formalin (brown adipose tissue, liver) and paraffin-embedded. 5-μm sections were stained by hematoxylin/eosin. Immunohistochemical staining for adipophilin (guinea pig polyclonal, Research Diagnostics, Inc.), immunofluorescent staining for proliferating cellular nuclear antigen (PCNA; mouse monoclonal, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and terminal deoxynucleotidytransferase-mediated UTP end labeling (TUNEL, Roche Applied Science) were performed. Alternatively, tissues were directly embedded in Tissue-Tek® O.C.T. Compound (Sakura Finetek, Inc., Torrance, CA), and 8-μm frozen sections were stained by Oil red O. Digital images were obtained using a Zeiss Axioskop optical microscope (Carl Zeiss International) and an Optronics Magnafire SP digital camera (Goleta, CA). The measurements feature of the Image-Pro Express software program (Media Cybernetics, Inc., Silver Spring, MD) was used to count the number of cells and to determine cell surface areas in the digital images.

**Lipid Extraction and Triglyceride Measurement**—Mouse liver was homogenized in 1 M NaCl. Liver tissue homogenate (100 mg/500 μl) was extracted with 3 ml of chloroform/methanol (2:1) and 0.5 ml of 1 M NaCl. The organic phase was collected, dried, and resuspended in 0.5 ml of Triton X-100/methanol (2:1). Muscle triglycerides were extracted as described previously (24). Triglycerides were measured using the GPO-Trinder kit (Sigma).

**RNA Isolation and Real-time RT-PCR**—RNA was isolated from epididymal white adipose tissue (eWAT) was isolated using TRizol (Invitrogen). The isolated RNA was subjected to DNase treatment and reverse-transcribed, and 100 ng of cDNA was used as template in a 25-μl real-time PCR reaction using an ICycler iQ System (Bio-Rad). Primers and 5′-FAM labeled probes for real-time detection were designed using the Primer Express software (PerkinElmer Life Sciences), purchased from Integrated DNA Technologies (Coralia, IA). Primer/probe sets (Table 1) were designed for aP2, Acrp30, CD36, FAS, GLUT4, LPL, PPARγ, RPL19, SREBP1c, and TNFα (GenBank accession numbers K02109, AF304466, L23108, AF127033, AB008453, BC003305, U10374, M62952, AF374266, and M38296, respectively). Amplification of the ribosomal housekeeping gene RPL19 was used as a control, and cycle thresholds were corrected relative to RPL19 expression.

**Protein Isolation and Western Blotting**—Whole cell extracts were prepared from adipose tissue that had been removed from mice and immediately frozen in liquid N2. The adipose tissue was pulverized and

### Table 1

| Gene      | Primers                          | Probe                       |
|-----------|----------------------------------|-----------------------------|
| aP2       | 5′-CGATCTTGAGTCCAGGTGATTT-3′      | 5′-CCCTGTTTGAGTCCAGGTGATTT-3′|
| Acrp30    | 5′-CGAGCTTCGTCCTGCCAGACAG-3′      | 5′-CGACGATCGTCTGCCAGACAG-3′  |
| CD36      | 5′-CTGCTCCGTCCTGCCAGACAG-3′       | 5′-GGACTTCCGTCCTGCCAGACAG-3′ |
| FAS       | 5′-CTGCTCCGTCCTGCCAGACAG-3′       | 5′-GGACTTCCGTCCTGCCAGACAG-3′ |
| GLUT4     | 5′-ATGGTTCTCTCCCATGAACT-3′        | 5′-CATCAGCCAGATCGTCTGCCAG-3′ |
| LPL       | 5′-TACCTCTCTCCCATGAACT-3′         | 5′-CATCAGCCAGATCGTCTGCCAG-3′ |
| PPARγ     | 5′-GGGAGGAGTTCTCCCATGAACT-3′      | 5′-CATCAGCCAGATCGTCTGCCAG-3′ |
| RPL19     | 5′-ATGGTTCTCTCCCATGAACT-3′        | 5′-GATGCCCTACAAAGTGTTCCATTA-3′|
| SREBP1c   | 5′-ATGGTTCTCTCCCATGAACT-3′        | 5′-CTACACGAGTTCTCCCATGAACT-3′|
| TNFa      | 5′-CTACACGAGTTCTCCCATGAACT-3′     | 5′-CTACACGAGTTCTCCCATGAACT-3′|

**Fig. 1. Generation of PPARKI mice.** A, construct design and recombination schematic. 1, targeting construct to introduce L466A mutation into PPARγ exon 6. Note the change of 3 nucleotides to create a novel Eco47III restriction site. 2, wild-type PPARγ locus. 3, PPARγ after homologous recombination. 4, PPARKI after breeding PPARKI mice to CMV-Cre mice to remove neomycin resistance cassette (neo<sup>+</sup>). TK, thymidine kinase. B, confirmation of heterozygosity at PPARγ by DNA sequencing. C, embryonic lethality of PPARKI homozygotes. From left to right, wild-type (+/+), PPARKI homozygotes (K/K), and PPARKI heterozygotes (+/K) at embryonic day 10.5–11.5.
homogenized in lysis buffer, and the protein concentration was determined by the DC protein assay (Bio-Rad). The extracts were separated by 12% SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (Amersham Biosciences). Immunodetection was carried out using the following antibodies in 5% milk/Tris-buffered saline with 0.05% Tween 20: rabbit SREBP-1 polyclonal (1:200) (Santa Cruz Biotechnology), rabbit PPAR\textsuperscript{\gamma}/H9253 polyclonal (1:2000) (Abcam, Inc., Cambridge, MA), and goat anti-rabbit horseradish peroxidase-conjugated IgG (1:10,000) (Cell Signaling, Beverly, MA). Proteins were visualized with an ECL Plus detection kit (Amersham Biosciences) according to the manufacturer's instructions.

**Indirect Calorimetry**—Oxygen consumption (VO\textsubscript{2}) and the respiratory exchange ratio were measured by an Oxymax indirect calorimeter (Columbus Instruments, Columbus, OH) with an air flow of 0.75 liter/min as described previously (25). VO\textsubscript{2} is expressed as the volume of O\textsubscript{2} consumed per kilogram of body weight per h. After 1 h, to allow for adaptation to the metabolic chamber, VO\textsubscript{2} was measured, starting at 10:00 in individual mice for 1 min at 15-min intervals for a total of 22 h under a consistent environmental temperature (22 °C). The respiratory exchange ratio is the ratio of the volume of CO\textsubscript{2} produced to the volume of O\textsubscript{2} consumed.

**Tail Cuff Blood Pressure Measurements**—Systolic blood pressure was determined with the use of the Visitech Systems BP-2000 blood pressure monitoring system, an automated device for measuring systolic blood pressure by tail cuff, as described previously (26). Eleven-month-old male and female mice were subjected to tail cuff blood pressure measurements prior to catheterization for clamp studies. Mice were trained for 3 consecutive days; base line systolic pressure was measured and averaged over 5 days.

**Hyperinsulinemic-Euglycemic Clamp and 2-Deoxyglucose Uptake Measurements**—Clamp studies were performed in chronically catheterized conscious mice in the Mouse Metabolic Phenotyping Center at Vanderbilt University Medical Center. Male mice, 10–11 months of age, had two catheters surgically implanted into the right jugular vein and left carotid artery as described previously (27). Five days post-surgery, animals were fasted for 6 h, and hyperinsulinemic-euglycemic clamp experiments with \([3H]\)deoxyglucose (2-DG) tracer were performed as described previously (27). After basal sampling at time 0, insulin was continuously infused at 4 milliunits kg\textsuperscript{-1} min\textsuperscript{-1} for the duration of the 2-h study. Blood glucose levels were measured from 8 l of blood every 5–10 min using a HemoCue glucose analyzer (Lake Forest, CA). Glucose was infused at a variable rate to maintain clamped blood glucose levels at 120–130 mg/dl. Tracer was added at \(t = 120\) min and continued until \(t = 145\) min, at which time the animals were anesthetized with an intravenous infusion of pentobarbital sodium. Tissues (soleus, gastrocnemius, superficial vastus lateralis, adipose tissue, liver, diaphragm, and brain) were excised and rapidly frozen in liquid nitrogen for subsequent glucose uptake analysis. \(2[3H]DG\) removal from the blood and uptake by various tissues was determined as described previously (27).

**Statistical Analysis**—Data are presented as means ± S.E. Comparisons between two experimental groups were performed using the two-tailed, unpaired Student’s \(t\) test; analysis of variance was used for multivariate comparisons.

**RESULTS**

Heterozygous PPARKI mice were viable and fertile. RT-PCR of mRNA from adipose tissue was used to verify equivalent
expression of both PPARγ alleles. Routine breeding of heterozygotes failed to produce pups homozygous for the L466A allele. Timed matings of heterozygotes demonstrated that PPARγ L466A homozygotes die in utero by embryonic day 10.5 (Fig. 1C). The PPARγ L466A mutant has no inherent transcriptional activity (22). The lack of homozygote viability is consistent with the embryonic death seen in homozygous PPARγ null animals (9).

Histological analysis of PPARKI eWAT and brown adipose tissue revealed altered adipocyte morphology (Fig. 2A).
PPARKI eWAT contains a heterogeneous population of adipocytes with a predominance of small and medium sized cells, whereas eWAT in wild-type (WT) mice is composed of a nearly homogenous population of larger adipocytes. PPARKI brown adipocytes contain large multiloculated fat vacuoles that were not present in WT mice (Fig. 2A). The number of PPARKI eWAT adipocytes per high powered field (×400) is 2-fold greater than that of WT eWAT (Fig. 2B), suggesting that the decreased size of the adipocytes is the cause of an overall reduction in fat mass. To investigate whether there is altered cellular proliferation or apoptosis in PPARKI eWAT, immunofluorescent staining for PCNA and TUNEL was performed. PCNA positivity (5.3 ± 4.8 and 4.0 ± 1.8) and TUNEL staining (1.5 ± 1.2 and 2.8 ± 1.6; number of nuclei per 100 4,6-diamidino-2-phenylindole-labeled nuclei) were not different between PPARKI and WT eWAT, respectively.

Real-time RT-PCR analysis was performed to investigate whether there is altered expression of adipocyte-specific genes in eWAT. The expression of adiponectin (Acrp30), aP2, CD36, GLUT4, and SREBP-1c mRNAs in PPARKI mice was 25–50% in eWAT. The expression of adiponectin (Acrp30), aP2, CD36, GLUT4, and SREBP-1c mRNAs in PPARKI mice was 25–50% in eWAT. The expression of adiponectin (Acrp30), aP2, CD36, GLUT4, and SREBP-1c mRNAs in PPARKI mice was 25–50% in eWAT relative to WT eWAT (Fig. 2D). This finding suggests decreased activation of SREBP-1 in PPARKI adipose tissue. The levels of PPARγ protein expression did not differ between PPARKI and WT. We also performed Western blot analysis of insulin-signaling proteins in adipose tissue 10 min after an intraperitoneal insulin injection. No difference in the protein levels of IRβ, IRS-1, IRS-2, Akt, or phospho-Akt was found between PPARKI and WT mice (data not shown).

PPARKI mice had normal birth weights. Weight gain was similar for PPARKI and WT littermates for the first 3 months. However, from 3 months of age, PPARKI mice gained less weight on either a standard (10 kcal % from fat) or high fat (HF) diet (45 kcal % from fat) than WT mice (Fig. 3A). These mice had reduced eWAT depots, directly correlating with total body weight. To investigate the cause of abrogated weight gain in PPARKI mice, food intake and oxygen consumption were evaluated. No significant changes in food intake (Fig. 3B) or oxygen consumption were detected (Fig. 3C).

In the fed state, PPARKI mice had higher serum FFA levels on a standard diet than WT mice. Serum FFAs were elevated in WT mice on a HF diet, but no further elevation was observed in PPARKI mice (Fig. 3D). An intraperitoneal injection of insulin (0.75 units/kg) lowered serum FFAs in fasting male mice (7–8 months old) by 30% in both WT (preinjection: 0.77 ± 0.12 mM, postinjection: 0.54 ± 0.21 mM) and PPARKI (preinjection: 0.96 ± 0.16 mM, postinjection: 0.64 ± 0.28 mM) mice. Serum adiponectin levels in PPARKI mice were significantly lower (p < 0.001) than those in WT mice on either a standard or HF diet (Fig. 3E). No difference in serum TNFα was detected (Fig. 3F), although mRNA expression for TNFα was decreased in PPARγ knock-in (KI) mice (Fig. 4A). Histological examination of the livers of 5-month-old male PPARKI mice showed macrovesicular fatty changes in midzonal and centrilobular locations, indicating hepatic steatosis. Hepatic steatosis becomes apparent as early as 4 months of age. With a HF diet (for 3 months), macrovesicular lipid accumulation was observed in both genotypes. Additional microvesicular fatty changes were seen around the central vein in PPARKI livers (Fig. 4A). The lipid accumulation was confirmed by Oil red O staining and adipophilin immunohistochemistry (data not shown). Inflammatory infiltrates were also detected in some PPARKI livers (not shown). The hepatic TG content (22.1 ± 5.2 mg/g liver) of PPARKI mice was 1.8-fold greater than that (12.3 ± 4.9) of WT mice (Fig. 4B). Although the hepatic steatosis appeared to be exacerbated by a HF diet in PPARKI livers, the hepatic TG content (24.9 ± 6.8) increased by only 13% when compared with a standard diet. WT mice exhibited a 39% increase in hepatic TG content (17.1 ± 4.6) on a HF diet. Muscle TG content was also measured, but there was no difference between PPARKI and WT mice (data not shown).

Hyperinsulinemic-euglycemic clamp studies and 2-[1H]DG tracer studies were used to further investigate insulin action in PPARKI mice. Four groups of male mice were studied at 11 months of age, having been subjected to a standard diet (10 kcal % from fat) or a high fat diet (30 kcal % from fat) for 8
months. Basal glucose and insulin levels were comparable in PPARKI and WT mice on a standard diet (Fig. 5A). PPARKI mice on a standard diet exhibited no apparent impairment in 2-DG uptake and do not exhibit a further increase in serum insulin from being clamped. B, box plot demonstrating the glucose infusion rates required to maintain blood glucose at 120–130 mg/dl during clamp studies. C, uptake of 2-DG into soleus, gastrocnemius (gastroc), and vastus muscles after clamp is significantly compromised in PPARKI mice on a HF diet. Values are expressed as mean ± S.E.; *, p < 0.05.

DISCUSSION

In this study, we created a knock-in mouse model (PPARKI) containing a dominant negative PPARγ L466A mutation, shown previously to inhibit wild-type PPARγ action in vitro. The compromised PPARγ function in PPARKI mice leads to a lipodystrophy with decreased expression of adipogenic genes, resulting in high circulating FFAs, low circulating adiponectin, hepatic steatosis, and HF diet-induced insulin resistance. The PPARKI mouse model confirms the importance of PPARγ in adipose tissue maintenance and insulin-resistant states (28).

The heterogeneity and predominance of smaller sized adipocytes may reflect a combination of loss of function and the dominant negative activity of the PPARγL466A allele. Smaller adipocytes were also described in the heterozygous PPARγ-deficient mouse model, and these smaller adipocytes were associated with enhanced insulin sensitivity (10). The morphological changes in PPARKI mice appear to be more severe, but these models have not been compared directly. The adipocytes in PPARKI mice could be smaller because they are newly formed or because they do not fully differentiate. PCNA immunostaining and TUNEL analysis did not reveal significant remodeling in PPARKI adipose tissue. Impaired adipocyte differentiation and function are suggested by the finding of decreased transcription of PPARγ target genes including adiponectin and SREBP-1 and the presence of inactive precursor SREBP-1 in PPARKI adipocytes.

The reduced storage of fat in adipose tissue depots is associated with less weight gain in PPARKI mice on either a standard or HF diet. Reduced weight gain and lipodystrophy in the setting of comparable food intake suggest the possibility of increased metabolism in the PPARKI mice, but no difference was detected by indirect calorimetry. A metabolic effect may be small, or other mechanisms may contribute to the reduced weight gain.

PPARγ is involved in the uptake and storage of non-esterified fatty acids within adipocytes, and this mechanism has been suggested as a partial explanation for the insulin-sensitizing effects of PPARγ agonists (29). Consistent with this role for PPARγ, PPARKI mice have increased serum FFA levels. Basal lipolysis of eWAT was no different in WT and PPARKI mice (data not shown), suggesting defective uptake or trapping of FFAs by adipose tissue. The reduced ability to store fat in adipose tissue may lead to ectopic storage of lipid in PPARKI mouse liver as revealed by macrovesicular centrilobular fatty changes and elevated hepatic TG content. Notably, a HF diet did not further elevate the serum FFAs or hepatic TG content, although histological features were more pronounced (new microvesicular fatty changes and occasional inflammatory infiltration). The chronic burden of fat deposition may therefore be hepatotoxic in PPARKI mice.

The insulin-resistant state in the PPARKI mouse model (129Sv/J genetic background) is strikingly similar to that seen in a fat-specific PPARγ knock-out (FKOγ) model, which was studied on a genetic background (C57BL6) known to be relatively susceptible to insulin resistance (13). The FKOγ mice exhibit progressive lipoatrophy and compromised adipocyte function because their adipose depots are deficient in PPARγ. FKOγ mice have fatty liver and increased plasma FFAs and TGs in the basal state, but muscle insulin sensitivity appears to be spared. HF feeding provokes overt insulin resistance and impaired glucose uptake into muscle, presumably secondary to altered fat cell function or the direct effects of FFAs and TGs on

|            | Basal Fasting Glucose (mg/dL) | Basal Glucose (mg/dL) | Experimental Insulin (ng/mL) |
|------------|-----------------------------|-----------------------|-------------------------------|
| WT         | 120.8 ± 14.4                | 2.14 ± 0.88           | 4.59 ± 4.05                   |
| KI         | 101.0 ± 15.2                | 1.56 ± 0.60           | 3.77 ± 1.17                   |
| WT-HF      | 129.0 ± 11.9                | 1.49 ± 0.63           | 2.63 ± 1.19                   |
| KI-HF      | 117.3 ± 21.5                | 3.23 ± 0.94           | 3.36 ± 0.80                   |

Fig. 5. PPARKI mice are insulin-resistant on a HF diet. A, table of basal glucose and basal and experimental insulin values for the 11-month-old male mice subjected to hyperinsulinemic-euglycemic clamp studies. PPARKI mice on a HF diet are hyperinsulinemic in the basal state and do not exhibit a further increase in serum insulin from being clamped. B, box plot demonstrating the glucose infusion rates required to maintain blood glucose at 120–130 mg/dl during clamp studies. C, uptake of 2-DG into soleus, gastrocnemius (gastroc), and vastus muscles after clamp is significantly compromised in PPARKI mice on a HF diet. Values are expressed as mean ± S.E.; *, p < 0.05.

Tail cuff blood pressure (B.P.) measurements on 11-month-old mice demonstrate that female PPARKI mice are overtly hypertensive on either a standard or HF diet (Fig. 6A). However, male PPARKI did not have hypertension (Fig. 6B).
muscle (30). Both the PPARKI mouse and the FKO mouse lack severe insulin resistance in skeletal muscle under basal conditions. These findings suggest that compromised PPARγ function in the absence of HF feeding is not sufficient to induce insulin resistance.

Muscle-specific PPARγ knock-out models are also characterized by high levels of FFAs and low circulating levels of adiponectin, and they exhibit insulin resistance that is ameliorated by high levels of FFAs and low circulating levels of adiponectin (20, 21).

The role of PPARγ in the regulation of insulin sensitivity in humans (20, 21). The identification of dominant negative PPARγ mutations in families with severe insulin resistance provides compelling support for the role of PPARγ in the regulation of insulin sensitivity in humans (20, 21).

The human P467L PPARγ mutation destabilizes helix 12 and inhibits the action of wild-type PPARγ in a dominant negative manner. This behavior is analogous to the properties of dominant negative thyroid receptor β mutants associated with the syndrome of resistance to thyroid hormone (31). We have previously shown that the L466A mutation studied here exerts stronger dominant negative activity than the P465L mouse mutant (P467L in humans) (22). In contrast to the L466A mutant (PPARKI), a P465L dominant negative PPARγ knock-in mouse model does not exhibit features of the metabolic syndrome (32). Hypertension and adipose tissue redistribution were found in the P465L knock-in mouse, but it is neither insulin-resistant nor dyslipidemic. The lack of insulin resistance in the P465L mouse may reflect its milder dominant negative activity. Of note, the PPARKI mouse was studied on a 129Sv/J genetic background. As the C57BL6 strain is known to be lacking in insulin resistance, perhaps because female mice are relatively protected from insulin resistance and diabetes in many genetic strains (34). Further studies using ovariectomized female PPARKI mice will help to clarify the hormonal contribution to this gender-specific effect.

In summary, heterozygous PPARKI mice express a dominant negative PPARγ protein that impairs adipocyte development and function. The small immature adipocytes are associated with reduced weight gain and an altered metabolic profile in PPARKI mice. The chronic burden of high circulating FFAs is associated with hepatic steatosis, increased hepatic triglyceride content, and insulin resistance in PPARKI mice subjected to HF diet feeding. The PPARKI mouse provides an in vitro model to examine the relationship between impaired PPARγ function and features of the metabolic syndrome and may be useful to study the ability of new pharmaceuticals to treat this syndrome.

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