Whole body overexpression of PGC-1α has opposite effects on hepatic and muscle insulin sensitivity

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Liang H, Balas B, Tantiwong P, Dube J, Goodpaster BH, O’Doherty RM, DeFronzo RA, Richardson A, Musi N, Ward WF. Whole body overexpression of PGC-1α has opposite effects on hepatic and muscle insulin sensitivity. Am J Physiol Endocrinol Metab 296: E945–E954, 2009. First published February 10, 2009; doi:10.1152/ajpendo.90292.2008.—Type 2 diabetes is characterized by insulin resistance, which is the primary disturbance responsible for increased glucose production. Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is a transcriptional coactivator that is thought to control adaptive responses to physiological stimuli. In liver, PGC-1α expression is induced by fasting, and this effect promotes gluconeogenesis. To examine whether PGC-1α is involved in the pathogenesis of hepatic insulin resistance, we generated transgenic (TG) mice with whole body overexpression of human PGC-1α and evaluated glucose homeostasis with a euglycemic-hyperinsulineemic clamp. PGC-1α was moderately (~2-fold) overexpressed in liver, skeletal muscle, brain, and heart of TG mice. In liver, PGC-1α overexpression resulted in increased expression of hepatocyte nuclear factor-4α and the gluconeogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. PGC-1α overexpression caused hepatic insulin resistance, manifested by higher glucose production and diminished insulin suppression of gluconeogenesis. Paradoxically, PGC-1α overexpression improved muscle insulin sensitivity, as evidenced by elevated insulin-stimulated Akt phosphorylation and peripheral glucose disposal. Content of myoglobin and troponin I slow protein was increased in muscle of TG mice, indicating fiber-type switching. PGC-1α overexpression also led to lower reactive oxygen species production by mitochondria and reduced IKK/IκB signaling in muscle. Feeding a high-fat diet to TG mice eliminated the increased muscle insulin sensitivity. The dichotomous effect of PGC-1α overexpression in liver and muscle suggests that PGC-1α is a fuel gauge that couples energy demands (muscle) with the corresponding fuel supply (liver). Thus, under conditions of physiological stress (i.e., prolonged fast and exercise training), increased hepatic glucose production may help sustain glucose utilization in peripheral tissues.

diabetes; phosphoenolpyruvate carboxykinase; glucose-6-phosphatase; gluconeogenesis

Type 2 diabetes is characterized by insulin resistance, which becomes manifest in the early stages of the disease (7). The main tissues that become resistant to insulin are liver, skeletal muscle, and fat (38). Hepatic insulin resistance is of critical importance, since it is the primary disturbance responsible for fasting hyperglycemia (23). Liver insulin resistance results in increased flux through the glycolytic and gluconeogenic pathways (14), which normally are inhibited by insulin (4, 10, 11). The accelerated rate of hepatic glucose production (HGP) leads to a rise in fasting plasma glucose concentrations (8). Although a number of molecular/biochemical mechanisms have been suggested to account for the development of hepatic insulin resistance (3, 41), the primary defect remains undefined.

Peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α is a transcriptional coactivator that coordinates the expression of genes that control diverse metabolic pathways in response to environmental and physiological changes (35). Under normal, ad libitum-fed conditions, PGC-1α expression is relatively low in liver compared with other tissues such as heart and brain, which rely mainly on aerobic metabolism for ATP production (26, 37). The gene expression of PGC-1α in liver increases with fasting (17, 48) and plays a central role in the regulation of gluconeogenesis (17, 22, 36, 48) by binding to and coactivating transcription factors, including hepatocyte nuclear factor (HNF)-4α, forkhead box O1A, and the glucocorticoid receptor, to coordinate the expression of rate-limiting gluconeogenic genes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) (36, 38).

PGC-1α expression in liver is upregulated in animal models of insulin resistance and diabetes (17, 45). Since PGC-1α promotes hepatic gluconeogenesis (36, 38), it has been postulated that PGC-1α may be responsible for the increases in HGP in individuals with type 2 diabetes (17, 35). Despite the large body of evidence from in vitro studies indicating that PGC-1α may play a role in the pathogenesis of insulin resistance in liver, it is not known whether increased PGC-1α signaling will cause hepatic insulin resistance in vivo. With the goal to understand the role of PGC-1α in gluconeogenesis and the development of hepatic insulin resistance, we generated transgenic (TG) mice moderately overexpressing PGC-1α and used the insulin-clamp technique to examine whole body glucose homeostasis in this model.

MATERIALS AND METHODS

Generation of PGC-1α TG mouse. To generate TG mice that overexpress PGC-1α ubiquitously, we selected a bacterial artificial chromosome (BAC) clone (CTD2238L2) that contains endogenous human PGC-1α gene and its regulatory sequences. The human

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PGC-1α BAC clone has ~100 kb of human PGC-1α gene sequence and ~80 kb upstream and ~20 kb downstream of regulatory sequences containing endogenous promoter and no other known genes in its range (Fig. 1A). The whole BAC clone DNA was amplified and then purified through cesium chloride. The purified PGC-1α BAC DNA was injected into the pronuclei of the fertilized eggs that were implanted into the uterus of pseudopregnant female mice. The founder PGC-1α TG mouse was generated on the C57Bl/6xDBA mixed background. The mice used in this study were backcrossed to C57Bl/6J two to four times. The genotype of the TG founders was determined using PCR to amplify PGC-1α BAC DNA (Fig. 1B), and expression of the human PGC-1α transgene was analyzed by RT-PCR specific for human PGC-1α mRNA (Fig. 1C).

Maintenance of mice. Animals were housed in an animal room maintained at 23°C with a 12:12-h light-dark cycle and fed standard laboratory chow and water ad libitum. Separate groups of wild-type (WT) and TG mice were fed a high-fat diet (HFD; D12331, Research Diets; 57% fat-derived calories) for 20 wk. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

RT-PCR and real-time PCR. Total RNA from tissues was isolated using TRizol reagent (Invitrogen, Carlsbad, CA). Two micrograms of RNA were reverse transcribed to cDNA (Ambion, Austin, TX) in 20 μl of total reaction volume. For identification of human PGC-1α mRNA expression by PCR, 1 μl of cDNA was used. The amplification was performed on a thermal cycler (Applied Biosystems, Foster City, CA) at 95°C for 5 min and then at 95°C for 30 s and 58°C for 30 s followed by 72°C for 45 s for 35 cycles. The resulting products were visualized on agarose gel. For real-time PCR, after determination by serial dilution, optimal amounts of cDNA solutions were mixed with SYBR Green PCR Master Mix (Applied Biosystems) and specific primers and run on a real-time PCR system (ABI 7900) at 90°C for 2 min, 95°C for 10 min, and then 95°C for 15 s followed by 60°C for 1 min for 40 cycles. The results were normalized to the level of β-actin expression before comparison of the relative levels of expression of TG and WT mice.

The following primers were used: 5'-gaagccccctgtgaatgtgctgc-3' (forward) and 5'-ctggtccatgatgcagttggtg-3' (reverse) for human PGC-1α transgene expression, 5'-acagaaatgctgctatgtcacagt-3' (forward) and 5'-tcagttgtacc-3' (reverse) for mouse and human PGC-1α, 5'-ccagttgagcagtttgctg-3' (forward) and 5'-gggggagggagagttttactg-3' (reverse) for GAPDH, 5'-gactgttgaaaaaggcaagaagatt-3' (forward) and 5'-ggaggagggagagtgttttatgta-3' (reverse) for G6Pase, 5'-ittcttagatagttggetttgg-3' (forward) and 5'-acagaaatgctgctacatgtg-3' (reverse) for medium-chain acyl-CoA dehydrogenase (MCAD), 5'-cgtcttagactgcactgactc-3' (forward) and 5'-ctgtgattatctgaatgctg-3' (reverse) for carnitine palmitoyltransferase (CPT)-1A (liver), 5'-aagtctgtagttggtctactctact-3' (forward) and 5'-ttgtgattatctgaatgctg-3' (reverse) for CPT-1B (muscle), 5'-tagatggagaagctgctgactg-3' (forward) and 5'-ctctctctcctctctctctct-3' (reverse) for cytochrome c, 5'-ccataacagagcaactgacaa-3' (forward) and 5'-tgatggaggaagctgctgactg-3' (reverse) for cytochrome c oxidase subunit II, and 5'-gggatgagagccagtgtacc-3' (forward) and 5'-ctctctctcctctctctctcttgc-3' (reverse) for cytochrome c oxidase subunit IV.

Western blot analysis. Total cellular proteins were extracted in RIPA buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS] containing 1 mM PMSF and a protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). The proteins were resolved on a 4–20% SDS-polyacrylamide gradient gel under denaturing conditions and then transferred to nitrocellulose membranes. Immunoblotting was performed using antibodies against the following proteins: PGC-1α and β-actin (Calbiochem, La Jolla, CA); histone, myoglobin, and tropinin I (Santa Cruz Biotechnology, Santa Cruz, CA); GAPDH (Alamo Laboratories, San Antonio, TX); phospho-Akt-Ser473, Akt, IκBα, and phospho-p38 MAPK (Cell Signaling, Danvers, MA); phospho-JNK (Promega, Madison, WI); and GLUT4 and GLUT1 (Millipore, Billerica, MA). Specific signals for the target proteins were visualized using enhanced chemiluminescence (ECL) reagent (GE Healthcare, Piscataway, NJ). The blots were analyzed and quantified using ImageQuant software.

Plasma chemistry. Tail blood was collected for plasma chemistry. A OneTouch Ultra blood glucose meter (LifeScan, Milpitas, CA) was used to measure glucose in 3- to 5-mo-old mice fasted for 18 h. Insulin levels were determined using a radioimmunoassay kit (Sigma-Aldrich, St. Louis, MO), and serum free fatty acids (FFAs) were determined using an NEFA assay kit (Wako Chemicals, Richmond, VA).

Euglycemic-hyperinsulinemic clamp. The euglycemic insulin clamp was performed in awake, unrestrained, chronically catheterized mice in combination with [3H]glucose infusion, as previously described (47). Female mice (24 wk average age) were fasted for 6 h before the insulin-clamp studies. Briefly, a prime-continuous infusion of regular insulin was administered at 18 μU·kg⁻¹·min⁻¹, and a variable infusion of a 10% glucose solution was started at time 0 and periodically adjusted to clamp the plasma glucose concentra-
tion at the fasting level. The insulin-clamp study included a 60-min baseline period for the assessment of basal glucose turnover rate and a 90-min euglycemic clamp period. Under steady-state conditions of fasting euglycemia, the rate of total body glucose disappearance equals the rate of total body glucose appearance and was calculated by dividing the infusion rate of [3-3H]glucose [disintegrations per minute (dpm)] by the steady-state plateau of [3-3H]glucose specific activity (dpm/mg). HGP was calculated by subtracting the exogenous glucose infusion rate from the rate of total body glucose appearance (47).

Glucose tolerance tests. A OneTouch Ultra blood glucose meter was used to measure tail blood glucose levels before and after (15, 30, 60, and 120 min) intraperitoneal administration of glucose (1.5 mg/g body wt) in 3- to 5-mo-old fasted female mice.

Diacylglycerol and ceramide content. Concentrations of diacylglycerol (DAG) and ceramide in mixed quadriceps muscle were determined using the Bradford protein assay reagent (Bio-Rad, Richmond, CA).

Mice were killed, and the skeletal muscles were rapidly excised and isolated from skeletal muscle as described by Makinen and Lee (28). The muscles were then homogenized in a Dounce glass homogenizer using 10–15 strokes, and the resulting homogenate was centrifuged at 600 g for 10 min. The supernatants were filtered through cheesecloth (Bellco Class, Vineland, NJ) and centrifuged at 14,000 g for 10 min. The resulting pellets were treated with washing buffer [100 mM KCl, 50 mM Tris-HCl, 5 mM MgCl₂, 1 mM EDTA, and 1 mM ATP (pH 7.4)] supplemented with 0.5% BSA and centrifuged at 7,000 g for 10 min. The mitochondrial pellets were washed in washing buffer without BSA, centrifuged at 3,500 g for 10 min, and then resuspended in a buffer containing 100 mM KCl and 50 mM MOPS (pH 7.44). The mitochondrial protein concentration was determined using the Bradford protein assay reagent (Bio-Rad, Richmond, CA).

Results of H₂O₂ from mitochondria was utilized as a measure of reactive oxygen species (ROS) production. Mitochondrial H₂O₂ release was measured using Amplex Red (N-acetyl-3,7-dihydroxyphenoxazine)-horseradish peroxidase (Molecular Probes, Eugene, OR) as previously described (29). Briefly, 80 μM Amplex Red reagent and 1 U/ml horseradish peroxidase were added to the mitochondria (40 μg protein) or to the H₂O₂ standard solution in 100 μl of reaction buffer [50 mM MOPS and 100 mM KCl (pH 7.44)] and incubated in Falcon 96-well microplates in the dark at 30°C. Fluorescence was followed at excitation wavelength of 530 nm and emission wavelength of 590 nm for 10 min in an automatic microplate reader (Labsystems, Helsinki, Finland). The rates of H₂O₂ production were determined in the absence (state 1) or presence of the respiratory substrates glutamate plus malate or succinate. In some experiments, specific inhibitors for complex I (rotenone) were used in combination with respiratory substrates to block electron transport to maximally stimulate H₂O₂ production.

Statistical analysis. Values are means ± SE. Data between groups were compared using unpaired Student’s t-test or one-way ANOVA, as appropriate. P < 0.05 was considered statistically significant. Statistical analysis was performed using SigmaStat software.

Results

Increased PGC-1α expression in TG mice. PGC-1α gene expression was measured in various tissues from ad libitum-fed mice by RT-PCR. Depending on the tissue, PGC-1α gene expression was ~1.5- to 2.5-fold higher in TG than WT animals (Fig. 2A). An experimental goal was to achieve a PGC-1α expression level well below (≥10-fold) the levels that have been reported to produce cardiomyopathy and skeletal muscle cell necrosis (24, 32). Increases in PGC-1α expression also were reflected at the protein level in liver and muscle (Fig. 2B). As expected, fasting for 18 h led to elevated expression of PGC-1α in liver, and this effect was accentuated in TG mice (Fig. 2C).

General characteristics of TG mice fed standard chow. The TG mice were grossly normal, and no premature death was...
overexpression led to increased HNF-4α/H9251PEPCK (Fig. 6B). Paradoxically, PGC-1α overexpression resulted in enhanced muscle insulin sensitivity, as evidenced by an increase in insulin-stimulated glucose disposal during the clamp (Fig. 4) and improved glucose tolerance during the glucose tolerance test (Fig. 5).

Expression of hepatic gluconeogenic enzymes and mitochondrial genes. To determine the molecular basis for the increased HGP observed in TG mice, we measured the gene expression of HNF-4α, which controls the expression of gluconeogenic enzymes such as PEPCK and G6Pase. PGC-1α overexpression led to increased HNF-4α gene expression (Fig. 6A), which was accompanied by an elevation in the expression of PEPCK (Fig. 6B) and G6Pase (Fig. 6C). In addition, PGC-1α overexpression was accompanied by elevated expression of mitochondrial proteins involved in oxidative phosphorylation, such as cytochrome c oxidase subunits II and IV and cytochrome c in liver (Fig. 6D).

Effects of PGC-1α overexpression in muscle. It has been postulated that PGC-1α mediates some of the adaptations that occur with physical training in muscle, such as switching from fast, more glycolytic, to slow, more oxidative, fibers (27). Consistent with this notion, content of myoglobin and troponin I slow protein, biomarkers for slow oxidative fibers, was elevated in muscle from TG mice (Fig. 7, A and B). In addition, PGC-1α overexpression resulted in increased gene expression of the oxidative phosphorylation proteins cytochrome c oxidase subunits II and IV in muscle (Fig. 7C). These changes in TG mice were accompanied by increased insulin signaling in muscle, manifested by elevated insulin-stimulated Akt phosphorylation (Fig. 7D). PGC-1α overexpression did not affect basal Akt (Fig. 7E), GLUT4 (Fig. 7F), and GLUT1 (Fig. 7G) protein content in muscle.

To further explore the mechanism by which PGC-1α overexpression leads to improved insulin action in muscle, we measured the phosphorylation (i.e., activity) of the MAPKs p38 and JNK, as well as the content of inhibitor κB (IκB)-α as an indication of the activity of IκB kinase (IKK) and NF-κB. Previous studies showed that the p38 MAPK (18), JNK (19), and IKK/NF-κB (6, 21, 42, 43) cascades are negative regulators of muscle insulin sensitivity. As shown in Fig. 8, p38 MAPK and JNK phosphorylation were unaffected in TG mice. In contrast, overexpression of PGC-1α resulted in elevated IκBα protein abundance (Fig. 8C). Because IκB sequesters NF-κB in the cytoplasm and because IκB abundance inversely correlates with NF-κB DNA-binding activity in muscle (1), increased IκB abundance is considered to indicate reduced IKK/NF-κB signaling.

Lipid content in liver. Considering that PGC-1α plays an important role in the regulation of lipid metabolism (25), we measured the concentrations of the lipid metabolites DAG and ceramide in the liver of standard chow-fed WT and TG mice. PGC-1α overexpression led to reduced DAG and ceramide (Fig. 9A) concentrations in the liver from TG mice compared with WT mice. This difference in hepatic lipid content between groups was not explained by differences in the gene expression of the fatty acid oxidative enzymes CPT-1 and MCAD (Fig. 9C).

Fig. 4. Whole body glucose disposal is increased in 6-mo-old female TG mice. Values are means ± SE in 5 TG and 7 WT mice. *P < 0.05 vs. WT.
Lipid content and ROS production in muscle. To determine the basis for reduced IKK/NF-κB signaling in muscle from TG mice, we measured the content of DAG and ceramide, as well as mitochondrial H2O2 production, because lipids and ROS can activate this pathway and, subsequently, impair insulin signaling (13). DAG and ceramide muscle content was similar between groups (Fig. 9B). However, overexpression of PGC-1α led to reduced H2O2 production by muscle mitochondria (Fig. 9D).

Effect of HFD. Plasma glucose concentrations (92 ± 6 and 91 ± 5 mg/dl, WT and TG, respectively) and body weights (data not shown) were similar in HFD-fed WT and TG mice. The HFD decreased hepatic PGC-1α levels by 22% and 15% in WT and TG mice, respectively (not shown). In muscle, HFD decreased PGC-1α content by 62% and 59% in WT and TG animals, respectively (not shown). Compared with WT mice fed standard chow, in which insulin caused near-total suppression of HGP (Fig. 3B), in HFD-fed WT mice, insulin reduced HGP by 86% (Fig. 10A). In addition, similar to animals fed standard chow, overexpression of PGC-1α (in TG mice) led to hepatic insulin resistance, manifested by a blunted insulin-mediated reduction in HGP (Fig. 10A). As expected, the HFD also caused peripheral insulin resistance in WT animals (Fig. 10B); whole body glucose disposal was 117 ± 4 and 93 ± 9 mg·kg⁻¹·min⁻¹ with standard chow and HFD, respectively (P < 0.05). However, overexpression of PGC-1α did not affect HFD-induced peripheral insulin resistance: whole body glucose disposal also decreased to 89 ± 8 mg·kg⁻¹·min⁻¹ with the HFD (Fig. 10B). In contrast to mice fed standard chow, in which PGC-1α overexpression led to a reduction in hepatic DAG and ceramide concentrations (Fig. 9A), upregulation of PGC-1α did not alter the content of these lipid metabolites in liver from HFD-fed TG mice (Fig. 10C). In addition, DAG and ceramide muscle content was similar in muscle from HFD-fed WT and TG mice (data not shown).

DISCUSSION

PGC-1α is an important regulator of the gluconeogenic metabolic pathway (17, 48). In this study, we provide direct in vivo evidence that upregulation of PGC-1α increases HGP. Rates of HGP are controlled by gluconeogenic enzymes such as PEPCK and G6Pase, and expression of these enzymes was elevated in the TG mice. This effect is likely due to PGC-1α-mediated increases in HNF-4α expression, because PGC-1α loses its capacity to stimulate PEPCK and G6Pase when HNF-4α is absent (38). Some insulin-resistant animal models, including ob/ob (48), liver-specific insulin receptor-knockout (LIRKO) (48), and db/db (45) mice, have been shown to have elevated PGC-1α expression in the liver (17, 45). However, it is not known (to the best of our knowledge) whether an elevation in PGC-1α expression in the liver causes hepatic insulin resistance in vivo, as assessed by a method such as the insulin clamp with Tritiated glucose, which provides direct, quantitative measurements...
of HGP. The present finding that upregulation of PGC-1α per se can cause hepatic insulin resistance suggests that increased expression of this transcriptional coactivator, as seen in ob/ob (48), LIRKO (48), and db/db (45) mice, may be responsible for the higher rates of HGP and liver insulin resistance in individuals with type 2 diabetes. Nevertheless, in the present study, we found that the HFD decreased PGC-1α expression in the liver. This finding argues against a role for PGC-1α in the pathogenesis of hepatic insulin resistance, at least in the HFD mouse model. Because PGC-1α expression is sensitive to fuel availability, the decrease in PGC-1α expression caused by the HFD could be caused by nutrient oversupply. Future studies are needed to clarify why the HFD differs from other mouse models of insulin resistance in regard to hepatic PGC-1α expression. Most importantly, it remains to be determined whether PGC-1α expression/function is abnormal in the liver of insulin-resistant individuals.

In contrast to the development of insulin resistance in the liver, TG mice overexpressing PGC-1α displayed improved peripheral (muscle) insulin sensitivity and signaling. In rodents (2, 15, 34, 46) and humans (33, 44), acute exercise induces PGC-1α gene expression in muscle. It has been proposed that this increase in muscle PGC-1α expression after prolonged exercise mediates the insulin-sensitizing effects (16). Consistent with the findings of Lin et al. (27), upregulation of PGC-1α increased the content of myoglobin I and troponin I slow protein in muscle, an indication of fast-to-slow fiber switching. However, we did not observe an increase in glucose transporter content (another typical adaptation to training) that occurred when PGC-1α was overexpressed in cultured muscle cells with use of an adenoviral vector (29). This discrepancy is likely due to differences in experimental systems (in vitro vs. in vivo) and the much higher levels of PGC-1α expression that were achieved with the viral vector than the modest (i.e., physiological) elevation observed in our TG mice.

To examine further the molecular basis for the improvement in insulin sensitivity/signaling in muscle from the TG mice, we examined whether PGC-1α upregulation affected the p38 MAPK, JNK, and IKK/NF-κB cascades. TG mice had increased abundance of the inhibitory protein IκBα.
which is an indication of decreased IKK/NF-κB signaling. Because the IKK/NF-κB pathway inhibits insulin action (6, 21, 42, 43), it is possible that the lower IKK/NF-κB activity contributed to the improvement in insulin action in the PGC-1α TG mice. We also measured the content of the intramyocellular lipid metabolites DAG and ceramide, since increased muscle content of these lipid metabolites has been shown to activate IKK/NF-κB signaling (5, 21). We had predicted lower DAG and ceramide content in muscle of TG mice; however, we did not observe differences in the content of these lipid metabolites between WT and TG mice. The IKK/NF-κB pathway is activated by other stimuli, including ROS (13). Indeed, PGC-1α overexpression decreased ROS production by muscle mitochondria, which may be the cause of lower IKK/NF-κB signaling.

Overexpression of PGC-1α leads to a reduction in the content of DAG and ceramides in the liver. Considering that PGC-1α plays a role in the regulation of mitochondrial fatty acid oxidative enzyme gene expression (25), we compared the expression of CPT-1 and MCAD in the liver of WT vs. TG mice. However, expression of these key fatty acid oxidative enzymes was similar between groups. It is possible that elevated PGC-1α expression leads to a reduced DAG and ceramide content through a decrease in the rate of synthesis of these metabolites, as opposed to an increase in the rate of oxidation. Further experimentation is required to determine the mechanism of action of PGC-1α on the lipid metabolic effects we have observed.

Another important observation derived from this study is that overexpression of PGC-1α decreased insulin sensitivity of the liver, despite a reduction in the content of DAG and ceramides, agents that are generally thought to be important mediators of insulin resistance (18, 40). Other investigators have also observed such a dissociation between the cellular content of these lipid metabolites and insulin sensitivity. Monetti et al. (31) described a mouse model in which overexpression of acyl-CoA:diacylglycerol acyltransferase-2 led to increases in the hepatic content of triacylglycerol, DAG, and ceramides without affecting the insulin sensitivity of the liver. It is also possible that the upregulation of gluconeogenic genes in the PGC-1α TG mice could have overcome any potential beneficial effect on insulin action caused by the reduction of DAG and ceramides.

The PGC-1α TG mice were challenged with the HFD to test whether overexpression of this transcriptional coactivator would ameliorate the deleterious effect of the HFD on peripheral insulin sensitivity. Nonetheless, parameters of insulin sensitivity (Fig. 10B) and signaling (not shown) were not different between HFD-fed WT and TG animals. Furthermore, PGC-1α overexpression did not affect DAG and ceramide levels in liver (Fig. 10C) and muscle (Fig. 10D) from HFD-fed mice. Similarly, PGC-1α overexpression did not alter phospho-JNK, phospho-p38, or IκBα content (not shown) in liver and muscle from HFD-fed mice. Because the degree of PGC-1α was modest, it is possible that the metabolic/cellular changes caused by the HFD, which is a fairly robust challenge, overwhelmed the improvements in insulin action in the TG mice.

By examining whole body glucose homeostasis in the PGC-1α TG mice using the insulin clamp-tritiated glucose technique, we found that overexpression of this transcriptional coactivator had a negative effect on hepatic insulin sensitivity but simultaneously improved insulin action in muscle. Although these results might appear to be somewhat paradoxical, they are consistent with the notion that PGC-1α exerts control of metabolic pathways in a tissue-specific manner (16, 35). Thus, PGC-1α acts as a fuel sensor that couples energy demands with the corresponding fuel supply. For example, under conditions of increased metabolic demand, such as fasting and exercise training, increased HGP would help sustain increased glucose utilization in peripheral tissues, primar-
ily muscle. The dichotomous effect of PGC-1α expression on liver and muscle insulin sensitivity also underscores the difficulty of targeting PGC-1α for the treatment of human disease, such as type 2 diabetes. Tissue-specific modulators would be required to stimulate PGC-1α signaling specifically in skeletal muscle, where enhanced insulin action is desired, without activating this pathway in liver, where it would have a deleterious effect on glycemia by promoting glucose production.

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Fig. 9. Diacylglycerol (DAG) and ceramide content in liver (A) and quadriceps muscle (B) from standard chow-fed animals. C: carnitine palmitoyltransferase (CPT-1A) and medium-chain acyl CoA dehydrogenase (MCAD) as measured by real-time PCR in liver and muscle. D: H2O2 production by mitochondria isolated from muscle. Succinate (succ), malate (mal), and glutamate (Glu) were used as substrates for respiration. Rote, rotenone. Values are means ± SE in 6 (4- to 6-mo-old male and female) mice per group. *P < 0.05 vs. WT.

Fig. 10. Under high-fat diet (HFD) conditions, insulin suppression of endogenous glucose production (A) and peripheral insulin sensitivity (B) during clamp are similar in TG and WT mice. DAG and ceramide levels in liver (C) and muscle (D) are similar in HFD-fed TG and WT animals. Values are means ± SE in 6 (6-mo-old male and female) mice per group.
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