Metformin and exercise reduce muscle FAT/CD36 and lipid accumulation and blunt the progression of high-fat diet-induced hyperglycemia

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Acute fatty acid (FA) exposure, as well as chronically increased FA exposure (e.g., in obesity or T2D), leads to increased GLUT4 (22, 29, 59) and improved insulin-stimulated glucose uptake (29), as well as peroxisome proliferator-activated coactivator-1α (PGC-1α) protein content (57) and increased mitochondrial biogenesis (59), which may restore normal lipid metabolism. Previous studies have shown that AMPK content is not downregulated in obese human skeletal muscle (54) and is capable of being activated during an acute exercise bout in muscle from subjects with T2D (41), suggesting that AMPK is a possible therapeutic target in T2D. The antiobesity agent metformin acutely activates AMPK in muscle (13, 20, 61) and liver (61). One week of metformin treatment in rats prevented acute lipid-induced insulin resistance and was shown to be associated with activation of AMPK in liver but not skeletal muscle (12). Four to ten weeks of metformin treatment activates AMPK in skeletal muscle of subjects with T2D (41), suggesting a time-dependent effect of metformin on regulation of skeletal muscle AMPK. Prospective studies have shown that lifestyle changes (diet, exercise) and metformin reduce the progression of T2D in subjects with impaired glucose tolerance (33). However, the muscle-specific mechanisms induced by metformin, as well as the involvement of AMPK, in correcting the abnormalities in muscle FA metabolism associated with insulin resistance and T2D are unclear.

The female Zucker diabetic fatty (ZDF) rat is a model of high-fat (HF) diet-induced T2D. Within 3–4 wk of being placed on a HF (48 kcal %fat) diet, these rats consistently develop diabetes that parallels the progression of T2D seen in humans (14). The female ZDF rat is a particularly attractive model for studying aspects of muscle metabolism involved in the development of T2D, because pancreatic β-cell function and blood insulin levels are well preserved (42, 43). Previous studies in the male ZDF rat have demonstrated that chronic AICAR treatment (44, 60) and exercise (44) blunt the progression of diabetes, but this may be associated with improvements in β-cell mass and morphology (44, 60) in addition to effects on skeletal muscle metabolism. It is unknown whether exercise...

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and metformin alter the progression of T2D via AMPK-related mechanisms in skeletal muscle of female ZDF rats. Therefore, the aims of the current study were to determine whether 8 wk of metformin and exercise interventions, alone or in combination, would 1) minimize the derangements in glucose homeostasis in the female ZDF rat and 2) be related to changes in skeletal muscle AMPK regulation and FA metabolism. Specifically, we hypothesized that metformin and exercise, alone or in combination, would improve hyperglycemia and insulin-stimulated glucose transport in skeletal muscle. Furthermore, we hypothesized that improvements in skeletal muscle glucose transport would be related to an increase in AMPK expression and a decrease in the abundance of plasma membrane FA transporters and improved partitioning of FA toward oxidation, ultimately leading to reduced DAG and ceramide accumulation.

MATERIALS AND METHODS

Animals

Female Zucker lean (+/−) or diabetic fatty (ZDF, fafa) rats were obtained from Charles River Laboratories (Senneville, QC, Canada) at 5 wk of age. The animals were housed individually in a controlled environment with a reversed 12:12-h light-dark cycle. Animals were fed standard rat chow (Purina Formula 5008), known to maintain normoglycemia in female ZDF rats (14) for a period of 5–7 days. Lean rats were maintained on standard rat chow, whereas ZDF rats were randomly assigned to either standard rat chow (16.7 kcal %fat, Purina Formula 5008) or HF diet (48 kcal %fat, Research Diets C13004 formula; New Brunswick, NJ) alone or with one of the following interventions: metformin (HF-Met), exercise (HF-Ex), or the combination of exercise and metformin (HF-E+M). Twelve animals were assigned to each group. All protocols were approved by the Animal Care Committee, University of Guelph.

Feeding and Training Protocol

Rats were fed their respective diets ad libitum for 8 wk. Metformin was dissolved in water (50 mg/ml), supplemented in the diet at an initial dose of 250 mg/kg body wt for 1 wk, and subsequently increased to 500 mg/kg body wt for the duration of the study. These metformin doses are comparable to those previously used (i.e., 100–500 mg/kg body wt) in various rodent models (3, 7, 12, 47, 51). Rats randomized to HF-Ex and HF-E+M were given two acclimation exercise sessions on a motorized treadmill at 13 m/min (0% grade) for 5 min, followed by 5 min of rest. Then, the rats were trained five days per week and began with the rats running for 15 min at 15 m/min on a 0% grade, gradually increasing to 2 h at 18–19 m/min on a 10% grade by the end of 4 wk, which was maintained for the remaining 4 wk. To prevent any acute effects of the last training bout, we carried out experimental procedures 48 h after the last training session. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (15 mg/kg body wt) before all procedures.

Blood and Tissue Sampling

Tail artery fasting blood was collected before the commencement of interventions and after 4 wk from animals anesthetized with isoflurane inhalant. At the completion of the 8 wk feeding and exercise protocols, rats were fasted overnight and blood was collected via cardiac puncture after the excision of all tissues. Blood was transferred to heparinized tubes and kept on ice; plasma was collected following centrifugation (10,000 g, 5 min). Fasting plasma insulin was assayed using a rat RIA kit (Linco, St. Charles, MO). Fasting plasma glucose was analyzed using a calibrated Ascencia Elite glucometer (Bayer).

To determine basal- and insulin-stimulated glucose transport and FA metabolism, we carefully dissected soleus muscles into longitudinal strips from tendon to tendon with a 27-gauge needle for incubation. Intact soleus muscles were removed for determination of lipid content and protein expression. It is important to note that analyses of protein expression of glucose (GLUT4) and FA transporters were determined in whole tissue and giant sarcolemmal vesicles prepared from red gastrocnemius (RG) muscle without any prior incubation to assess chronic protein expression caused by the interventions.

Basal- and Insulin-Stimulated Skeletal Muscle Glucose Transport

Soleus strips were equilibrated for 30 min in 2 ml of pregassed (95% O2-5% CO2) Krebs-Henseleit buffer (KHB; 0.1% BSA, 30°C) containing 8 mM glucose and 32 mM mannitol, in the absence or presence of insulin (10 μU/ml, maintained in all subsequent steps), in a gentle shaking bath. Muscle strips were washed (2 × 10 min) with glucose-free KHB (4 mM pyruvate, 36 mM mannitol). Solei were then incubated for 20 min (insulin) or 40 min (basal) in KHB [4 mM pyruvate, 8 mM 3-0-[3H]methyl-D-glucose (800 μCi/mmol), 28 mM [14C]mannitol (60 μCi/mmol)]. Muscles were blotted, weighed, and digested (95°C, 10 min, 1 ml of NaOH). Glucose transport was calculated from a 200-μl aliquot of muscle digest to quantify intracellular 3-O-[3H]methyl-D-glucose.

Skeletal Muscle FA Metabolism

Soleus strips were equilibrated in 2 ml of pregassed (95% O2-5% CO2) KHB (4% BSA, 30°C), with 1 mM palmitate and 5 mM glucose in a gentle shaking bath for 30 min. Muscles were incubated for an additional 60 min with the addition of 0.5 μCi/ml [1-14C]palmitate to determine exogenous palmitate oxidation and incorporation into triacylglycerol (TAG) as outlined previously (50).

Whole Tissue Lipid Content Analysis

Soleus muscle (~50–80 mg) was freeze-dried, powdered, and cleaned of any visible connective tissue, also without prior incubation. Individual reactive lipids (DAG and ceramide) were extracted and measured using gas-liquid chromatography as described previously (9). Because of tissue limitations and previous data suggesting that neutral lipids most likely do not play a role in insulin-signaling abnormalities in skeletal muscle (8, 9), whole tissue TAG was not analyzed in these samples.

Preparation of Giant Sarcolemmal Vesicles

Giant sarcolemmal vesicles were prepared from oxidative RG muscle in the absence of acute insulin stimulation as described previously (10) and were frozen at −80°C until analyzed for sarcolemmal membrane-associated FA and glucose transport protein expression.

Preparation of Tissue Lysates for Western Blotting and Enzyme Activity Assays

Muscle tissue (~50 mg, soleus or RG) was homogenized (5,000 μl/g tissue, 1:5 dilution) in ice-cold buffer suitable for protein extraction and preserving phosphorylation states of proteins as described previously (50).

Western Blot Analyses

Fifty micrograms of whole tissue lysate protein or 10 μg of giant sarcolemmal vesicle protein [for fatty acid translocase (FAT/CD36, plasma membrane-bound fatty acid binding protein (FABPpm), GLUT4] were solubilized in 4X Laemmli’s buffer, boiled (95°C, 5 min), resolved by SDS-PAGE, and wet-transferred to polyvinylidene difluoride membranes [1–1.5 h, 100 V; Ser79-phosphorylated
acetyl-CoA carboxylase (pACC); 8–15 h, 25–40 V, 4°C). The membranes were blocked for 1 h and then incubated with the specific primary antibodies for Thr172-phosphorylated AMPK (pAMPK; Cell Signaling), total AMPKα (tAMPK; Cell Signaling), AMPKα1 (Upstate), AMPKα2 (Upstate), pACC (Cell Signaling), GLUT4 (Chemicon), sarcollemmal FABPpm (gift from Dr. J. Calles-Escandon, Wake Forest University School of Medicine), FAT/CD36 (gift from Dr. N. N. Tandon, Otsuka Maryland Medicinal Laboratories), and PGCl –α (Calbiochem). After incubation with appropriate secondary antibody, the immune complexes were detected using the enhanced chemiluminescence method and quantified with densitometry.

Measurement of Oxidative Enzyme Activity in Soleus Muscle Homogenates

Citrate synthase (CS; 37°C, 412 nm) and β-hydroxyacyl-CoA dehydrogenase activity (β-HAD; 37°C, 340 nm) were determined spectrophotometrically from soleus muscle lysates diluted 1:20 (34, 52).

Calculations and Statistics

To calculate palmitate oxidized or incorporated into lipid pools (nmol/g wet wt), we used the specific activity of the incubation buffer (dpm radiolabeled palmitate/nmol total palmitate). Soleus muscle glucose transport (nmol glucose analog (dpm radiolabeled palmitate/nmol total palmitate). Soleus muscle glucose transport (nmol glucose analog (dpm radiolabeled palmitate/nmol total palmitate).

Results are presented as means ± SE. Two-way ANOVA were performed for body mass, fasting plasma glucose, and insulin (time × treatment). For all end-point measurements, one-way ANOVA were performed to analyze significant differences with Fisher least significant difference post hoc analyses. Statistical significance was accepted at P ≤ 0.05, with note of any trends (0.05 < P ≤ 0.10) marked in parentheses.

RESULTS

Body Composition

Body mass of all ZDF groups was increased compared with lean rats at weeks 0 (+50%, P < 0.01), 4 (70–80%, P < 0.001), and 8 (+80–90%, P < 0.001; Table 1). The final body mass of all ZDF groups was not different at the end of the trial.

Plasma Insulin and Glucose

Insulin. There were no initial differences in fasting plasma insulin among any of the groups (Table 1). By week 4, plasma insulin levels were higher than at week 0 in all ZDF HF-fed rats (P < 0.05) except for HF-E+M; however, the increase in plasma insulin was greatest in rats fed the HF diet alone (i.e., no intervention). After 8 wk, all HF rats were hyperinsulinemic compared with lean rats (P < 0.001).

Glucose. At week 0, there were no differences in fasting plasma glucose among any of the groups (Table 1). By week 4, fasting plasma glucose was increased in HF-fed rats compared with lean (+146%) and control rats (+80%), whereas the rise in fasting plasma glucose was attenuated in HF-Ex (+80%) and HF-E+M rats (+60%, P < 0.05). By week 8, HF rats developed hyperglycemia (24 ± 2.1 mM), which was greater than in lean (+193%, P < 0.001) and control rats (+44%, P < 0.001). In all interventions, the progression of hyperglycemia was attenuated with a reduction in fasting plasma glucose (−26–60%, P < 0.01), suggesting a blunting in the progression of diabetes.

Skeletal Muscle Basal and Insulin-Stimulated Glucose Transport and GLUT4 Protein Expression

There were no significant differences in soleus muscle basal glucose transport among any of the groups (Fig. 1A). All ZDF groups exhibited impaired insulin-stimulated glucose transport (−40–80%, P < 0.001) compared with lean rats. In addition, HF (−32%, P < 0.001) and HF-Met rats (−35%, P < 0.001) exhibited impaired insulin-stimulated glucose transport compared with control, HF-Ex (P = 0.068), and HF-E+M rats. Insulin-stimulated glucose transport was partially normalized in HF-Ex and HF-E+M rats and was not different from that in control rats (Fig. 1B). Both HF-Ex and HF-E+M rats exhibited increases in RG whole muscle (HF-Ex: +23–26%, P < 0.05; HF-E+M: +31–35%, P < 0.01; Fig. 2A) and sarcolemmal membrane-associated GLUT4 protein content (HF-Ex: +45–55%, P < 0.05; HF-E+M: +70–80%, P < 0.001; Fig. 2B). Soleus whole muscle GLUT4 protein expression was similar to

Table 1. Body weight, fasting plasma insulin, and glucose from lean and ZDF rats on control or HF diet

<table>
<thead>
<tr>
<th>ZDF</th>
<th>Lean</th>
<th>C</th>
<th>HF</th>
<th>HF-Met</th>
<th>HF-Ex</th>
<th>HF-E+M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
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<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>100±1</td>
<td>146±5*</td>
<td>146±4*</td>
<td>153±6*</td>
<td>137±5*</td>
<td>139±4*</td>
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<tr>
<td>Week 4</td>
<td>160±2</td>
<td>274±4*</td>
<td>286±8*</td>
<td>290±7*</td>
<td>285±3*</td>
<td>273±4*</td>
</tr>
<tr>
<td>Week 8</td>
<td>188±3</td>
<td>356±6*</td>
<td>345±8*</td>
<td>350±7*</td>
<td>339±3*</td>
<td>337±4*</td>
</tr>
<tr>
<td>Fasting plasma insulin, ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>0.3±0.1</td>
<td>2.6±2.5</td>
<td>2.2±0.5</td>
<td>1.4±0.3</td>
<td>1.8±0.3</td>
<td>3.0±0.8</td>
</tr>
<tr>
<td>Week 4</td>
<td>0.4±0.1</td>
<td>3.4±0.4</td>
<td>13.9±1.4*</td>
<td>7.0±1.3*</td>
<td>7.7±0.8*</td>
<td>2.5±0.4*</td>
</tr>
<tr>
<td>Week 8</td>
<td>1.9±0.3</td>
<td>13.2±0.8*</td>
<td>16.2±1.6*</td>
<td>17.2±2.2*</td>
<td>18.1±1.2*</td>
<td>14.2±1.3*</td>
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<td>Fasting plasma glucose, mM</td>
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</tr>
<tr>
<td>Week 0</td>
<td>2.6±0.7</td>
<td>6.7±0.8</td>
<td>5.5±0.5</td>
<td>7.8±3.3</td>
<td>6.6±0.9</td>
<td>6.6±1.1</td>
</tr>
<tr>
<td>Week 4</td>
<td>4.1±1.2</td>
<td>5.6±0.5</td>
<td>10.1±2.9*</td>
<td>7.1±1.3*</td>
<td>5.6±0.6*</td>
<td>6.5±0.5*</td>
</tr>
<tr>
<td>Week 8</td>
<td>8.3±1.10b*</td>
<td>13.3±1.4*</td>
<td>24.2±2.1*</td>
<td>17.7±2.7*</td>
<td>11.1±0.9*</td>
<td>14.7±1.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. ZDF, Zucker diabetic fatty rats; C, control; HF, high-fat diet; HF-Met, high-fat diet with metformin treatment; HF-Ex, high-fat diet with treadmill exercise; HF-E+M, high-fat diet with both metformin and exercise. *P ≤ 0.05, significantly different from lean. †P ≤ 0.05, significantly different from control. ‡P ≤ 0.05, significantly different from HF. §P ≤ 0.05, significantly different from HF-Met. ¶P ≤ 0.05, significantly different from HF-Ex. ‖P ≤ 0.05, significantly different from week 0. 0P ≤ 0.05, significantly different from week 4.
that in RG muscle, with significant increases observed with both HF-Ex and HF-E+M (Fig. 2C).

**Skeletal Muscle FA Metabolism and Oxidative Capacity**

All ZDF groups exhibited depressed exogenous FA oxidation (−24–43%, \( P < 0.01 \); Fig. 3A) and increased TAG esterification (47–51%, \( P < 0.01 \); Fig. 3B) compared with lean rats. Surprisingly, no differences in FA oxidation were observed among the ZDF (control or HF) groups.

There were significant increases in CS activity (Table 2) in all ZDF groups compared with lean rats (+30–38%, \( P < 0.05 \)). Within the HF-fed groups, CS activity was further increased with both HF-Ex (+12–15%, \( P < 0.05 \)) and HF-E+M (+21–24%, \( P < 0.05 \)). There was a trend toward an increase in \( \beta \)-HAD activity in control diet-fed ZDF rats compared with lean rats (Table 2, \( P = 0.08 \)). \( \beta \)-HAD activity was suppressed with HF and HF-Met (−20–32%, \( P < 0.001 \)) compared with control. With both exercise interventions, \( \beta \)-HAD activity was increased 20–30% above HF and HF-Met (\( P < 0.05 \)), being restored to levels not different from those of lean rats.

**Skeletal Muscle AMPK Signaling**

There were no significant differences in total AMPK protein expression or phosphorylation (Table 2). There were modest increases in AMPK\( \alpha \)1 protein expression with HF-Met (+30%, \( P < 0.05 \)) and in AMPK\( \alpha \)2 protein expression in HF-Ex (+12–17%, \( P < 0.05 \)) and HF-E+M (+15–21%, \( P < 0.05 \)) compared with HF and HF-Met rats. Furthermore, there were no significant differences in ACC phosphorylation among groups; however, PGC-1\( \alpha \) protein content tended to increase in HF-Met rats (+12–15%, \( P = 0.06 \)) and was increased in...
Skeletal Muscle FAT/CD36 and FABPpm Protein Expression

All interventions resulted in significant reductions in whole muscle FAT/CD36 protein expression compared with HF-fed rats. No changes in whole muscle or sarcolemmal FABPpm were observed in the ZDF groups (Fig. 4, C and D).

Skeletal Muscle Lipid Content

Diacylglycerol. Increased total DAG content was observed in HF-fed rats compared with both lean (+72%, \( P < 0.001 \)) and control rats (+20%, \( P = 0.028 \)) due to increases in total saturated (\( P < 0.001 \) vs. lean and control), monounsaturated, and polyunsaturated (\( P < 0.01 \) vs. lean) DAG content (Table 3). Reductions in total DAG content were observed with HF-Met (−53%, \( P < 0.001 \)) and both HF-Ex and HF-E+M rats (−110–130%, \( P < 0.001 \)) compared with HF-fed rats. Interestingly, total DAG content was also decreased in all HF interventions (HF-Met, HF-Ex, HF-E+M, \( P < 0.005 \)) compared with control rats on a low-fat diet. There were further reductions in total DAG content in both HF-Ex (\( P = 0.021 \)) and HF-E+M (\( P = 0.008 \)) compared with the HF-Met intervention alone. Linear regression revealed a significant negative correlation between soleus muscle DAG and GLUT4 protein expression (\( r = 0.63, P < 0.001 \)) in ZDF rats.

Ceramide. Increased total ceramide content was observed in HF-fed rats (+70–111%, \( P < 0.001 \) vs. lean and control), due to increases in total saturated, monounsaturated, and polyunsaturated ceramide content (\( P < 0.001 \)), as well as a number of individual ceramide species (\( P < 0.05 \); Table 4). Reductions in total ceramide were observed with HF-Met (−68%, \( P < 0.001 \)) and both HF-Ex and HF-E+M rats (−109–225%, \( P < 0.001 \)) compared with HF-fed rats. Further reductions in total ceramide content were observed with combined HF-E+M rats compared with control and HF-Met rats (−90%, \( P < 0.01 \)). Furthermore, a synergistic effect was observed given that HF-E+M demonstrated less total ceramide species compared with HF-Ex (−55%, \( P = 0.05 \)). There were significant reductions in total saturated (\( P < 0.001 \)), monounsaturated (\( P < 0.001 \)), and polyunsaturated (\( P < 0.05 \)) ceramide content in HF-Met, HF-Ex, and HF-E+M interventions. Linear regression revealed a significant negative correlation between soleus muscle ceramide and GLUT4 protein expression (\( r = 0.50, P < 0.001 \)) in ZDF rats.

DISCUSSION

In the present model of high-fat induced T2D, hyperglycemia was accompanied by impaired insulin-stimulated glucose transport in skeletal muscle of HF-fed ZDF rats. Metformin, exercise, and their combination attenuated the progression of hyperglycemia in HF-fed rats. These beneficial effects were related to enhanced skeletal muscle insulin-stimulated glucose transport and increased GLUT4 protein expression with exercise-based interventions (HF-Ex, HF-E+M) but not with metformin on its own. Surprisingly, no intervention increased FA oxidation or decreased TAG synthesis. However, FAT/CD36 protein expression was reduced with all interventions and was likely at least partially responsible for the robust reduction in DAG and ceramide contents. Finally, modest increases in PGC-1α, CS, and β-HAD with exercise and isoform-specific increases in AMPK were observed. Metformin increased...
AMPKα1, whereas exercise interventions normalized AMPKα2 with increased PGC-1α protein expression, suggesting differential effects on skeletal muscle AMPK for metformin compared with exercise training interventions.

Effects of Metformin and Exercise on the Prevention of Hyperglycemia and Skeletal Muscle Insulin Resistance

Relatively few studies have utilized the female ZDF rat as a diabetic model. We confirmed previous findings (14) that female ZDF rats fed a HF-diet develop overt fasting hyperglycemia and hyperinsulinemia. In HF-fed (48 kcal %fat) rats, improvements in hyperglycemia with metformin were not related to improvements in skeletal muscle insulin-stimulated glucose transport or changes in GLUT4 protein expression. The exact mechanism by which metformin exerts its antidiabetic effects is poorly understood. The main site of metformin action may be the liver, via a reduction in hepatic glucose output through inhibition of gluconeogenesis (24). However, limited evidence also suggests that metformin may have direct effects on skeletal muscle. Specifically, metformin has been shown to activate AMPK (13, 61) and stimulate glucose transport in isolated rat extensor digitorum longus muscle (61) and enhance insulin-stimulated glucose transport in soleus muscle from healthy Sprague-Dawley rats (7). Stimulation of skeletal muscle insulin-stimulated glucose transport with metformin has been demonstrated in insulin-resistant human subjects (21) but not in subjects with newly diagnosed diabetes (30).

Acute exercise, as well as chronic training, improves muscle insulin sensitivity by increasing GLUT4 protein expression (27, 29) and translocation to the plasma membrane (18). Exercise training has been shown to improve insulin-stimulated glucose transport in obese Zucker rats (11, 28) up to 7 days after the last exercise bout (11). In the current study, the development of skeletal muscle insulin resistance was blunted, as indicated by improved insulin-stimulated glucose transport, as well as whole muscle and plasma membrane-associated GLUT4 protein expression. Increases in whole muscle GLUT4 protein expression with exercise training may be a key mechanism by which the muscle compensates for defects in muscle insulin signaling (27). Collectively, our findings suggest that metformin and exercise may have distinct effects on glucose metabolism (i.e., different tissues, liver and skeletal muscle), each of which ultimately result in reduced hyperglycemia. However, although metformin did not ultimately improve maximal insulin-stimulated glucose uptake in isolated soleus, we point out that this is only representative of one major fiber type and, furthermore, that metformin still resulted in decreased muscle ceramide and DAG content. Thus we caution that our data not be overinterpreted to indicate that metformin affects only the liver, and not skeletal muscle.

Effects of Metformin and Exercise on Skeletal Muscle FA Metabolism, AMPK Signaling, and Oxidative Capacity

Evidence is accumulating that strongly suggests that defects in skeletal muscle FA metabolism are involved in the pathogenesis of insulin resistance in obesity. Impairments in FA oxidation have been observed in various models of obesity, including male ZDF rats (19, 25), and in obese humans (31, 32). Isolated soleus muscle from female ZDF rats demonstrate reduced acute exogenous FA oxidation and an increase in TAG esterification compared with lean rats. Surprisingly, although other antidiabetic agents such as the thiazolidinedione troglitazone increase exogenous FA oxidation in male ZDF rats (25), no intervention in this study affected in vitro FA metabolism in this model. It should be realized that in this study FA metabolism was assessed in quiescent, isolated soleus strips. It is entirely possible that stimulation of the muscle’s metabolic rate (contraction, AICAR) may have revealed an improved capacity to direct FA toward oxidation in the exercise or even the metformin interventions. Thus we cannot rule out the possibility that in vivo, altered FA partitioning may be partially responsible for improved insulin-stimulated glucose transport. Interestingly, maximal activities of both CS and β-HAD but not CS activity. However, in both cases, endurance training further increases their activities. Importantly, this is the first study to demonstrate that total and sarcomemal expression of the putative FA transporter FAT/CD36 is increased in HF-fed female ZDF rats, which is in agreement with previous studies in male ZDF rats (10) and is associated with increased rates of FA transport (6, 10). Fur-
thermore, levels of FAT/CD36 were negatively associated with sarcolemmal GLUT4 protein content. FAT/CD36 can be translocated to the sarcolemmal membrane in the presence of various stimuli, including insulin (35), AICAR, and contraction (37), and regulates skeletal muscle FA uptake. Sarcolemmal FAT/CD36 protein expression is also increased with obesity and T2D in humans and is thought to be a mechanism involved in increased FA uptake and accumulation of lipids in muscle (6). Rates of FA transport were not measured in the current study; however, a strong correlation between sarcolemmal FA transporter abundance and FA transport measured in vesicles has been previously demonstrated (10). Thus the observed reductions in FAT/CD36 were likely at least partially responsible for the robust reductions in reactive DAG and ceramide in response to the metformin and exercise interventions. It should be realized that FA uptake also has a significant diffusive component, and altered synthesis/degradation of lipid pools were not assessed, which might affect DAG and ceramide content.

DAG and ceramide contents are increased during acute lipid exposure that induces insulin resistance (4, 26, 48, 55) and have previously been shown to be elevated in obese states (1, 9) and to disrupt the insulin-signaling pathway (26, 49, 56). Both acute exercise (16) and chronic training (9, 17) reduce DAG and ceramide content and are associated with increased insulin sensitivity (9). In our study, both metformin and exercise prevented the HF-diet induced accumulation of most saturated, monounsaturated, and polyunsaturated DAG and ceramide species in skeletal muscle. Stearoyl-CoA desaturase 1 (SCD1) catalyzes the desaturation of individual lipid species, namely, saturated fatty acyl-CoA (palmitoyl-CoA and stearoyl-CoA) to monounsaturated fatty acyl-CoA (oleoyl-CoA) and may be a contributing factor to obesity and insulin resistance (15). In male ZDF rats (58) and obese humans (23), an increase...
in Scdl gene expression has been observed, and in myocytes cultured from obese humans, increased Scdl expression and activity are correlated with low rates of FA oxidation, increased TAG, and increased monounsaturated lipid species (23). Although we did not measure Scdl protein expression in this study, it is likely that Scdl is elevated, leading to the approximately twofold increase in oleoyl (C18:1) DAG and ceramide compared with lean, insulin-sensitive rats.

Although the metformin intervention on its own reduced DAG and ceramide content, it did not improve the insulin-stimulated glucose transport in vitro. We have no explanation for this apparent dissociation between reduced DAG/ceramide content and improved insulin sensitivity. Thus it is reasonable to infer that a reduction in hepatic glucose production may have been a contributor to the blunting of hyperglycemia by metformin in this model.

### Table 4. Soleus muscle ceramide content from lean and ZDF rats on control or HF diet

<table>
<thead>
<tr>
<th></th>
<th>Lean C</th>
<th>HF</th>
<th>HF-Met</th>
<th>HF-Ex</th>
<th>HF-E + M</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saturated</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>14:0</td>
<td>111 ± 16</td>
<td>116 ± 11</td>
<td>222 ± 27</td>
<td>106 ± 10</td>
<td>58 ± 6</td>
</tr>
<tr>
<td>16:0</td>
<td>208 ± 18</td>
<td>259 ± 17</td>
<td>413 ± 42</td>
<td>220 ± 17</td>
<td>183 ± 9</td>
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<tr>
<td>18:0</td>
<td>200 ± 15</td>
<td>265 ± 18</td>
<td>441 ± 43</td>
<td>242 ± 21</td>
<td>207 ± 13</td>
</tr>
<tr>
<td>20:0</td>
<td>15 ± 1</td>
<td>22 ± 3</td>
<td>25 ± 2</td>
<td>16 ± 1</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>22:0</td>
<td>18 ± 2</td>
<td>26 ± 4</td>
<td>41 ± 6</td>
<td>20 ± 1</td>
<td>18 ± 2</td>
</tr>
<tr>
<td><strong>Total saturated</strong></td>
<td>576 ± 51</td>
<td>712 ± 76</td>
<td>1168 ± 126</td>
<td>674 ± 60</td>
<td>547 ± 44</td>
</tr>
<tr>
<td><strong>Monounsaturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:1</td>
<td>11 ± 1</td>
<td>15 ± 2</td>
<td>24 ± 3</td>
<td>16 ± 2</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>18:1</td>
<td>66 ± 7</td>
<td>106 ± 18</td>
<td>160 ± 18</td>
<td>76 ± 8</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>24:1</td>
<td>32 ± 4</td>
<td>38 ± 5</td>
<td>57 ± 5</td>
<td>33 ± 3</td>
<td>20 ± 3</td>
</tr>
<tr>
<td><strong>Total monounsaturated</strong></td>
<td>104 ± 12</td>
<td>129 ± 14</td>
<td>208 ± 29</td>
<td>127 ± 8</td>
<td>89 ± 6</td>
</tr>
<tr>
<td><strong>Polyunsaturated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2</td>
<td>54 ± 10</td>
<td>92 ± 15</td>
<td>148 ± 30</td>
<td>91 ± 13</td>
<td>85 ± 11</td>
</tr>
<tr>
<td>20:4</td>
<td>43 ± 6</td>
<td>63 ± 9</td>
<td>145 ± 30</td>
<td>87 ± 10</td>
<td>84 ± 14</td>
</tr>
<tr>
<td>20:5</td>
<td>19 ± 2</td>
<td>17 ± 3</td>
<td>38 ± 4</td>
<td>15 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>22:6</td>
<td>35 ± 2</td>
<td>31 ± 6</td>
<td>43 ± 6</td>
<td>31 ± 3</td>
<td>22 ± 3</td>
</tr>
<tr>
<td><strong>Total polyunsaturated</strong></td>
<td>128 ± 18</td>
<td>165 ± 24</td>
<td>330 ± 67</td>
<td>216 ± 23</td>
<td>181 ± 30</td>
</tr>
<tr>
<td><strong>Total ceramide</strong></td>
<td>807 ± 72</td>
<td>996 ± 98</td>
<td>1,706 ± 188</td>
<td>1,017 ± 81</td>
<td>817 ± 72</td>
</tr>
</tbody>
</table>

Values are means ± SE in nmol/g dry wt ceramide content. *P ≤ 0.05, significantly different from lean. **P ≤ 0.05, significantly different from control. "P ≤ 0.05, significantly different from HF. \(^*\)P ≤ 0.05, significantly different from HF-Met. \(^*\)P ≤ 0.05, significantly different from HF-Ex. Letters in parentheses denote a trend (0.05 < \(P \leq 0.10\).
In this study, modest stimulatory effects on AMPK, PGC-1α, and oxidative capacity were observed. Indeed, isoform-specific increases in AMPK were observed with metformin (AMPKα1), and AMPKα2 was normalized to levels observed in lean rats with exercise. Recent studies suggest that AMPK is not the sole factor regulating FA oxidation, because AMPK activation is only associated with increases in FA oxidation during moderate intensity exercise in males and not females (46), and the robust acute exercise-induced activation of AMPKα2 is abolished after training in humans (38). PGC-1α, a transcriptional coactivator involved in the regulation of GLUT4 and mitochondrial biogenesis (2), was increased with all three interventions; modest increases in CS and β-HAD activities occurred only with exercise. Thus, given the relatively modest changes in markers of oxidative capacity, coupled with the lack of noted increase in FA oxidation ex vivo, it is difficult to ascertain the impact of an increased capacity to oxidize FA on improved hyperglycemia or muscle insulin sensitivity in this study. However, these changes do not appear to be as profound as the decreased expression of FA transporters and the decrease in intramuscular lipids.

In conclusion, the progression of high-fat induced hyperglycemia was attenuated with metformin, exercise, and their combination. Only the exercise-based interventions increased muscle sarcocemmal GLUT4 content and improved insulin-stimulated glucose transport. However, reductions in sarcocemmal FAT/CD36 and intramuscular DAG and ceramide content occurred with all interventions and are likely to be partially responsible for the alleviation of high-fat diet-induced insulin resistance. Modest increases in CS and β-HAD activity (exercise training), AMPK isoform-specific protein content, and PGC-1α protein content (metformin and exercise) also may have been related to attenuating the development of hyperglycemia. Interestingly, despite the reduced content of DAG and ceramide, metformin did not improve insulin-stimulated glucose uptake in soleus muscle, suggesting that metformin’s effects on muscle may not be critical for reducing hyperglycemia in this animal model.

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GRANTS

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REFERENCES

REDUCED LIPID ACCUMULATION WITH METFORMIN AND EXERCISE


