Metabolic Adaptations in the Absence of Perilipin

INCREASED β-OXIDATION AND DECREASED HEPATIC GLUCOSE PRODUCTION ASSOCIATED WITH PERIPHERAL INSULIN RESISTANCE BUT NORMAL GLUCOSE TOLERANCE IN PERILIPIN-NULL MICE

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Targeted disruption of the lipid droplet protein, perilipin, in mice leads to constitutional lipolysis associated with marked reduction in white adipose tissue as a result of unbridled lipolysis. To investigate the metabolic adaptations in response to the constitutive lipolysis, we studied perilipin-null (plin−/−) mice in terms of their fatty acid oxidation and glycerol and glucose metabolism homeostasis by using dynamic biochemical testing and clamp and tracer infusion methods. plin−/− mice showed increased β-oxidation in muscle, liver, and adipose tissue resulting from a coordinated regulation of the enzymes and proteins involved in β-oxidation. The increased β-oxidation helped remove the extra free fatty acids created by the constitutive lipolysis. An increase in the expression of the transcripts for uncoupling proteins-2 and -3 also accompanied this increase in fatty acid oxidation. Adult plin−/− mice had normal plasma glucose but a reduced basal hepatic glucose production (46% that of plin+/+). Insulin infusion during low dose hyperinsulinemic-euglycemic clamp further lowered the glucose production in plin−/− mice, but plin+/+ mice also showed a 36% decrease (p < 0.007) in glucose disposal rate during the low dose insulin clamp, indicating peripheral insulin resistance. However, compared with plin+/+ mice, 14-week-old plin−/− mice showed no significant difference in glucose disposal rate during the high dose hyperinsulinemic clamp, whereas 42-week-old plin−/− mice displayed significant insulin resistance on high dose hyperinsulinemic clamp. Despite increasing insulin resistance with age, plin−/− mice at different ages maintained a normal glucose response during an intraperitoneal glucose tolerance curve, being compensated by the increased β-oxidation and reduced hepatic glucose production. These experiments uncover the metabolic adaptations associated with the constitutional lipolysis in plin−/− mice that allowed the mice to continue to exhibit normal glucose tolerance in the presence of peripheral insulin resistance.

Obesity is a growing health problem with significant associated morbidity and mortality. Although obesity can result from environmental factors and a sedentary life-style, recent findings suggest that susceptibility to obesity is to a large extent genetically determined (1). One approach to study obesity and its associated morbidity is to investigate genes and pathways involved in the regulation of lipid metabolism and body fat deposition. A fat cell protein, perilipin, has recently been found to play a key role in determining body habitus as its absence leads to a lean and obesity-resistant phenotype in mice (2, 3). Perilipin belongs to a class of proteins found exclusively at the limiting surface of storage droplets in adipocytes and in steroi-dogenic cells (4–7). It coats the lipid droplets and protects triglycerides from the lipolytic action of hormone-sensitive lipase (6). Hormone-sensitive lipase catalyzes the breakdown of triacylglycerols and the release of free fatty acids and glycerol from adipose tissue to the circulation (8). The amount of hormone-sensitive lipase expressed in perilipin-null (plin−/−) mice appeared to be normal (2). Substantial evidence indicates that lipolysis is under the control of protein kinase A-mediated phosphorylation-dephosphorylation of perilipin in response to different lipolytic agents (9, 10).

Observations in plin−/− mice indicate that the absence of this lipid droplet protein is associated with a marked reduction in stored adipose mass, normal body weight despite increased food intake, increased muscle mass, and resistance to diet-induced and genetic obesity (2, 3). plin−/− mice have increased metabolic rate and oxygen consumption and constitutively activated basal lipolysis but attenuated stimulated lipolytic activity. We hypothesized that with the constitutively active lipolysis, there would be a compensatory increase in utilization of the released free fatty acids by increased tissue β-oxidation and that this would result in a switch from glucose utilization to fatty acid utilization for energy needs. This switch could then affect glucose tolerance in these mice and may underlie some of the reported glucose intolerance and hyperinsulinemia in plin−/− mice (3). To test this hypothesis, we studied the metabolism of plin−/− mice with special attention to the lipid and glucose handling in the liver and skeletal muscle by using tracer and euglycemic-hyperinsulinemic clamp techniques.

MATERIALS AND METHODS

Animals—plin−/− mice in C57BL/6J background at F6–F8 generation were generated and genotyped as described previously (2). plin−/− and plin+/+ mice, weighing 18–26 g, were used in this study unless otherwise mentioned. Wild-type (plin+/+) and homozygous knockout mice (plin−/−) were housed and bred in a pathogen-free barrier facility (Baylor College of Medicine). Animals were housed under controlled temperature and lighting (22–24 °C; 12-h light-dark cycle) with free access to food and water. All experiments were done following approval of the protocol by the animal care research committee of the Baylor College of Medicine.

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Glucose Tolerance Test and Insulin Tolerance Test—We performed intraperitoneal (IP) insulin tolerance test (ITT) and IP glucose tolerance test (GTT) at 8, 14, 28, and 42 weeks for male mice (weighing 20–26 g) and at 8, 14, 26, and 38 weeks for female mice (weighing 18–22 g). For GTT, we injected intraperitoneally 1.5 g of glucose/kg following a 12-h fast as described (2, 11). For ITT, we administered an intraperitoneal injection of regular insulin (1 unit/kg body weight) (Humulin R) to mice after an overnight fast. We measured blood glucose levels at different time points by using a glucometer (Life Scan, Milpitas, CA).

Insulin and Lactate Measurements—We measured insulin levels by using a mouse enzyme-linked immunosorbent assay kit from Crystal Chemical, Chicago, and circulating lactate by using a kit from Sigma following the manufacturers’ instructions.

Pyruvate Tolerance Test—Overnight fasted (16 h) mice were injected IP with pyruvate (2 g/kg), and the glucose level was measured before and after injection at different time points (12).

β-Oxidation of Soleus Muscle, Adipocytes, and Liver Homogenate—We isolated soleus muscle from the hind limb of 4-h fasted mice. We cleaned and weighed it and measured β-oxidation as described by Alam and Saggerson (13). In brief, each soleus muscle strip was incubated with oxygenated Krebs-Henseleit buffer at 37 °C containing 4% fatty acid-free bovine serum albumin complexed with [14C]palmitic acid (Amersham Biosciences) for 1 h. Reaction was started with 60% chloric acid, and the released CO2 was trapped by hyamine hydroxide at the top of a hanging center well, and the radioactivity in CO2 was digested with collagenase at 37 °C for 1 h. The primary adipocytes were then washed extensively and used for β-oxidation measurements in the absence and presence of insulin (4 μM). The rate of oxidation of palmitate was calculated from the DNA content of the isolated adipocytes. β-Oxidation in liver homogenate was measured as described previously (15). In brief, 10% liver homogenate was prepared in ice-cold homogenization buffer and filtered through a 70-μm nylon membrane. We incubated the homogenate in incubation buffer containing carnitine, NAD, ATP, cytochrome c oxidase, and one unit of enzyme A, and [14C]palmitic acid complexed with fatty acid-free bovine serum albumin for 1 h, trapped the CO2 released, and quantified the radioactivity as described above. In some samples, antimycin (5 μM) and rotenone (1 μM) were used to inhibit completely the β-oxidation in the homogenate.

Adipokine Measurements—We measured plasma leptin by using an enzyme-linked immunosorbent assay kit (Crystal Chemical, Mitsubishi, Minneapolis, MN) and resistin by using a radioimmunoassay kit (Amersham Biosciences) for 1 h. Reaction was stopped with 60% perchloric acid in some samples to maximally stimulate β-oxidation. To measure β-oxidation in adipose tissue, we isolated adipocytes from epididymal fat pads as described (14). Briefly, the fat pads were removed, minced, and digested with collagenase at 37 °C for 2 h. The primary adipocytes were then washed extensively and used for β-oxidation measurements in the absence and presence of insulin (4 μM). The rate of oxidation of palmitate was calculated from the DNA content of the isolated adipocytes. β-Oxidation in liver homogenate was measured as described previously (15).

Note: The abbreviations used are: IP, intraperitoneal; ITT, insulin tolerance test; GTT, glucose tolerance test; GCMS, gas chromatography-mass spectrometry; WAT, white adipose tissue; BAT, brown adipose tissue; ACC, acetyl-CoA carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; GPR, glucose production rate.

Isotope Tracers—We used the tracers [6,6-2H2]glucose (isotopic purity, 99 atom %) and [2-13C]glycerol (isotopic purity 99 atom %) purchased from Cambridge Laboratories. We dissolved them in normal saline solution and sterilized them by filtration just before use.

Metabolic Studies Using Stable Isotopes—We anesthetized mice with avertin and exposed the right jugular vein via a midline neck incision. We inserted a microcannula into the jugular vein, threaded it to the mouse, and anchored it to the skin with silk sutures percutaneously. The free end of the cannula was tunneled subcutaneously to the top of the head, where it was exteriorized through a skin incision. The mice were allowed to recover for 4 days with normal access to food and water. After an overnight fast, the awake, unstressed, and chronically catheterized mice received a prime (150 μmol/kg) constant rate infusion of [6,6-2H2]glucose and [2-13C]glycerol (2.5 μmol/min/kg) aiming at an isotopic enrichment of 1–2 ppm. We collected blood samples via tail vein before infusion and at hourly intervals during infusion, immediately separating and freezing the plasma for analysis later.

Sample Preparation—We used cold acetone to treat the plasma sample to precipitate the protein. We prepared the penta-acetate derivative of glucose and triacetate derivative of glycerol by using pyridine and acetic anhydride (18, 19). Isotopic enrichments were measured by gas chromatography-mass spectrometry as described in detail below.

High Dose and Low Dose Hyperinsulinemic-Euglycemic Clamp Studies—We anesthetized and cannulated the mice as described above. Animals were allowed to recover for 3–4 days. We then administered to overnight-fasted, conscious mice a primed infusion (10 μCi) and then a constant rate intravenous infusion (0.1 μCi/min) of high pressure liquid chromatography-purified [6,6-2H2]glucose (PerkinElmer Life Sciences), using a syringe infusion pump (KD Scientific). For determination of basal glucose production, we collected blood samples from the tail vein after 50, 55, and 60 min of labeled glucose infusion. After 60 min, mice were primed with regular insulin (bolus 40 milliunits/kg body weight) followed by a 2-h insulin infusion (3 milliunits/kg/min). Simultaneously, we infused 10% glucose using another infusion pump at a rate adjusted to maintain the blood glucose level at 100–140 mg/dl (low dose hyperinsulinemic-euglycemic clamp). Blood glucose concentration was determined every 10 min by a glucometer (LifeScan, NJ). At the end of a 120-min period, we collected blood (100, 110, and 120 min) to measure the hepatic glucose production and peripheral glucose disposal rates (20, 21). A high dose hyperinsulinemic-euglycemic clamp was then started using 10 milliunits/kg/min insulin infusion and continued for 120 min to measure in vivo glucose utilization according to the method of Fujita et al. (22) with slight modification. We monitored the plasma glucose level every 3 min. At the end of the 120 min, we collected blood to measure the glucose disposal rate (20, 21) and calculated the total body glucose infusion rate.

Statistical Methods—Plasma was separated and deproteinized using equal volumes of barium hydroxide and zinc sulfate, dried to remove H2O, resuspended in water, and counted in scintillation fluid using a liquid scintillation counter (Beckman Instruments, Palo Alto, CA). We determined insulin concentrations by using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chemical Inc., Chicago) and plasma glucose by GPO trinder kit (Sigma). We calculated whole body glucose and basal glucose production (mg/kg/min) by dividing the [3-3H]glucose infusion rate by the plasma glucose-specific activity corrected to the body weight. During the clamp, hepatic glucose production was calculated as the difference between the tracer-derived rate of glucose appearance and the infusion rate of glucose.

GCMS Measurements—[6,6-2H2]glucose and [2-13C]glycerol were analyzed by gas chromatography-mass spectrometry (GCMS) with an HP5890 (GC)/5989B (MS) (Agilent Technologies, Palo Alto, CA). The GC column used was medium polar SPB 17, 30 m × 0.25 mm × 0.25 μm (Supelco, Bellefonte, PA). The positive chemical ionization mode with methane as reaction gas was applied with selective monitoring of m/z 159 corresponding to unlabeled glycerol, m/z 160 corresponding to [1-13C]glycerol. For [2-13C]glycerol, the m/z 160 monitoring mode was used with selected monitoring of m/z 331–333, reflecting unlabeled glycerol (m/z 331), single labeled glucose derived from [2-13C]glycerol (m/z 332), and double labeled glucose derived from [6,6-2H2]glucose (m/z 333) (23). To correct for possible errors in the GCMS measures, we prepared two different standard curves that were analyzed with the samples. These standard curves were prepared by mixing unlabeled glucose and glycerol, respectively, with [6,6-2H2]glucose (mole ratio 0–6%) and [2-13C]glycerol (mole ratio 0–20%), respectively.

Calculations for Tracer Studies—Total plasma glucose appearance rates were calculated from 6,6-2H1, enrichment of glucose during steady states using established isotope dilution Equation 1 (24, 25),
and rotenone (\[2-13\text{C}\]glycerol tracer) is an indicator of lipolysis. In addition, the fraction of glycerol enrichment in plasma glucose reflected by the incorporation of glycerol into glucose was calculated as shown in Equation 3, where \(E_{\text{p}}\) is the isotopic enrichment of the [2-\text{\textsuperscript{13}C}]glycerol tracer, \(E_{\text{p}}\) is the isotopic enrichment of [2-\text{\textsuperscript{13}C}]glycerol in plasma, \(I\) is the infusion rate of [6,6-\text{\textsuperscript{13}H}]glucose (\(\mu\text{mol/kg/min}\)) and glyceraldehyde 3-phosphate dehydrogenase (GPDH) was glucose appearance rate. Under steady state conditions, glucose appearance rate = glucose disappearance (utilization) rate (Equation 2).

\[
\text{GPR} = \text{total glucose} \times \frac{(E_{\text{p}}/E_{\text{p}})}{I} \quad \text{(Eq. 1)}
\]

Total glyceraldehyde 3-phosphate, was calculated as shown in Equation 3,

\[
\frac{(E_{\text{p}}/E_{\text{p}})}{I} \times \text{glycerol flux to glucose} = \frac{(\text{\textsuperscript{13}C} \text{ enrichment in glucose})}{(\text{\textsuperscript{13}C} \text{ enrichment in glycerol})} \times \text{glycerol } \text{R}_{\text{a}} \text{ (Eq. 4)}
\]

The reason for multiplication by \(\frac{1}{2}\) is that glucose is a 6-carbon unit, whereas glyceraldehyde 3-phosphate is a 3-carbon unit. The fraction of glyceraldehyde 3-phosphate converted to glucose = glyceraldehyde 3-phosphate/fructose-1,6-bisphosphate.

Glucose Uptake by Isolated Soleus Muscles—We measured glucose uptake in isolated soleus muscle as described (28) with little modification. Briefly, we isolated soleus from overnight fasted mice and first incubated it for 1 h at 37 °C in KRB buffer (NaCl (118.5 mm), KCl (4.7 mm), CaCl\(_2\) (2.4 mm), MgCl\(_2\) (1.3 mm), NaHCO\(_3\) (25 mm), and NaH\(_2\)PO\(_4\) (2 mm)) supplemented with 8 mm glucose or 2-deoxyglucose, 8 mm mannitol, and 0.1% albumin. All buffers were continuously gassed with 95% O\(_2\), 5% CO\(_2\). At the end of preincubation, we transferred the muscle to fresh KRB containing 8 mm glucose and 0 or 1000 micromol/ml insulin and continued incubation for 20 min at 37 °C. The muscle was then rinsed in KRB containing 16 mm mannitol at 30 °C for 10 min. We then measured glucose uptake in 2 ml of KRB containing 8 mm 2-deoxyglucose and 2 μCi/ml 2-deoxy-O-[\text{\textsuperscript{13}C}]glucose and 8 mm mannitol with 0.3 μCi/ml 1,2-[\text{\textsuperscript{3}H}]glucose and 8 mm mannitol in the presence of insulin (0 or 1000 micromol/ml) at 30 °C for 15 min. The muscle was then blotted on filter paper and immediately frozen in liquid nitrogen until analysis of 2-deoxyglucose uptake was performed as described (29).

RESULTS

\textit{plin}\(^{-/-}\) mice are lean due to constitutional lipolysis. However, their plasma-free fatty acid level is not significantly different from that of wild-type littermates (2), which suggests that the free fatty acids produced must be utilized at an accelerated rate in \textit{plin}\(^{-/-}\) mice compared with \textit{plin}\(^{+/+}\) controls. To test this hypothesis, we measured various parameters related to β-oxidation in the skeletal muscle, adipocyte and liver of these mice.

Increased β-Oxidation in Isolated Soleus Muscle, Adipocyte and Liver Homogenates of \textit{plin}\(^{-/-}\) Mice—We measured the rate of β-oxidation in isolated soleus muscle (predominantly composed of oxidative red fibers) and found that it was 30% higher (\(p < 0.001\)) in \textit{plin}\(^{-/-}\) mice compared with \textit{plin}\(^{+/+}\) littermates (Fig. 1a). There was no difference in the rate of glutamine synthesis between \textit{plin}\(^{-/-}\) and \textit{plin}\(^{+/+}\) mice when the soleus muscle was maximally stimulated by incubation with a catecholamine (epinephrine). These findings indicate that the constitutionally increased basal lipolysis in \textit{plin}\(^{-/-}\) mice is associated with an increase in fatty acid oxidation in skeletal muscle in the basal state.

In addition to the soleus muscle, \textit{plin}\(^{-/-}\) mice also show a similar adaptive change in the liver. When we measured the rate of β-oxidation in liver homogenates, we found it to be ~60% higher in \textit{plin}\(^{-/-}\) mice (\(p < 0.02\)) compared with that of \textit{plin}\(^{+/+}\) controls (Fig. 1b). We next measured the rate of β-oxidation of the liver homogenate in the presence of rotenone and antimycin, inhibitors of complex I and complex III of the electron transport chain, respectively. We found a complete inhibition of β-oxidation of liver homogenates isolated from \textit{plin}\(^{-/-}\) and \textit{plin}\(^{+/+}\) mice in the presence of these inhibitors, indicating that although the \textit{plin}\(^{-/-}\) mice exhibit an increased basal β-oxidation, the process was still under the control of the electron transport chain as it exists in wild-type mice. We also measured β-oxidation in isolated adipocytes from epididymal white fat. We found a 75% higher rate of β-oxidation per adipocyte isolated from \textit{plin}\(^{-/-}\) mice compared with wild-type mice (\(p < 0.0002\)) (Fig. 1c). As expected, insulin inhibited the rate of β-oxidation in both \textit{plin}\(^{-/-}\) and wild-type mice, but more so in the \textit{plin}\(^{-/-}\) group.

Increased Expression of Oxidation Enzyme-related Genes in \textit{plin}\(^{-/-}\) Mice—To examine the mechanism of the increased β-oxidation in \textit{plin}\(^{-/-}\) mice, we studied the mRNA expression of enzymes involved in β-oxidation and its regulation. The rate-limiting step in the β-oxidation of fatty acids is the entry of fatty acyl-CoA into the mitochondria via the carnitine acyltransferase/translocase shuttle located in the mitochondrial membrane. Carnitine palmitoyltransferase-1 (CPT-1) is a major enzyme involved in this transport. In \textit{plin}\(^{-/-}\) mice expression of CPT-1 transcript is markedly and significantly upregulated in white adipose tissue (WAT), brown adipose tissue (BAT), and liver and tended to be higher in skeletal muscle and heart (Fig. 2a). The key allosteric inhibitor of CPT-1 activity is malonyl-CoA, which is synthesized from acetyl-CoA by acetyl-CoA carboxylases (ACC). ACC2 mRNA expression was significantly down-regulated in WAT, BAT, liver and skeletal muscle, and ACC1 mRNA, in WAT, BAT, and skeletal muscle of
plin−/− mouse (Fig. 2a). This down-regulation would be expected to result in a decrease in malonyl-CoA, relieving the inhibition of the rate-limiting transport of fatty acyl-CoA into the mitochondria. Indeed, Acc2−/− mice also exhibit a similar expected increase in the rate of β-oxidation and a decreased body fat phenotype (30). Among several different dehydrogenases that participate in the complete degradation of fatty acids in mitochondria (for a review see Ref. 31), medium chain acyl-CoA dehydrogenase (MCAD), long chain acyl-CoA dehydrogenase (LCAD), and medium chain acyl-CoA dehydrogenase (MCAD), dehydrogenase enzymes involved in the degradation of fatty acids based on their number of carbon atom present in the fatty acid. c, Northern blot analysis of β-thiolase and 3-hydroxyacyl-CoA dehydrogenase (LCHAD), enzymes directly involved in β-oxidation. d, Northern blot of uncoupling protein mRNAs. WT, wild type; KO, knockout.

To determine whether adipocyte factors may play a role in the metabolic adaptations, we measured fasting plasma leptin, adiponectin, and resistin levels in plin−/− and wild-type mice. We found that plasma leptin level tended to be lower in plin−/− mice (2.26 ± 0.38 ng/ml in plin−/− versus 3.64 ± 0.58 ng/ml in plin+/+ mice), but the difference was not significant (p > 0.05). Relative plasma adiponectin level determined by Western blotting was increased (264 ± 15%) in plin−/− mice relative to that in control (100 ± 23%) plin+/+ mice (p < 0.01). Plasma resistin was also slightly but significantly increased in plin−/− mice (7.56 ± 0.24 ng/ml in plin−/− versus 6.84 ± 0.21 ng/ml in plin+/+ mice, p < 0.03).

Perilipin Ablation Affects Overall Glucose Homeostasis in plin−/− Mice—We next turned our attention to addressing whether the change in energy substrate metabolism impacted the glucose homeostasis of the plin−/− mice. We performed IP-GTT on plin−/− mice and their plin+/+ littermates at different ages. GTT curves were indistinguishable between plin−/− and plin+/+ mice in 8- (data not shown) and 14-week-old mice in terms of both blood glucose and plasma insulin response (Fig. 3, a and b). Subsequently, at 28 weeks and again 42 weeks, there was a significant increase in plasma insulin both at base line and at various times after the glucose challenge, indicating the presence of increased insulin resistance in plin−/− mice compared with plin+/+ controls as they age (Fig. 3, c and d). Most interestingly, despite the appearance of insulin resistance with age, the plasma glucose response remained completely normal in plin−/− mice (Fig. 3, e and f).

To examine further the role of insulin resistance in glucose homeostasis in these mice, we performed IP-ITT on plin−/− mice to assess their whole body insulin sensitivity and hepatic glucose production, which contribute to the recovery from the insulin-induced hypoglycemia. There was no significant difference in the degree or rate of the hypoglycemic response in male and female between the plin−/− and plin+/+ littermates at 14 weeks (Fig. 4, a and b), suggesting the absence of significant insulin resistance at this age. Most interestingly, there was a slight delay in the recovery of plasma glucose after 120 min, which suggests a possible reduced glucose production in plin−/− mice. As these mice aged, at 28 weeks and again at 42

FIG. 2. Northern blots of proteins and enzymes involved in energy and fatty acid metabolism of WAT, BAT, liver, muscle, and heart of plin+/+ and plin−/− mice. We fasted 14-week-old mice for 4 h and extracted total RNA from different tissues (see "Materials and Methods"). Total RNA (20 μg) was subjected to formaldehyde/agarose gel electrophoresis and blotted. 28 S RNA staining of individual RNA samples is shown. Representative blots are shown. Blots were performed on three or more mice per tissue with similar results. a, Northern blot of CPT-1, ACC1, and ACC2. b, Northern blot of very long chain acyl-CoA dehydrogenase (VLCAD); long chain acyl-CoA dehydrogenase (LCAD), and medium chain acyl-CoA dehydrogenase (MCAD), dehydrogenase enzymes involved in the degradation of fatty acids based on their number of carbon atom present in the fatty acid. c, Northern blot analysis of β-thiolase and 3-hydroxyacyl-CoA dehydrogenase (LCHAD), enzymes directly involved in β-oxidation. d, Northern blot of uncoupling protein mRNAs. WT, wild type; KO, knockout.
weeks, the plasma glucose concentration in response to ITT became grossly abnormal in plin:\(-/-\) mice. The plasma glucose level of these mice was resistant to the hypoglycemic action of IP insulin appearing within 15 min of treatment and lasting throughout the 120-min period (Fig. 4, c and d). Therefore, despite the presence of normal blood glucose concentration at baseline and after a glucose load, plin:\(-/-\) mice developed evidence of insulin resistance with age.

Glycerol Homeostasis in plin:\(-/-\) Mice—WAT isolated from plin:\(-/-\) mice displayed constitutional lipolysis (2, 3), and the lipolytic product glycerol is a source of substrate for gluconeogenesis. However, the total amount of WAT contributing to lipolysis is markedly reduced as plin:\(-/-\) mice are very lean (2, 3). We examined glycerol homeostasis in vivo by infusion of \([2-^{13}C]\)glycerol in fasting plin:\(-/-\) and plin:\(+/+\) mice. The infusion led to a 2.74-fold increase in the \(2-^{13}C\) isotopic enrichment of glycerol in plin:\(-/-\) mice compared with plin:\(+/+\) littermates (Fig. 5a); there were no differences in plasma glycerol level before and after \([2-^{13}C]\)glycerol infusion. From the enrichment and infusion rates we calculated the endogenous glycerol \(R_e\) (see “Materials and Methods”), which is an indicator of the rate of lipolysis. We found that the \(in\ vivo\) glycerol \(R_e\) was 22.5 ± 2.6 \(\mu\)mol/kg/min in plin:\(-/-\) mice and 67.01 ± 1.36 \(\mu\)mol/kg/min in plin:\(+/+\) littermate controls. Therefore, total body lipolysis of plin:\(-/-\) mice was 3-fold lower than that in plin:\(+/+\) mice. This is interesting as isolated WAT of plin:\(-/-\) mice was shown to have elevated basal lipolysis compared with WAT of plin:\(+/+\) mice (2, 3). However, the markedly reduced mass of WAT in plin:\(-/-\) mice compared with plin:\(+/+\) mice could provide an explanation for the low total body lipolysis in plin:\(-/-\) mice. We isolated and weighed the epididymal fat pad of 14-week-old mice in which we performed the stable isotope experiments, as this fat depot is the most discrete WAT that can be cleanly isolated and accurately weighed. The fat pad weighed 4.68 ± 0.08 mg/g in plin:\(-/-\) mice compared with 22.65 ± 2.9 mg/g in plin:\(+/+\) mice, i.e. the mass of plin:\(-/-\) WAT was 4.8-fold higher than that of plin:\(-/-\) WAT. Therefore, in the face of a marked (4.8-fold) reduction in WAT mass, the less substantial (3-fold) reduction in lipolysis is consistent with an increased rate of lipolysis per unit of WAT mass in plin:\(-/-\) mice.

As the amount of glycerol produced by plin:\(-/-\) mice during steady state is reduced compared with plin:\(+/+\) mice, we calculated the amount of glycerol converted to glucose in the liver in the two types of animals (see “Materials and Methods”). We found that the amount of glucose derived from glycerol was 5–6-fold lower in plin:\(-/-\) mice compared with plin:\(+/+\) littermates (Fig. 5b), indicating that the reduced total body produc-
Basal glucose production was significantly lower in 14-week-old mice, respectively. Results are expressed as the means ± S.D. (n = 4 of each genotype). c, mass isotopomer enrichment in glucose after a 5-h infusion [6,6-2H2]glucose. Glucose infusions were measured at 0–5 h to check the steady state. Steady state was reached at 3 h. d, GPR. Whole body basal glucose production rate was measured after 5 h of continuous infusion [6,6-2H2]glucose for plin−/− and plin+/+ mice. Basal glucose production was significantly lower in plin−/− mice than in plin+/+ mice; *, p < 0.0008. Results are expressed as mean ± S.D. (n = 4 of each genotype), pyruvate tolerance test. e and f, blood glucose at different time points after injection of pyruvate in 14- and 42-week-old overnight fasted mice. Values (mean ± S.D.) are plotted in percent of basal glucose value in five mice/group. p values are as follows: *, p < 0.01; **, p < 0.002; ***, p < 0.009; ****, p < 0.001; ******, p < 0.03, respectively.

Reduction Glucose Production and Gluconeogenesis in plin−/− Mice—In addition to quantifying glucose production from glycerol, we measured total body glucose production in plin−/− and plin+/+ mice by infusing [6,6-2H2]glycerol in these animals (see "Materials and Methods"). Under steady state conditions, isotopic enrichment of 2H2 in plasma was 60% higher in plin−/− mice (Fig. 5c), amounting to a 42% lower in vivo glucose production (GPR (mg/kg/min), 18.7 ± 3.7 and 31.43 ± 2.51 in plin−/− and plin+/+ mice, respectively, p < 0.0008) in plin−/− compared with plin+/+ mice (Fig. 5d). Plasma lactate level was lower in plin−/− mice than in plin+/+ mice (11.6 ± 1.2 mg/dl in plin−/− mice versus 13.2 ± 1.8 mg/dl in plin+/+ mice, p > 0.05), although the difference was not significant. To analyze gluconeogenesis further in plin+/+ and plin−/− mice, we examined the effect of pyruvate treatment on glucose homeostasis in these mice (33). Injection of the gluconeogenic substrate pyruvate in 14- and 42-week-old mice after an overnight fast led to a significantly higher plasma glucose level in both groups of mice, but the plin+/+ mice displayed a greater increase in plasma glucose levels than plin−/− mice (Fig. 5, e and f), and the effect was more pronounced in the older (42-week) than the younger (14-week) animals, suggesting that perilipin ablation impairs gluconeogenesis, which became more severe with age.

To dissect further some of the factors that contribute to normal plasma glucose levels in the presence of insulin resistance in plin−/− mice, we performed euglycemic-hyperinsulinemic clamp experiments, and we measured the glucose production rate using [3-3H]glucose infusion (see "Materials and Methods") in plin−/− and plin+/+ mice at 14 weeks and again at 42 weeks of age. This approach also allowed us to estimate whole body glucose disposal from residual glucose appearance during clamp, a reflection of peripheral insulin resistance. Whole body glucose uptake under hyperinsulinemic conditions is a measure of muscle insulin sensitivity as skeletal muscle contributes to most of the insulin-stimulated glucose uptake (34).

Fourteen-week-old plin−/− mice displayed a significant reduction (p < 0.008) in basal hepatic glucose production rate (Fig. 6a) in comparison with plin+/+ mice (GPR (mg/kg/min), 19.4 ± 3.5 and 35.39 ± 1.54 in plin−/− and plin+/+ mice, respectively). Both plin−/− and plin+/+ mice had suppressed glucose production during the low dose insulin clamp (Fig. 6b), with the rate remaining lower after insulin treatment in the plin−/− animals. plin−/− mice showed a 36% decrease (p < 0.007) in glucose disposal rate during the low dose insulin clamp (Fig. 6c). In the high dose clamp study glucose disposal and infusion rates were not significantly different (p = 0.31 and p = 0.14) between plin−/− and plin+/+ mice (Fig. 6, d and e). These results indicate that plin−/− mice have a mild peripheral insulin resistance as evidenced by the decreased glucose.
disposal in the low dose but not the high dose insulin clamp conditions.

We repeated the above measurements in 42-week-old mice, and we observed similar differences between plin-/- and plin+/- mice in basal glucose production rate, as well as in glucose production rate and glucose disposal rate under low dose hyperinsulineic clamp conditions (Fig. 7, a–c) as we had detected in the 14-week-old mice. The data for the high dose hyperinsulineic clamp were exaggerated in the older mice compared with the younger (14-week) animals such that the 42-week-old plin-/- mice displayed a significantly lower glucose disposal rate (Fig. 7d) and lower glucose infusion rate (Fig. 7e), compared with plin+/- littermates, corroborating the observed hyperinsulinaemia and deteriorating insulin resistance that was also evident during GTT and ITT in aging mice that lacked perilipin. Moreover, at 42 weeks high dose insulin suppressed hepatic glucose production completely in plin+/- mice, but it had only a small effect on plin-/- mice, which indicates that 42-week-old plin-/- mice are insulin-resistant.

To investigate further the mechanism of the low hepatic glucose production, we used Northern blot analysis to examine mRNA expression of various enzymes involved in hepatic gluconeogenesis. Phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase, fructose-1,6-diphosphatase, and pyruvate carboxylase are key enzymes that regulate hepatic gluconeogenesis. Among these enzymes, we found that the steady state levels of the mRNAs for PEPCK and fructose-1,6-diphosphatase in the liver were lower in plin-/- mice compared with those in plin+/- littermates (Fig. 7f). Thus, down-regulation of the expression of the two gluconeogenic enzymes contributed to the reduced hepatic glucose production in plin-/- mice.

To confirm peripheral insulin resistance, we measured in vitro glucose uptake by soleus muscle isolated from plin+/- and plin-/- mice. Basal glucose uptake was not different in the two genotypes at 14 weeks (1.61 ± 0.22 μmol/g/h for plin-/- mice versus 1.81 ± 0.15 μmol/g/h for plin+/- mice); insulin treatment increased glucose uptake in both genotypes, but the increase was moderately less in plin-/- mice (14.04 ± 0.32 μmol/g/h in plin-/- mice versus 16.9 ± 0.55 μmol/g/h in plin+/- mice after insulin treatment, p < 0.01) (Fig. 8a) consistent with the data from the hyperinsulineic euglycemic clamp experiment. We also found that there was also no difference in basal glucose uptake in the 42-week-old mice of both genotypes (1.52 ± 0.63 μmol/g/h for plin-/- mice versus 2.7 ± 0.36 μmol/g/h for plin+/- mice); however, with insulin treatment, glucose uptake was stimulated in both groups of 42-week-old mice, but the insulin-stimulated glucose uptake was more than 60% less in plin-/- mice (8.056 ± 0.43 μmol/g/h in plin-/- mice versus 19.8 ± 0.57 μmol/g/h in plin+/- mice after insulin treatment, p < 0.002). Moreover, insulin-stimulated increase in glucose uptake was essentially more in 42-week-old plin+/- mice in comparison with 14-week-old plin+/- mice,

Fig. 7. Effect of low dose and high dose hyperinsulineic-euglycemic clamp on glucose production, uptake, and disposal rate, calculated based on [3-3H]glucose infusion. The experiments were performed in conscious 42-week-old male plin-/- and plin+/- mice (see "Materials and Methods"). In 42-week-old mice, a and b, glucose production rate (GPR) at basal and low dose insulin clamp; c and d, rate of glucose disposal (GDR) in plin-/- and plin+/- mice at low and high dose insulin clamp. GPR were low and significantly (*, p < 0.03) different at basal (a) but not in low dose clamp. GDR was significantly different in high dose clamp; **, p < 0.002 (d) but not in low dose clamp (c). Glucose infusion rate (GIR) (e) and glucose production rate (f) in high dose clamp also showed significant difference; ***, p < 0.001, and ****, p < 0.0001, respectively, during high dose clamp. All values are expressed as mean ± S.D. (n = 5). Northern blot of gluconeogenic enzymes (g), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (GP), fructose-1,6-diphosphatase (FDP), and pyruvate carboxykinase (PC); 28 S RNA staining is presented at the bottom. Blots were from 20 μg of total liver RNA. A representative blot is shown. Similar results were obtained from three or more mice per genotype. Densitometric scan showed that only PEPCK expression decreased significantly in plin+/- mice, PEPCK (knockout (KO), 34.5 ± 7.4; wild type (WT), 67.95 ± 11; p < 0.04), glucose-6-phosphatase (GP) (knockout, 59.34 ± 23.9; wild type, 65.91 ± 26.7); fructose-1,6-diphosphatase (FDP) (knockout, 65.91 ± 26.7; wild type, 40.5 ± 11.3); pyruvate carboxylase (PC) (knockout, 50.19 ± 16.4; wild type, 56.41 ± 9.6).
whereas the insulin-stimulated increase in glucose uptake was less in 42-week-old plin−/− mice in comparison with 14-week-old plin−/− mice (Fig. 8, a and b). This difference in insulin-stimulated glucose uptake between plin−/− and plin+/+ mice accounts for the substantially lower glucose disposal rate in plin−/− mice after insulin treatment (Fig. 7d).

DISCUSSION

plin−/− mice produced from two independent laboratories were found to be lean, with markedly reduced WAT that exhibited activated basal lipolysis (2, 3). These mice had increased oxygen consumption and were resistant to diet-induced obesity. In this study we examined the basis for the increased oxygen consumption and energy dissipation. We found that ACC expression was reduced in plin−/− mice; reduction in ACC2 expression would down-regulate mitochondrial malonyl-CoA, releasing its inhibition of CPT-1 activity, thereby stimulating β-oxidation. The increased plasma adiponectin level could be one factor that stimulated β-oxidation (35, 36). We also found an increase in the mRNA level of a number of enzymes involved in the β-oxidation pathway in the plin−/− mice (Fig. 2). Furthermore, there was also increased expression of UCP2 and UCP3 mRNA in different tissues of plin−/− mice (Fig. 2d), which could partly account for the increased energy dissipation via uncoupling in oxidative phosphorylation. The occurrence of these adaptive changes in energy metabolism in tissues that do not normally express perilipin indicates the importance of cross-talks between different tissues that have been documented repeatedly (37–39).

Increased β-oxidation is often associated with increased insulin sensitivity (40, 41). In the plin−/− mice, there is increased β-oxidation but no major change in insulin sensitivity when the animals are young. However, with time, despite the persistence of increased β-oxidation, they acquire insulin resistance as these mice age. Loss of body fat has been found to be associated with insulin resistance in other mouse models, leading to glucose intolerance when compensatory mechanisms fail (42–44). plin−/− mice appear to also develop the same metabolic perturbation as they age, although they continue to maintain normal plasma glucose. Both plin+/+ and plin−/− mice have normal insulin sensitivity at the young ages of 8 (data not shown) and 14-weeks by GTT and ITT (Fig. 4). However, experiments using low dose insulin clamp revealed significant insulin resistance in the 14-week-old plin−/− mice compared with plin+/+ mice of the same age (Fig. 6c). As the animals age further, they develop deterioration in their insulin resistance, as evidenced by increased plasma insulin during GTT and impaired glucose response during ITT. Most interestingly, plin−/− mice maintain a normal blood glucose response during GTT throughout the study period (at least up to 42 weeks, Fig. 3). In contrast, Tansey et al. (3) found that plin−/− mice develop elevated blood glucose response during GTT at 14 weeks of age. The difference in phenotype may be related to the difference in genetic background of the animals examined.

Tansey et al. (3) studied mice that were F2 intercrosses of 129/SvEv Tac and C57BL/6J, whereas we used in our analysis mice that were F6–F8 C57BL/6J backcrosses.

One mechanism by which the plin−/− mice kept their blood glucose response normal despite the appearance of insulin resistance, as evidenced by a decreased insulin-induced glucose uptake in skeletal muscle, was via a reduction in hepatic gluconeogenesis, as documented by stable isotope infusion (Fig. 5) as well as hyperinsulinemic-euglycemic clamp experiments (Figs. 6 and 7). Most interestingly, the plasma resistin level in the plin−/− mice was increased compared with the plin+/+ animals. Resistin has been reported to regulate fasting blood glucose levels in mice (33), and the mild increase in resistin levels could be part of a compensatory mechanism in regulating glucose homeostasis in these animals. Adiponectin is another adipokine whose plasma level is stimulated with loss of perilipin, which could represent another compensatory response mounted by the affected animals in an attempt to overcome the insulin resistance associated with plin−/− mice.

Both we (2) and Tansey et al. (3) showed that WAT isolated from plin−/− mice display up-regulated basal lipolysis. However, despite the increased lipolysis per unit of adipose mass, these mice were very lean, and the markedly reduced adipose mass was reflected by a significantly lower total body lipolysis as measured by isotopic glycerol infusion analysis (Fig. 5a). There was thus a reduced gluconeogenic substrate flux as the glycerol-derived gluconeogenesis was reduced substantially. In addition, we found that injection of the gluconeogenic substrate pyruvate into plin−/− mice also led to a lower glucose production in these animals compared with wild-type animals, indicating the impairment of steps downstream of pyruvate in the gluconeogenic pathway. At least part of this impairment is caused by the down-regulation of two key enzymes, PEPCK and fructose-1,6-diphosphatase, in the pathway.

In conclusion, we show that the ablation of perilipin leads to an increase in fatty acid oxidation and mild insulin resistance. However, we also observed a decreased hepatic glucose production as an adaptive response, which appears to help protect them from gluconeolysis intolerance. The uncovering of these different adaptive responses using multiple complementary biochemical and physiological measurements provides further insight into mechanisms that prevent obesity and glucose intolerance.

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REFERENCES

1. Barsh, G. S., Farooqi, I. S., and O’Rahilly, S. (2000) Nature 404, 644–651
2. Martinez-Botas, J., Anderson, J. B., Tessier, D., Lapillonne, A., Chang, B. H. J., Quast, M. J., Gorenstein, D., Chen, K.-H., and Chan, L. (2000) Nat. Genet. 26, 474–479
3. Tansey, J. T., Satalydy, C., Grasl-Gray, J., Roash, D. L., Zee, J. V., Gavrilova, O., Reitman, M. I., Deng, C.-X., Li, C., Kimmel, A.-R., and Lomedo, C. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 6494–6499
4. Greenberg, A. S., Egan, J. J., Wêk, S. A., Garty, N. B., Blanchette-Mackie,
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E. J., and Londos, C. (1991) J. Biol. Chem. 266, 11341–11346
5. Servetnick, D. A., Braassema, D. L., Gruia-Gray, J., Kimmel, A. R., Wollf, J., and Londos, C. (1995) J. Biol. Chem. 270, 16970–16973
6. Londos, C., Braassema, D. L., Schultz, C. J., Segrest, J. P., and Kimmel, A. R. (1999) Science Cell Dev. Biol. 10, 51–58
7. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402
8. Holm, C., Osterlund, T., Laurell, H., and Contreras, J. A. (2000) Annu. Rev. Nutr. 20, 365–393
9. Souza, S. C., Mulim, K. V., Liscum, L., Lien, P., Yamamoto, M. T., Schaffer, E. J., Dallal, G. E., Wang, X., Kraemer, F. B., Obin, M., and Greenberg, A. S. (2002) J. Biol. Chem. 277, 8267–8272
10. Souza, S. C., Mitoose de Vargas, L., Yamamoto, M. T., Lien, P., Franciosa, M. D., Moss, L. G., and Greenberg, A. S. (1998) J. Biol. Chem. 273, 24965–24969
11. Chang, B. H.-J., Liao, W., Nakamuta, M., Mack, D., and Chan, L. (1999) J. Biol. Chem. 274, 6051–6055
12. Miyake, K., Ogawa, W., Matsumoto, M., Nakamura, T., Sakase, H., and Kasaiga, M. (2002) J. Clin. Investig. 110, 1483–1491
13. Alam, N., and Saggerson, E. D. (1998) Biochem. J. 334, 233–243
14. Hong, R., Zarnowski, M. J., Cushman, S. V., and Lodish, H. F. (2003) J. Biol. Chem. 278, 47588–47593
15. Ma, K., Cabrero, A., Saha, P. K., Kojima, H., Li, L., Chang, B. H.-J., Paul, A., and Chan, L. (2002) J. Biol. Chem. 277, 34658–34661
16. Jones, J. P., and Dohm, G. L. (1997) Am. J. Physiol. 273, E682–E687
17. Lau, P. P., Villanueva, H., Kobayashi, K., Nakamura, M., Chang, B. H.-J., and Chan, L. (2002) J. Biol. Chem. 277, 34658–34661
18. Andrade, A., Almeida, J., and Guedes, V. (2003) J. Physiol. 273, 4059–4065
19. Fjeld, C. R., Cole, F. S., and Bier, D. M. (1992) J. Biol. Chem. 268, 10859–10866
20. Kim, J. K., Gavrilova, O., Chen, Y., Reitman, M. L., and Shulman, G. I. (2000) J. Physiol. 273, 46445–46452
21. Sunehag, A., Ewald, U., and Gustafsson, J. (1999) J. Clin. Investig. 103, 550–557
22. Fujita, Y., Kojima, H., Hidaka, H., Fuijii, M., Kashiyami, A., and Kikkawa, R. (1998) Diabetologia 41, 1459–1466
23. Sunehag, A., Ray, M., Chan, H., and Shulman, G. I. (2000) J. Clin. Investig. 105, 1005–1015
24. Bougneres, P. F. (1987) Biomed. Res. 8, 87–96
25. Bougneres, P. F., Hillman, L. S., and Bier, D. M. (1982) J. Clin. Investig. 70, 292–270
26. Patel, D., and Kalhan, S. (1992) Pediatr. Res. 31, 52–58
27. Bumang, J. C., Michael, M. D., Winnay, J. N., Hayashi, T., Hersch, D., Acelli, D., Goodyear, L. J., and Kahn, C. R. (1998) Mol. Cell 2, 559–569
28. Hilemann, M., Ploug, T., Hellensten, Y., and Galbo, H. (1999) Am. J. Physiol. 277, E208–E214
29. Abu-Elheiga, L., Matsuz, M. M., Abo-Hashema, K. A. H., and Waki, S. J. (2001) Science 291, 2613–2616
30. Komay, W. H., Dommes, V., and Schulz, H. (1995) Prog. Lipid Res. 34, 267–342
31. Argyropoulos, G., and Harper, M. E. (2002) J. Appl. Physiol. 92, 2187–2198
32. Banerjee, R. R., Rangwala, S. M., Shapiro, J. S., Rich, A. S., Rha, W., Qian, Y., Wang, J., Rajala, M. W., Pociak, A., Scherer, P. E., Steppan, C. M., Ahima, R. S., Oh, S. K., Rotelli, L., and Lazar, M. A. (2004) Science 303, 1195–1198
33. Kim, J. K., Zisman, A. F., Fillmore, J. J., Peroni, O. D., Kotani, K., Perret, P., Zeng, H., Dong, J., Kahn, C. R., Kahn, B. B., and Shulman, G. I. (2001) J. Clin. Investig. 108, 153–160
34. Yamauchi, T., Kamon, J., Waki, I., Imai, Y., Shimonaw, N., Hori, K., Uchida, S., Ito, T., Takekawa, K., Matsui, J., Takata, M., Eto, K., Terasaki, Y., Komada, K., Tsunoda, M., Murakami, K., Oishi, N., Naitoh, T., Yamaamura, K., Uyana, Y., Fuguel, P., Kimura, S., Nagai, R., and Kadowaki, T. (2003) J. Biol. Chem. 278, 2461–2468
35. Pajvani, U. B., Hawkin, M., Combs, T. P., Rajala, M. W., Dobbler, T., Berger, J. P., Wagner, J. A., Wu, M., Kropp, A., Xiang, A. H., Utschneider, K. M., Kahn, S. E., Olsfky, J. M., Buchanan, T. A., and Scherer, P. E. (2004) J. Biol. Chem. 279, 12152–12162
36. Abel, E. D., Peroni, O., Kim, J. K., Kim, Y. B., Boss, O., Hadro, E., Minnemann, T., Shulman, G. I., and Kahn, B. B. (2001) Nature 409, 729–733
37. Shepherd, P. R., Gruzi, L., Tozzo, E., Yang, H., Leach, F., and Kahn, B. B. (1993) J. Biol. Chem. 268, 22243–22246
38. Minokoshi, Y., Kahn, C. R., and Kahn, B. B. (2003) J. Biol. Chem. 278, 33609–33612
39. Yechoor, V. K., Patti, M. E., Saccone, R., and Kahn, C. R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 10587–10592
40. Chou, C. J., Ha, M., Matsui, K., Aliprantis, O., Vinson, C., Gavrilova, O., and Reitman, M. J. (2002) J. Biol. Chem. 277, 24484–24489
41. Chom, C. J., Haluzik, J., Gregory, C., Ditz, K. R., Vinson, C., Gavrilova, O., and Reitman, M. J. (2002) J. Biol. Chem. 277, 24484–24489
42. Shimomura, I., Hammer, B. E., Richardson, J. A., Ikemoto, S., Bashmakov, Y., Goldstein, J. L., and Brown, M. S. (1998) Genes Dev. 12, 3182–3194
43. Moitra, J., Mason, M. M., Olive, M., Krylov, D., Gavrilova, O., Marcus-Samuels, B., Feigenbaum, L., Lee, E., Aoyama, T., Schell, N., Reitman, M. L., and Vinson, C. (1998) Genes Dev. 12, 3168–3181
44. Reitman, M. L. (2002) Annu. Rev. Nutr. 22, 459–482