

Paradoxical Coupling of Triglyceride Synthesis and Fatty Acid Oxidation in Skeletal Muscle Overexpressing DGAT1

Li Liu,¹ Xiaojing Shi,¹ Cheol Soo Choi,² Gerald I. Shulman,² Katherine Klaus,³ K. Sreekumaran Nair,³ Gary J. Schwartz,⁴ Yiyang Zhang,⁵ Ira J. Goldberg,¹ and Yi-Hao Yu¹

OBJECTIVE—Transgenic expression of diacylglycerol acyltransferase-1 (DGAT1) in skeletal muscle leads to protection against fat-induced insulin resistance despite accumulation of intramuscular triglyceride, a phenomenon similar to what is known as the “athlete paradox.” The primary objective of this study is to determine how DGAT1 affects muscle fatty acid oxidation in relation to whole-body energy metabolism and insulin sensitivity.

RESEARCH DESIGN AND METHODS—We first quantified insulin sensitivity and the relative tissue contributions to the improved whole-body insulin sensitivity in muscle creatine kinase (MCK)-DGAT1 transgenic mice by hyperinsulinemic-euglycemic clamps. Metabolic consequences of DGAT1 overexpression in skeletal muscles were determined by quantifying triglyceride synthesis/storage (anabolic) and fatty acid oxidation (catabolic), in conjunction with gene expression levels of representative marker genes in fatty acid metabolism. Whole-body energy metabolism including food consumption, body weights, oxygen consumption, locomotor activity, and respiration exchange ratios were determined at steady states.

RESULTS—MCK-DGAT1 mice were protected against muscle lipotoxicity, although they remain susceptible to hepatic lipotoxicity. While augmenting triglyceride synthesis, DGAT1 overexpression also led to increased muscle mitochondrial fatty acid oxidation efficiency, as compared with wild-type muscles. On a high-fat diet, MCK-DGAT1 mice displayed higher basal metabolic rates and 5–10% lower body weights compared with wild-type littermates, whereas food consumption was not different.

CONCLUSIONS—DGAT1 overexpression in skeletal muscle led to parallel increases in triglyceride synthesis and fatty acid oxidation. Seemingly paradoxical, this phenomenon is characteristic of insulin-sensitive myofibers and suggests that DGAT1 plays an active role in metabolic “remodeling” of skeletal muscle coupled with insulin sensitization. *Diabetes* 58:2516–2524, 2009

From the ¹Department of Medicine, Preventive Medicine and Nutrition, Columbia University, New York, New York; the ²Departments of Internal Medicine and Cellular and Molecular Physiology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut; the ³Endocrine Research Unit and Department of Laboratory Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota; the ⁴Department of Medicine & Neuroscience, Diabetes Research and Training Center, Albert Einstein College of Medicine, New York, New York; and the ⁵Department of Pediatrics, Naomi Berrie Diabetes Center, Columbia University, New York, New York.

Corresponding author: Yi-Hao Yu, yy102@columbia.edu.

Received 11 August 2008 and accepted 16 July 2009. Published ahead of print at <http://diabetes.diabetesjournals.org> on 12 August 2009. DOI: 10.2337/db08-1096.

C.S.C. is currently affiliated with the Laboratory of Cellular & Molecular Physiology and Metabolism, Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, Incheon, Korea.

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Despite the observation that higher fat content in skeletal muscle is associated with insulin resistance as commonly seen in obesity and type 2 diabetes, increased muscle fat content is also associated with exercise training, in which it is “paradoxically” coupled with enhanced insulin sensitivity (the “athlete paradox”) (1–3). In fact, higher fat content is also seen in “oxidative” (with greater fatty acid oxidation capacity), slow-twitch type I myofibers compared with “glycolytic,” fast-twitch type II myofibers. Interestingly, type I myofibers appear to be inherently more insulin sensitive than type II myofibers (4). In human skeletal muscle, insulin-stimulated glucose transport directly correlates with the percentage of oxidative type I myofibers (5–7). Furthermore, exercise enhances muscle insulin sensitivity and is associated with increased muscle fat content and/or mitochondrial fatty acid oxidation capacity (1,3,8,9). In contrast, a decrease in oxidative, and conversely an increase in glycolytic, capacity is characteristic of skeletal muscle seen in sedentary obese individuals (10,11).

Exercise increases myocytic diacylglycerol acyltransferase (DGAT) activity, and transgenic overexpression of DGAT1 in skeletal muscle in mice is sufficient to replicate the exercise paradox characterized by increased muscle fat content coupled with increased muscle insulin sensitivity (3). Exercise also increases skeletal muscle DGAT1 expression and improves insulin sensitivity in humans (12). DGAT1 catalyzes the last step of triglyceride synthesis from diacylglycerol (DAG) and fatty acyl-CoA. By channeling fatty acid substrates into the triglyceride synthesis pathway, DGAT1 effectively decreases levels of muscle DAG and ceramide (3), two fatty acid derivatives that are believed to be causative in the development of obesity-related insulin resistance (3,13–17). In sedentary humans or rodents with fat-induced insulin resistance, higher muscle triglyceride content is also associated with elevated myocytic DAG and/or ceramide levels as a result of fatty acid overload (12,13,15); lipid metabolites and storage products are both increased in this substrate-driven process. Increased fatty acid load and consequent increases in intramyocellular fatty acid metabolites are lipotoxic and cause muscle insulin resistance (18). Increased myocytic DAG, for example, causes insulin resistance via activation of protein kinase C θ (13,14) leading to serine phosphorylation of insulin receptor substrate (IRS)-1 (13). Thus, while increased DAG is causal, high myocytic triglyceride content is not always a marker for muscle insulin resistance, as long as lipotoxic fatty acid metabolites are kept at low levels (3).

Given the parallel increases in both muscle triglyceride stores and fatty acid oxidation activity as exemplified by oxidative type I myofibers or the exercise-trained muscle model, we hypothesized that triglyceride synthesis and fatty acid oxidation capacities are intrinsically interconnected and both are upregulated in insulin-sensitive muscles. The present study demonstrates upregulation of both triglyceride synthesis and fatty acid oxidation capacities in the insulin-sensitive, DGAT1 overexpressing muscle model. This study also suggests that DGAT1 overexpression is a cause of such a “paradoxical” change in fat metabolism. We postulate that this coordinated change enables the myocytes to keep the intracellular levels of fatty acid metabolites below a potentially lipotoxic level. By channeling lipotoxic fatty acid metabolites into storage (as triglyceride) and into a pathway for terminal oxidation, such a change may lessen fatty acid-induced insulin resistance that is often associated with acquired obesity (3,12,13,19).

RESEARCH DESIGN AND METHODS

Mice, diets, and exercise. MCK-DGAT1 transgenic mice and wild-type littermates were fed standard rodent diet or high-fat diet (HFD) as previously described (3). The swimming exercise regimen was the same as described (3). Male mice were used in this study. Animal protocols were approved by the Columbia University Institutional Animal Care and Use Committee.

Hyperinsulinemic-euglycemic clamps. Three-month-old mice were fed HFD or standard rodent diet for 8 weeks before the clamp study. The clamp was conducted in awake mice as previously described (19). All procedures were approved by the Yale University Institutional Animal Care and Use Committee. Briefly, after an overnight fast, [^3H]-glucose (high-performance liquid chromatography purified; Perkinelmer) was infused at 0.05 $\mu\text{Ci}/\text{min}$ for 2 h to assess the basal glucose turnover. Hyperinsulinemic-euglycemic clamp was conducted for 120 min with primed/continuous infusion of human insulin (Novo Nordisk; 105 pmol/kg prime, 15 pmol \cdot kg $^{-1}$ \cdot min $^{-1}$ infusion), keeping plasma glucose at \sim 6.7 mmol/l (basal level). [^3H]-glucose was infused at 0.1 $\mu\text{Ci}/\text{min}$ throughout the clamp to estimate insulin-stimulated whole-body glucose fluxes. Calculations of hepatic glucose output (HGO), whole-body glycolysis, and whole-body glycogen synthesis were also as described (20). To assess muscle glucose uptake, separate groups of mice were clamped and 2-deoxy-D-[1- ^{14}C]glucose (2-[^{14}C]DG; Perkinelmer) was injected as a bolus at the 75th min of the clamp as described (20).

Mitochondrial fatty acid oxidation. Four-month-old mice pretreated with HFD for 8 weeks were used for these experiments. Mitochondria were isolated from muscle tissues using Mitochondria Isolation Kits (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. Mitochondrial fatty acid oxidation was performed by measuring the rate of $^3\text{H}_2\text{O}$ production from the substrate [^3H]palmitate present in excess (final concentrations: 0.2 mmol/l palmitate with 10 $\mu\text{Ci}/\text{ml}$ of 9,10(n)-[^3H]palmitate and 1.25% BSA) in a mitochondria incubation medium (containing 20 μg mitochondria protein) as described (21).

Mitochondrial DNA copy number, ATP production rate, citrate synthase, β -hydroxyacyl-CoA dehydrogenase, and carnitine palmitoyl transferase I activities. Four-month-old mice pretreated with HFD for 8 weeks were used for these experiments. Mitochondrial DNA copy number was measured using quantitative real-time PCR as previously described (22). Mitochondrial DNA for NADH dehydrogenases 1 and 4 (ND1 and ND4) and nuclear DNA for β -actin were coamplified within the same reaction wells (for primer sequences, see supplemental Table 1, available in an online appendix at <http://diabetes.diabetesjournals.org/cgi/content/full/db08-1096/DC1>). Mitochondrial ATP production rates were measured in isolated mitochondria through time-course monitoring of bioluminescence in response to a luciferin-luciferase ATP-monitoring reagent (Biotherma, Haninge, Sweden) as described (23). Maximal activity of citrate synthase was measured in muscle homogenates by photospectrometry as described (24). β -hydroxyacyl CoA dehydrogenase (HAD) activity was also measured in muscle homogenates by following spectrophotometrically the conversion of acetoacetyl-CoA to β -hydroxybutyryl-CoA by β -HAD as described (25). Total carnitine palmitoyl transferase (CPT) activity was assayed radiochemically in isolated mitochondria by following the conversion of palmitoyl-L-carnitine from palmitoyl-CoA and radiolabeled L-carnitine as described (25,26). CPT-I activity was calculated from the total CPT activity after subtraction of CPT-II activity, which was measured in the presence of 0.2 mmol/l malonyl-CoA that inhibits CPT-I.

Transmission electron microscopy. Four-month-old mice pretreated with HFD for 8 weeks were used. Muscles were isolated, fixed with 2.5% glutaraldehyde in 0.1M Sorenson's buffer (PH 7.2), and treated with 1% OsO $_4$ also in Sorenson's buffer for 1 h. After dehydration and embedment in Lx-112 (Ladd Research Industries), thin sections (60 nm) were cut using a MT-7000 ultramicrotome, stained with uranyl acetate and lead citrate, and examined under a JEOL JEM-1200 EXII electron microscope. Pictures were captured by an ORCA-HR digital camera (Hamamatsu) and recorded with an AMT Image Capture Engine.

Indirect calorimetry. Twenty-week-old weight-matched male MCK-DGAT1 mice and wild-type littermates that had been on a HFD for 8 weeks were singly housed in metabolic chambers. After 2–4 days of adaptation, oxygen consumption, locomotor activity, and respiration exchange ratio were measured continuously over at least 5 days during 12:12 light-dark cycles, using a CLAMS (Columbus Instruments, Columbus, OH) open-circuit indirect calorimetry system as previously described (27).

RT-PCR, Western blot, DGAT activity, and triglyceride contents. Four-month-old mice with 8-week HFD pretreatment were used for these experiments. RT-PCR (3,28) and Western blot (3,29) were as described. Gene-specific primer sets are listed in supplemental Table 1. Primary antibodies to pyruvate dehydrogenase kinase (PDK)4, uncoupling protein (UCP)-3, CD36, and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were obtained from Abcam (Cambridge, MA). DGAT activity was measured in vitro using [^{14}C]-labeled palmitoyl-CoA as previously described (29,30). The method to quantify muscle triglyceride content was as described (3,29).

Statistical analysis. Statistical differences were first analyzed by ANOVA to determine overall treatment effects (genotype or exercise), followed by post hoc comparisons between concerned study groups (Statistica V6.0, StatSoft, OK). A two-tailed P value of <0.05 was used to indicate statistical significance. For indirect calorimetry measurements, ANOVA repeated measures (Prism, GraphPad Software, San Diego, CA) were used. Post hoc analyses were conducted using Tukey post hoc or Bonferroni comparisons. Data are expressed as means \pm SE.

RESULTS

MCK-DGAT1 transgenic mice demonstrate improved resistance to diet-induced lipotoxicity, but they remain susceptible to hepatic insulin resistance on HFD. Using glucose tolerance and insulin tolerance tests, we showed previously that MCK-DGAT1 mice were protected against HFD-induced insulin resistance (3). Ex vivo 2-deoxyglucose uptake in skeletal muscles isolated from HFD-fed animals demonstrated that insulin sensitivity was greater in DGAT1-overexpressing muscles than in the wild-type muscles (3). In this study, whole-body and tissue insulin sensitivities were assessed in vivo by hyperinsulinemic-euglycemic clamps in combination with radioisotope-labeled glucose infusions. Three-month-old male MCK-DGAT1 and wild-type mice were fed HFD for 8 weeks (WT-HF and DGAT1-HF, respectively) before the clamp study. Age- and sex-matched wild-type mice on standard rodent diet (WT-NC) were used as baseline control. The major findings are depicted in Fig. 1, and other energetic and clamp parameters are summarized in supplemental Table 2. As expected, WT-HF mice showed marked insulin resistance with the mean whole-body glucose infusion rate at only \sim 16 mg \cdot kg $^{-1}$ \cdot min $^{-1}$ (Fig. 1A). DGAT1-HF mice were more insulin sensitive than WT-HF mice, achieving a mean whole-body glucose infusion rate of \sim 29 mg \cdot kg $^{-1}$ \cdot min $^{-1}$ (Fig. 1A). However, the baseline whole-body glucose infusion rate in the control WT-NC mice was \sim 45 mg \cdot kg $^{-1}$ \cdot min $^{-1}$ (Fig. 1A), indicating that DGAT1 overexpression provided \sim 45% protection against HFD-induced insulin resistance. Transgenic mice were not protected from HFD-induced hepatic insulin resistance. Compared with a $>50\%$ suppression of HGO in WT-NC mice during the clamping, insulin-mediated suppression of HGO was completely absent in both WT-HF and DGAT1-HF mice (Fig. 1B). To directly assess muscle insulin sensitivity under the same clamping

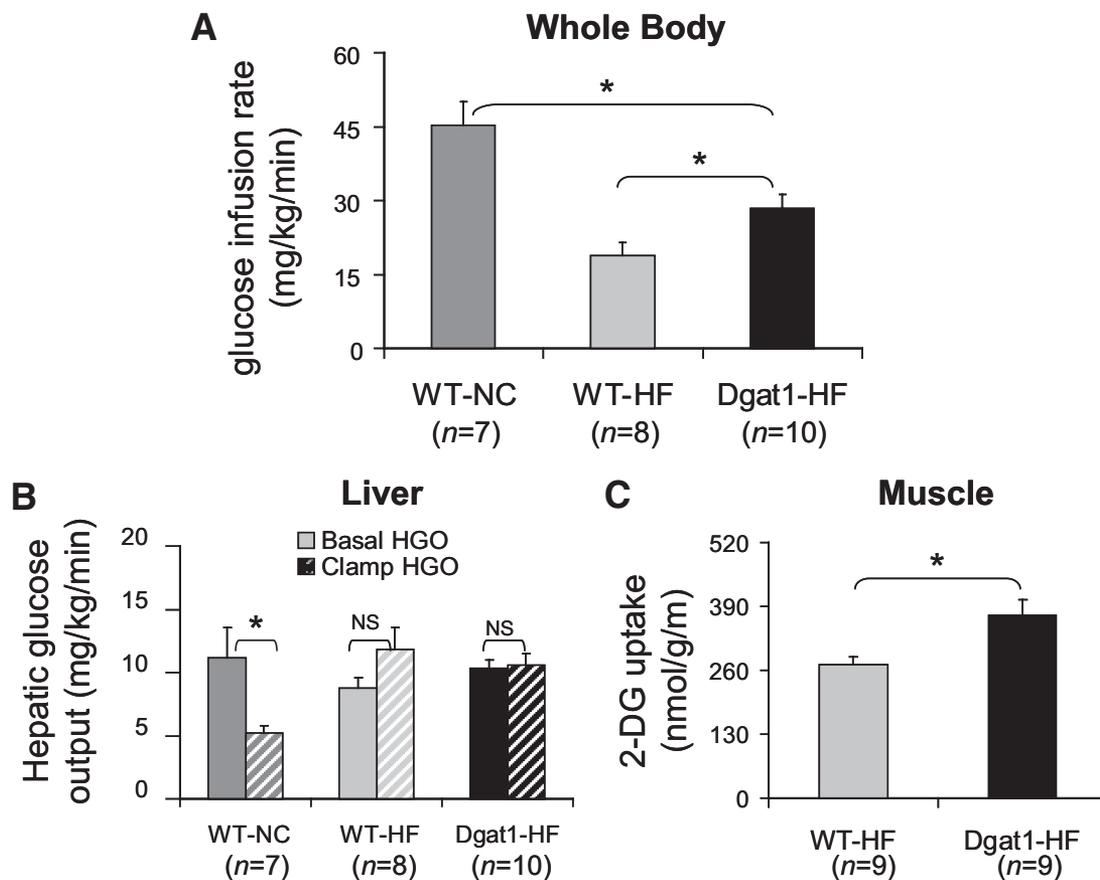


FIG. 1. Whole-body, hepatic, and muscle insulin sensitivity assessed by hyperinsulinemic-euglycemic clamp. **A:** Steady-state whole-body glucose infusion rates in age-, sex-, genetic background-, and breeding environment-matched wild-type mice on standard rat diet (WT-NC), wild-type mice pretreated with ad lib HFD feeding for 8 weeks (WT-HF), and MCK-DGAT1 mice pretreated with ad lib HFD feeding for 8 weeks (DGAT1-HF). **B:** Steady-state HGO before (basal HGO) and after (clamp HGO) insulin infusion at the rate of $3 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in WT-NC, WT-HF, and DGAT1-HF mice under the same clamp conditions as in **A**. **C:** 2-Deoxyglucose uptake in skeletal muscle after bolus injection of the [^{14}C]-labeled 2-deoxyglucose under the same clamp conditions as in **A** and **B**. Data are presented as means \pm SE; *P* and *n* are as indicated; **P* < 0.05. 2-DG, 2-deoxyglucose.

conditions, a bolus infusion of 2-deoxy-D-[^{14}C]glucose was given. Uptake of 2-deoxy-D-[^{14}C]glucose in skeletal muscle was $\sim 30\%$ greater in DGAT1-HF mice than in WT-HF mice (Fig. 1C), confirming that increased lipotoxicity tolerance in skeletal muscle was the physiological basis for the protection of MCK-DGAT1 mice, albeit incomplete, against HFD-induced whole-body insulin resistance.

DGAT1 overexpression in skeletal muscle leads to improved mitochondrial fatty acid oxidation efficiency as well as increased triglyceride synthesis and storage. As expected, muscle DGAT activity was threefold increased in MCK-DGAT1 (Fig. 2A), which was associated with a $\sim 70\%$ increase in muscle triglyceride content (Fig. 2B). To characterize skeletal muscle fatty acid oxidation capacity, we first determined mitochondrial DNA (mtDNA) copy number by assessing the ratio of mtDNA ND1 and ND4 to the nuclear DNA β -actin gene in soleus and anterior tibial muscles. There were 30–45% fewer mitochondria per copy genome DNA in DGAT1-overexpressing tibial and soleus muscles than those of the wild-type controls (Fig. 2C). However, analyses of mitochondrial ATP production rates (MAPR) per unit muscle protein revealed no reduction in transgenic mice as compared with wild-type mice (Fig. 2D). Maximal tissue citrate synthase activity was significantly greater in the DGAT1-overexpressing soleus muscle than the wild-type counter-

part was, although no significant difference was detected in tibialis anterior (Fig. 2D). Maximal tissue β -HAD activity per unit muscle protein was significantly greater in the transgenic muscle than the wild-type muscle (Fig. 2E). Similarly, mitochondrial CPT-I activity per unit mitochondrial protein was significantly greater in the DGAT1-overexpressing muscle than the wild-type muscle (Fig. 2E). The unchanged or potentially enhanced mitochondrial oxidative capacity in the presence of fewer mitochondria suggested a higher mitochondrial efficiency in DGAT1-overexpressing muscles. Direct examination of soleus muscles by electron microscopy confirmed that fewer, but bigger, mitochondria were present in the DGAT1-overexpressing muscle (Fig. 2F) than in the wild-type muscle (Fig. 2G). Additionally, in vitro measurement of maximal fatty acid oxidation rates demonstrated a 20% increase in fatty acid oxidation capacity in the MCK-DGAT1 muscle compared with the wild-type muscle ($1,217$ vs. $1,019 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ mitochondrial protein, *P* < 0.01; Fig. 2H).

Upregulation of PDK4 and UCP3 in skeletal muscle of MCK-DGAT1 transgenic mice. mRNA levels of marker genes in fat metabolism were examined by real-time PCR in soleus muscle from 4-month-old male wild-type and MCK-DGAT1 transgenic mice after 8-week HFD feeding (Fig. 3A). DGAT1 mRNA levels were approximately threefold as high in MCK-DGAT1 mice as in wild-type mice (*P* <

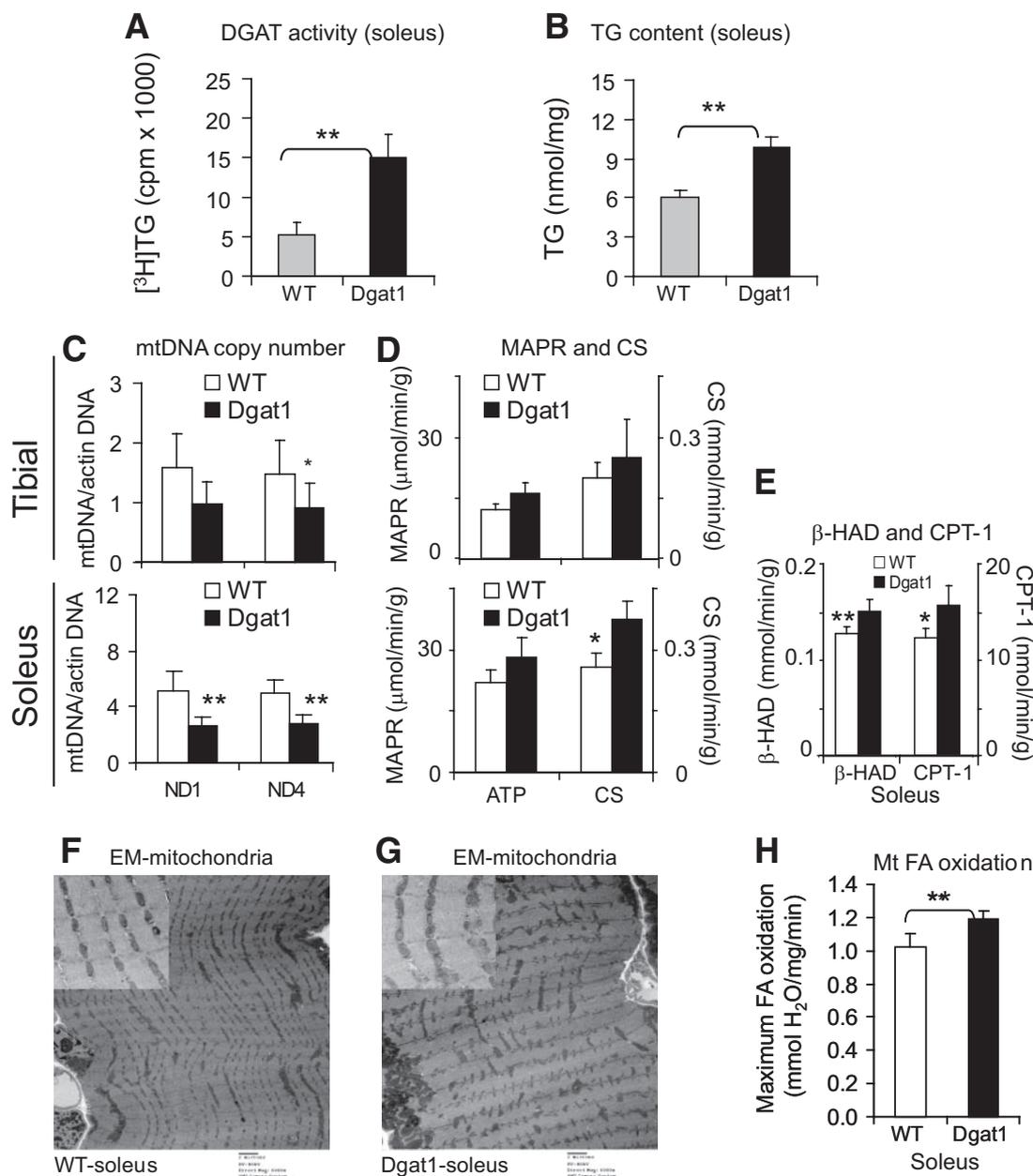


FIG. 2. Assessment of anabolic and catabolic fatty acid metabolism in skeletal muscle of MCK-DGAT1 and control wild-type mice. Male mice pretreated with 8-week HFD as described in Fig. 1. **A:** DGAT activity in soleus muscles isolated from the 4-month-old wild-type and MCK-DGAT1 mice ($n = 4$, each group). **B:** Triglyceride content in soleus muscle from the wild-type ($n = 5$) and MCK-DGAT1 ($n = 6$) mice. **C:** Mitochondrial copy numbers as determined by PCR quantification of ND1 or ND4 as mitochondrial DNA (using β -actin as reference for nuclear DNA) in anterior tibial and soleus muscles of the wild-type ($n = 7$) and MCK-DGAT1 ($n = 6$) mice. **D:** MAPR (determined in isolated mitochondria) and citrate synthase activity (measured in muscle homogenates) of the anterior tibial and soleus muscles from the wild-type ($n = 7$) and MCK-DGAT1 ($n = 6$) mice. **E:** β -HAD activity (measured in muscle homogenates) and CPT-1 activity (measured in isolated mitochondria) of the soleus muscles from the wild-type and MCK-DGAT1 mice ($n = 6$, each group). **F** and **G:** Representative low- and high-power (*inserts*) views of the electron microscopic fields of the soleus muscle from the wild-type (**F**) and MCK-DGAT1 (**G**) mice. Quantification of $6000\times$ EM micrographs using an image analysis system (Imagine-Pro Plus 5.0, Media Cybernetics) showed that wild-type and DGAT1 mice have 149 ± 3.6 and 98 ± 7.7 mitochondria per 10×10 micron muscle area, respectively ($P < 0.001$). The average mitochondrial size is $34,432 \pm 4,246$ pixels for wild-type and $62,418 \pm 938$ pixels for DGAT1 mice ($P = 0.014$). **H:** Maximal mitochondrial fatty acid oxidation rates measured in isolated mitochondria of the soleus muscles from the wild-type and MCK-DGAT1 mice ($n = 6$, each group). Values are expressed as means \pm SE; NS, no statistical significance ($P > 0.05$); * $P < 0.05$, ** $P < 0.01$ (n as indicated). CS, citrate synthase; FA, fatty acid; 28S, 28S rRNA; TG, triglyceride; WT, wild type.

0.05) as expected, and mRNA levels for PDK4 and UCP3 were doubled in MCK-DGAT1 compared with wild-type mice ($P < 0.05$). There was also a trend of approximate doubling of mRNA levels for CD36 ($P = 0.051$), CPT1 ($P = 0.120$), ACC2 ($P = 0.072$), and GLUT4 ($P = 0.117$) in MCK-DGAT1 mice. No significant changes were detected in mRNA levels for DGAT2, fatty acids, ACS1, ADRP, sterol regulatory element-binding protein-1c, peroxisome

proliferator-activated receptor (PPAR)- α , PPAR δ , PPAR γ , peroxisome proliferator-activated receptor γ coactivator- β , CRAT, ECHS1, UCP1, UCP2, and PEPCK.

PDK4 is believed to play a critical role in regulating the fuel partitioning between fat and glucose oxidation (31,32). To further understand the changes in PDK4 expression in MCK-DGAT1 muscles, we compared a group of DGAT1-overexpressing mice with a group of wild-type

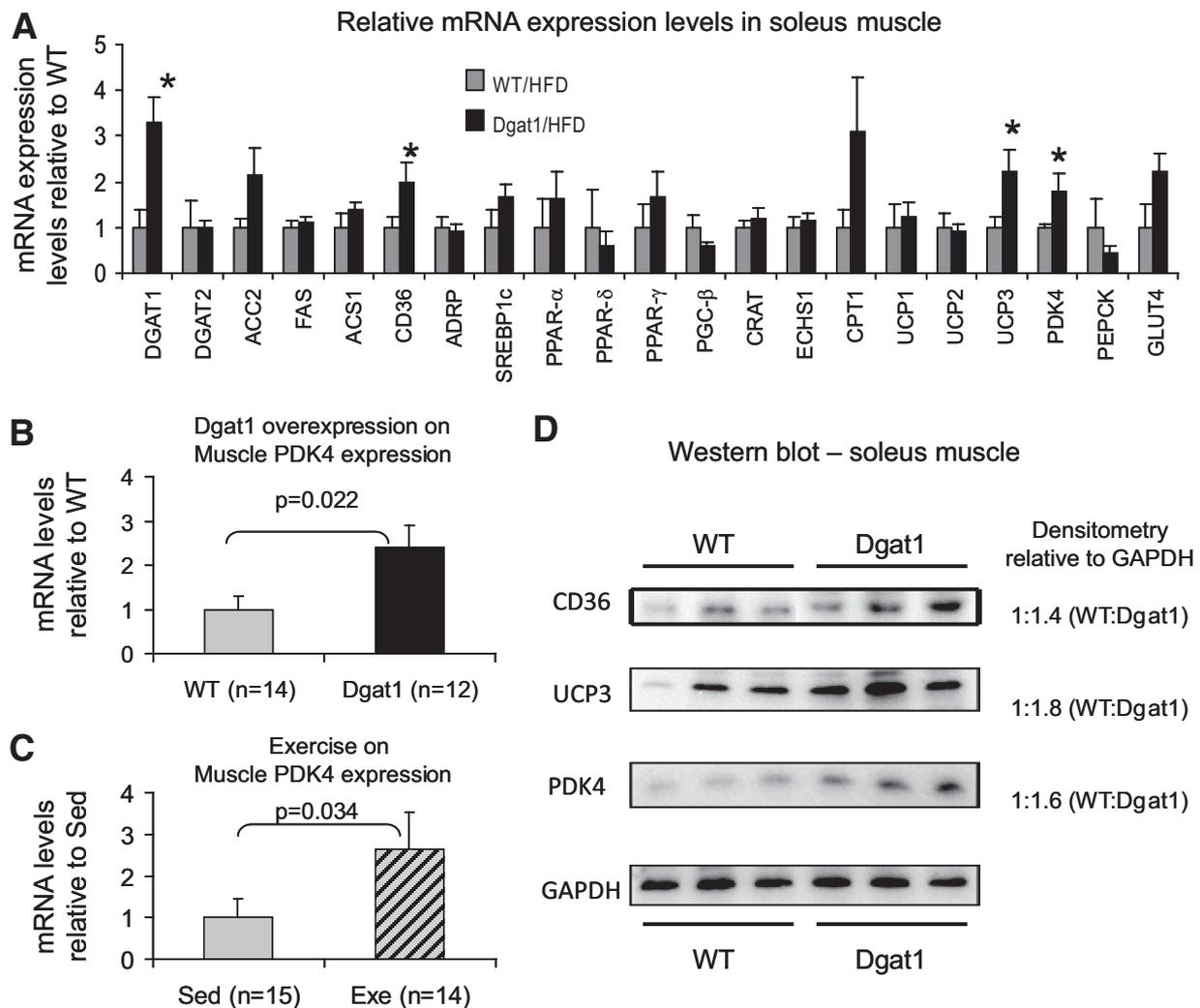


FIG. 3. Effects of DGAT1 overexpression and exercise on relative levels of gene expression in soleus muscle. **A:** Gene expression levels were measured by RT-PCR in soleus muscles isolated from wild-type and MCK-DGAT1 mice pretreated with 8-week HFD as described in Fig. 1, using primer sets listed in suppl. Table 1. Gene abbreviations are the same as listed in suppl. Table 1. * $P < 0.05$ ($n = 5-7$ in each group). **B:** Relative gene expression levels of PDK4 in soleus muscles from HFD-pretreated wild-type versus MCK-DGAT1 mice. **C:** Relative gene expression levels of PDK4 in soleus muscles from sedentary (Sed) versus exercised (Exe) wild-type mice. The swimming exercise regimen is as previously described (3); P values and n are as indicated. **D:** Western blot analysis of CD36, UCP3, and PDK4 in soleus muscle from the 8-week HFD-pretreated wild-type and MCK-DGAT1 mice. WT, wild type.

mice after 1-week swimming exercise (3). Using sedentary wild-type mice as a baseline control, both DGAT1 overexpression and exercise led to ~ 1.5 -fold increases ($P < 0.05$) in PDK4 mRNA levels in skeletal muscle (Fig. 3B and C), providing two independent cases in which upregulation of PDK4 mRNA expression was dissociated from glucose intolerance. Western blot of CD36, UCP3, and PDK4 showed 1.4- to 1.8-fold increases of these proteins in DGAT1-overexpressing muscle relative to wild-type muscle (Fig. 3D).

MCK-DGAT1 mice display increased energy expenditure without changes in food intake. To determine the physiological significance of the above in vitro findings and to help interpret the physiological consequences of the increased triglyceride synthesis and increased mitochondrial fatty acid oxidation capacity (and higher expression levels of the uncoupling protein UCP3) in DGAT1-overexpressing skeletal muscle, we measured whole-body energy metabolism in vivo. Four-month-old weight- and adiposity-matched (Fig. 4A) male MCK-DGAT1 and wild-type mice were analyzed in metabolic chambers after

8-week HFD feeding. MCK-DGAT1 mice appeared less physically active than wild-type mice (Fig. 4B), although the changes seemed to be unrelated to food-seeking behavior, as food intake was the same between MCK-DGAT1 and wild-type mice (Fig. 4G). In contrast to the changes in physical activity, oxygen consumption rates were significantly higher in the transgenic mice than in wild-type mice (Fig. 4C, wild-type vs. MCK-DGAT1, $P < 0.05$, repeated-measures ANOVA). No significant difference was detected in respiration exchange rates (RER) under these conditions (Fig. 4D). Further experimental maneuver with two consecutive 24-h measurements of V_{O_2} during fasting followed by refeeding revealed that the difference in energy expenditure was accentuated during refeeding. As expected, energy expenditure reduced and increased in response to prolonged fast and refeeding, respectively, in both wild-type and MCK-DGAT1 mice (Fig. 4E). However, the increase during refeeding was markedly greater in the transgenic mice than in wild-type mice ($P < 0.05$, repeated-measures ANOVA) (Fig. 4F). As was during regular ad lib feeding, no difference in food intake was observed during

higher than that of the WT-HF mice. No differences in weight were observed between wild-type and MCK-DGAT1 mice on standard rodent diet (data not shown).

DISCUSSION

In this study, we first demonstrated *in vivo* that although skeletal muscle of MCK-DGAT1 mice was more resistant to developing HFD-induced lipotoxicity, their liver was as susceptible for lipotoxic insulin resistance as that of wild-type mice. Under hyperinsulinemic-euglycemic clamp conditions, MCK-DGAT1 mice were ~45% protected against HFD-induced insulin resistance at the whole-body level compared with wild-type mice. The greater whole-body insulin sensitivity in HFD-fed MCK-DGAT1 mice (compared to HFD-fed wild-type mice) was associated with ~30% higher uptake of bolus-injected 2-deoxyglucose in skeletal muscle under the same feeding and clamp conditions, whereas insulin suppression of HGO was similarly impaired in these mice, indicating that liver did not contribute to the improved whole-body insulin sensitivity.

We showed that although triglyceride synthesis and storage capacities were increased in DGAT1-overexpressing muscle, mitochondrial fatty acid oxidation efficiency was also improved. The latter was initially recognized by decreased mitochondrial copy number in combination with the uncompromised muscle citrate synthase activity and increased maximal β -HAD and CPT-I activities in DGAT1-overexpressing muscles. This was then confirmed by increased maximal mitochondrial fatty acid oxidation capacity per unit mitochondrial protein in DGAT1-overexpressing muscles. Interestingly, MAPR per unit mitochondrial proteins was similar in the two genotypes, suggesting that the increased fatty acid oxidation efficiency does not lead to increased energy efficiency. Gene expression levels of muscle PDK4 and UCP3, normalized to β -actin expression levels, were ~100% greater in HFD-fed MCK-DGAT1 mice than in age-, sex-, and diet-matched wild-type mice. Although increased PDK4 has been associated with greater fuel partition into fatty acid oxidation (31,32), higher levels of UCP3 may also signify increased use of fat as fuel substrate and/or conversion of fat to thermal energy (33,34). It is plausible that the lack of increase in MAPR in DGAT1-overexpressing muscle, in the presence of increased fatty acid oxidation rates, is because of increased uncoupling of oxidative phosphorylation leading to increased heat production. Although heat production was not directly measured, whole-body 24-h V_{O_2} production rates were greater in DGAT1-HF than in WT-HF mice in the absence of increased physical activity in the former. The increased energy expenditure in MCK-DGAT1 mice was further substantiated by the modest but significant lower body weight in the transgenic mice relative to wild-type mice without any difference in energy intake; long-term follow-up of the growth curve showed that MCK-DGAT1 mice weighed 5–10% less than wild-type mice on an *ad lib* HFD feeding regimen, whereas food consumption was the same in both groups. The reduced body weight may contribute to the improved insulin sensitivity shown in this study. However, the small weight difference appears to result from changed fuel metabolism and is unlikely a primary cause of the observed protection against lipotoxicity. Marked differences in insulin sensitivity were seen between “weight-matched” wild-type and MCK-DGAT1 mice and between isolated DGAT1 muscle

and wild-type muscle in *ex vivo* experiments, where weight was not a factor (3).

Insulin resistance is associated with mitochondrial dysfunction in type 2 diabetes (23,35,36) and in the offspring of parents with type 2 diabetes (37). Reduced mitochondrial function and insulin resistance occur in aging in association with reduced mitochondrial DNA copy number (23). However, the issue regarding mitochondrial deficiency and insulin resistance is unresolved (38). The present study does not support the hypothesis that reduction in mitochondrial DNA copy number *per se* results in insulin resistance. In our model, despite a reduction in mitochondrial number, mitochondrial ATP production is maintained (and heat production probably increased) because of increased fatty acid oxidation efficiency. These findings suggest that mitochondrial quality is more critical. In this regard, a recent study by Koves *et al.* demonstrated that stressed mitochondria with greater but incomplete fatty acid oxidation are only detrimental to insulin sensitivity (39). The present study further suggests that greater fatty acid oxidation capacity associated with increased uncoupling activity may also be important in maintaining insulin sensitivity. Of course, maintaining adequate oxidative phosphorylation is necessary for muscle contractility, particularly during exercise.

We have previously shown that skeletal muscle overexpressing DGAT1 behaved similarly to exercise-trained muscle in that it had higher triglyceride content but was more insulin sensitive, a phenomenon referred to as the “athlete paradox” or “exercise paradox.” The decreased myocytic DAG and ceramide levels with increased myocytic triglyceride content are the expected effects of DGAT1 actions (3). By converting fatty acyl-CoA and DAG to triglyceride, DGAT1 is believed to be directly responsible for lowering the levels of the lipotoxic fatty acid derivatives. The key finding of the present study is that DGAT1-overexpressing muscle, like exercised muscle, had increased fatty acid oxidation capacity, which was associated with increased steady-state metabolic rates at the whole body level and a smaller HFD-induced weight gain compared with wild-type mice. Importantly, because these findings are made in the muscle-specific DGAT1 transgenic mice, DGAT1 overexpression must be primarily responsible for these paradoxical changes. Because increased DGAT1 expression is also observed with exercise, we further postulate that DGAT1 may play a key mediatory role in exercise-induced augmentation of both triglyceride synthesis and, indirectly, mitochondrial fatty acid oxidation. Together, the increased anabolic (triglyceride synthesis/storage) and catabolic (fatty acid oxidation/degradation) capacities may both help remove fatty acid intermediates that can be overproduced during fatty acid overload. Reducing intramyocellular DAG levels to below the “threshold of lipotoxicity” is believed necessary to prevent the induction of a cascade of signaling events including activation of PKC isoforms and Jun NH₂-terminal kinase-1 and inhibition of IRS-1, Akt, and GLUT4 in HFD-fed mice (3).

Parallel upregulated triglyceride synthesis and fatty acid oxidation seemed perplexing at the first glance because anabolic and catabolic pathways usually undergo changes in the opposite directions, determined by cellular substrate levels and energy status. However, examination of typical myofibers does show interdependency between the two aspects of fatty acid metabolism: myofibers that have a high triglyceride content are typically the “oxidative”

type characterized by a high mitochondrial capacity, whereas those with low triglyceride content (and high glycogen content) are typically “glycolytic.” Importantly, compared with glycolytic myofibers, oxidative myofibers with greater capacities for triglyceride synthesis and fatty acid oxidation appear to be intrinsically more insulin sensitive (4). The present study provided an example of the “paradoxical” coupling of lipid synthesis and oxidation in the DGAT1-overexpressing muscles.

Clearly, these are not the usual reciprocal regulations of the two aspects of the fatty acid metabolism driven by the fatty acid substrate availability. DGAT1 overexpression in skeletal muscle increased the capacities of both triglyceride synthesis and fatty acid oxidation and enabled the myocytes to endure fat overload without developing significant lipotoxicity or insulin resistance. However, instead of increasing mitochondrial densities, DGAT1-overexpressing muscle appeared to have greater mitochondrial fatty acid oxidation efficiency. Whether the improved fatty acid oxidation is coupled with increased fatty acid transport (as suggested by upregulation of CD36 and CPT-1) and/or potentially increased de novo fatty acid synthesis will require further investigation. A “futile cycle” of fatty acid metabolism that consists of increased fatty acid oxidation coupled with increased de novo fatty acid synthesis has been proposed as a thermogenic mechanism in muscle (40).

PDK is a key regulator of fuel partitioning between fatty acid and glucose oxidation, and PDK4 is the major isoform in muscle (41,42). PDK is acutely regulated, in general, by small molecular effectors generated from fatty acid β oxidation. Gene expression of PDK4 also responds to fuel substrate availability and insulin levels (43–46). Additionally, PDK4 gene expression appears to be modulated by several more complex biological processes, such as insulin resistance or exercise. Insulin resistance is generally associated with upregulation of PDK4 (43,45), which may be because of elevated fatty acid overload (47,48) or deficient/impaired insulin action (45,46,48). While promoting muscle insulin sensitivity, exercise also “paradoxically” upregulates PDK4 gene expression in skeletal muscle (49,50). Therefore, it appears that a different mechanism for upregulating PDK4 is involved in exercise-trained skeletal muscle and that this mechanism is not substrate driven (49). Exercise upregulates PDK4 and fatty acid oxidation but does not inhibit insulin-stimulated glucose oxidation. In the present study, we showed that DGAT1 overexpression produced a result similar to exercise with regard to PDK4 upregulation, increased fatty acid oxidation, and insulin sensitivity, suggesting that the change in PDK4 expression in this model is also not a secondary response to reduced insulin actions. It is currently unclear, in either the exercise model or the DGAT1-overexpression model, what mediates upregulation of PDK4 gene expression and whether increased mitochondrial fatty acid oxidation is directly caused by upregulated PDK4.

In summary, transgenic expression of DGAT1 in skeletal muscle results in a series of “paradoxical” metabolic changes often seen in the exercise-trained muscle, including increased triglyceride synthesis, increased fatty acid oxidation, and preserved insulin sensitivity during the challenging of fat overload. Although a high triglyceride content and PDK4 expression level may serve as markers of insulin resistance in sedentary and obese individuals, the same parameters may reflect a greater capacity for

fat metabolism. In the latter situation, insulin sensitivity is preserved because of reduced levels of intracellular fatty acid derivatives. Because DGAT1 is induced in exercise, and because the MCK-DGAT1 model presents a set of metabolic features similar to the exercise model, we hypothesize that DGAT1 mediates a significant part of exercise-induced metabolic remodeling in skeletal muscle.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health (NIH) Grants DK-60530, DK-071866, DK-063034, HL-73029, and HL45095. Contributions also came from New York Obesity Research Center Grant DK-026687 and Albert Einstein College of Medicine Diabetes Research and Training Center Grant NIH DK-020541.

No potential conflicts of interest relevant to this article were reported.

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