A single prior bout of exercise protects against palmitate-induced insulin resistance despite an increase in total ceramide content

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Thrush AB, Harasim E, Chabowski A, Gulli R, Stefanyk L, Dyck DJ. A single prior bout of exercise protects against palmitate-induced insulin resistance despite an increase in total ceramide content. Am J Physiol Regul Integr Comp Physiol 300: R1200–R1208, 2011. First published February 16, 2011; doi:10.1152/ajpregu.00091.2010.—Ceramide accumulation has been implicated in the impairment of insulin-stimulated glucose transport in skeletal muscle following saturated fatty acid (FA) exposure. Importantly, a single bout of exercise can protect against acute lipid-induced insulin resistance. The mechanism by which exercise protects against lipid-induced insulin resistance is not completely known but may occur through a redirection of FA toward triacylglycerol (TAG) and away from ceramide and diacylglycerol (DAG). Therefore, in the current study, an in vitro preparation was used to examine whether a prior bout of exercise could confer protection against palmitate-induced insulin resistance and whether the pharmacological [50 μM fumonisin B1 (FB1)] inhibition of ceramide synthesis in the presence of palmitate could mimic the protective effect of exercise. Soleus muscle of sedentary (SED), exercised (EX), and SED in the presence of FB1 (SED+FB1) were incubated with or without 2 mM palmitate for 4 h. This 2-mM palmitate exposure impaired insulin-stimulated glucose transport (−28%, P < 0.01) and significantly increased ceramide, DAG, and TAG accumulation in the SED group (P < 0.05). A single prior bout of exercise prevented the detrimental effects of palmitate on insulin signaling and caused a partial redistribution of FA toward TAG (P < 0.05). However, the net increase in ceramide content in response to palmitate exposure in the EX group was not different compared with SED, despite the maintenance of insulin sensitivity. The incubation of soleus from SED rats with FB1 (SED+FB1) prevented the detrimental effects of palmitate and caused a redirection of FA toward TAG accumulation (P < 0.05). Therefore, this research suggests that although inhibiting ceramide accumulation can prevent the detrimental effects of palmitate, a single prior bout of exercise appears to protect against palmitate-induced insulin resistance, which may be independent of changes in ceramide content.

skeletal muscle; diacylglycerol; triacylglycerol

IT IS WELL KNOWN THAT EXERCISE improves whole body insulin sensitivity (7, 12) and protects against high-fat diet-induced insulin resistance (26). However, the mechanism by which this occurs is not well understood. Skeletal muscle of obese rodents and humans is characterized by an increase in triacylglycerol (TAG) content (5, 28) that is associated with insulin resistance in this population. Paradoxically, muscle TAG is also increased in endurance-trained athletes that are highly insulin sensitive (15), suggesting that TAG is not directly causal in insulin resistance. Recent evidence has suggested that preferentially storing lipid as TAG may protect against lipid-induced insulin resistance (23, 26). The mechanism behind the protective effect of TAG storage is not well understood but may involve directing FA away from more reactive lipid pools, namely ceramide and diacylglycerol (DAG). Ceramide and DAG have both been implicated in the development of insulin resistance associated with obesity and/or lipid exposure (9, 18, 19, 35, 37).

Ceramide is a sphingolipid that has been implicated in impaired insulin signaling induced by saturated fatty acid (FA)-induced insulin resistance (8, 9, 18). Ceramides inhibit insulin-stimulated glucose transport by causing the dephosphorylation of Akt and preventing its translocation to the cell membrane (16, 29). Ceramides are increased in skeletal muscle of obese rodents and humans and accumulate following acute exposure to saturated FA (1, 21, 28, 32). Furthermore, the pharmacological inhibition of enzymes involved in ceramide synthesis prevents ceramide accumulation following saturated FA exposure and the ensuing impairment in insulin signaling (9, 18). DAG has also been implicated in lipid-induced insulin resistance from polyunsaturated FA exposure (19). DAG stimulates the serine phosphorylation and inhibition of the insulin receptor and insulin receptor substrate-1 through the activation of DAG-responsive PKC isoforms (19, 37).

There is mounting evidence suggesting that preferentially storing FA as TAG, rather than as ceramide or DAG, may protect against lipid-induced insulin resistance. In support of this, the overexpression of diacylglycerol acyltransferase-1 (DGAT-1; converts DAG to TAG) protects against high-fat diet-induced insulin resistance (23). Furthermore, in a hallmark study by Schenk and Horowitz (26), a prior bout of exercise in humans was shown to protect against the insulin-desensitizing effects of an overnight lipid infusion by causing a redirection of lipids toward TAG and away from ceramide and DAG (26). In the current study, we used an in vitro, isolated muscle preparation to specifically examine the importance of altering lipid storage as a means by which prior exercise can protect against FA-induced insulin resistance. We also expanded on prior research by focusing on the importance of ceramide accumulation in palmitate-induced insulin resistance by comparing the effects of a prior bout of exercise to the pharmacological inhibition of ceramide synthesis [fumonisin B1 (FB1)]. Specifically, we examined whether a prior bout of exercise could confer protection against the insulin resistance that would otherwise be induced by a 4-h palmitate incubation by diverting FA away from ceramide and whether FB1 in the presence of palmitate could mimic the protective effect of exercise. The isolated soleus muscle preparation allowed us to

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assess the specific effects of palmitate on muscle metabolism and insulin response independent of potential systemic influences. We hypothesized that a single prior bout of exercise and the presence of FB1 would prevent the insulin-desensitizing effects of palmitate, and this would potentially occur through a redistribution of lipids toward TAG and away from ceramide and DAG.

**MATERIALS AND METHODS**

Female Sprague-Dawley rats (Charles River, Saint Constant, Quebec) weighing 90–110 g were used for all experiments. Rats were housed in a controlled environment on a 12:12-h reverse light-dark cycle and were allowed free access to standard chow and water. Animals arrived 1 wk prior to experimental procedures to allow for acclimatization and familiarization with the treadmill. Ethical consent for all experimental procedures was obtained from the Animal Care Committee at the University of Guelph.

**Study Design**

Rats were separated into three different groups in this study, 1) sedentary (SED), 2) exercise (EX), and 3) sedentary + 50 μM fumonisins B₁ (SED+FB₁). In the SED+FB₁ group, soleus muscle from SED animals was incubated with 50 μM FB₁, which specifically inhibits ceramide synthase, and thus ceramide synthesis (36). The concentration of FB₁ has previously been shown by our laboratory (20) and others (9) to prevent palmitate-induced and palmitate-resistin-induced impairments in glucose transport in muscle.

Within each group, experiments were conducted to assess basal (unstimulated) and insulin-stimulated glucose transport, insulin signaling, FA oxidation, and lipid accumulation. For glucose transport and insulin signaling experiments, one leg was designated as palmitate-free (0 mM palmitate) and the other as 2 mM palmitate, and within each leg, each soleus strip underwent basal and insulin-stimulated treatment. For lipid and Western blot analysis, palmitate-free and 2-mM palmitate conditions were paired within one leg. In the SED+FB₁ group, all soleus strips were incubated with or without 2 mM palmitate in the presence of FB₁ (SED+FB₁).

**Treadmill Familiarization**

Prior to the experimental day, animals underwent two treadmill acclimatization sessions on a motorized treadmill on consecutive days, followed by a two-day rest period. The first training session was conducted at 10–15 m/min, 5% grade, for 10 min. During the second training session, speed was gradually increased to 20 m/min for a total time of 15 min.

**Experimental Day**

On the day of the experiment, food was removed from the rats ~3 h prior to anesthetization. Rats in the EX group began to exercise 2 h following food removal. For the first 20 min of exercise, speed was gradually increased to 25 m/min, where it was maintained for the remaining 30 min for a total exercise duration of 50 min. Upon completion of exercise, rats were removed from the treadmill and immediately anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/g body mass). Rats in the SED and SED+FB₁ group were also anesthetized at this time. Following anesthesia, muscle tissue was removed. Animals were euthanized upon completion of tissue sampling via intracardiac injection of pentobarbital sodium.

**Soleus Incubations**

Using a 27-gauge needle, we carefully dissected soleus muscles into two longitudinal strips from tendon to tendon. Soleus muscle strips were then placed into 2-ml pregassed (95% O₂, 5% CO₂) medium 199 (M199) preincubation buffer supplemented with 8 mM glucose, 4% BSA, and 14.3 μU/ml insulin, for 20 min. Soleus muscles were then transferred into 3 ml of the incubation buffers for 4 h. Incubations buffers were the same as preincubation buffers with or without 2 mM palmitate, and with the addition of 50 μM FB₁. All incubations were conducted in 20-ml vials, in pregassed buffers at 30°C in a shaking water bath.

**Tissue Viability**

To maintain viability, soleus muscle samples were gassed continuously during the 4-h incubation, and the incubation buffer was changed after 2 h. The preincubation and incubation buffers contained 14.3 μU/ml of insulin, to maintain viability. Pilot experiments demonstrated that this low level of insulin did not alter basal or subsequent insulin-stimulated glucose transport (data not shown). Viability was confirmed through the spectrophotometric measurement of ATP and phosphocreatine (PCr) content (3), as previously described (6). Muscle ATP (0 min, 17.4 ± 0.7; 4 h, 16.6 ± 0.6 μmol/g dry wt) and PCr (0 min, 35.5 ± 4.9, 4 h, 34.8 ± 2.6 μmol/g dry wt) was maintained during 4 h of incubation. We confirmed soleus recruitment during the acute exercise protocol by measuring muscle glycogen content. Muscle glycogen content was significantly reduced from 100.6 ± 20.3 mmol/kg dry mass (preexercise) to 45.2 ± 5.3 mmol/kg dry mass (postexercise).

**Basal and Insulin-Stimulated Skeletal Muscle Glucose Transport**

Following 4 h of incubation under the previously described conditions, basal (0 mU/ml) and insulin-stimulated (insulin, 10 mU/ml) glucose (3-O-methylglucose) transport was assessed, as described elsewhere in more detail (24, 34). Briefly, muscle samples were transferred into 2 ml of pregassed modified M199 buffer containing 8 mM glucose and 32 mM mannitol in the absence and presence of 10mU/ml insulin. Basal and insulin-stimulated conditions were maintained in all subsequent steps. Muscles were washed (2 × 10 min) with glucose-free M199 buffer containing 4 mM pyruvate and 36 mM mannitol and subsequently incubated for 40 min (basal) or 20 min (insulin) in M199 containing 8 mM 3-O-[³H]methyl-D-glucose (0.8 μCi/ml) and 32 mM [¹⁴C] mannitol (0.3 μCi/ml). [¹⁴C] mannitol was used to estimate extracellular volume to determine intracellular 3-O-[³H]methyl-D-glucose content. Glucose transport was determined as the accumulation of intracellular 3-O-[³H]methyl-D-glucose, i.e., total glucose accumulation corrected for extra cellular content. Linearity of glucose transport was confirmed, in a separate set of experiments, over a range, including 5, 10, 20, and 30 min under basal conditions (r² = 0.96; P = 0.015) and a range, including 5, 10, 20, and 30 min under insulin-stimulated conditions (r² = 0.985; P = 0.0072).

**Western Blot Analysis**

In the final 15 min of the 4-h incubation, samples were transferred into fresh pregassed buffer, with or without 10 mU/ml insulin to measure the phosphorylation state and/or protein content of insulin-signaling proteins. Muscle samples were then removed from the buffer, blotted and immediately frozen, and stored in liquid nitrogen. Muscle samples were homogenized using a whole muscle preparation, and the protein content was measured using the commercially available BSA protein assay (Fishers Scientific, Ottawa, ON, Canada). Total Akt (Cell Signaling Technology, Danvers, MA), Ser-phosphorylated and Thr-phosphorylated Akt (Ser473 Akt and Thr308 Akt, respectively; Abcam, Cambridge, MA), total Akt substrate 160 (AS160; Millipore, Lake Placid, NY), and phosphorylated AS160 (Thr642 AS160; Medicorp, Montreal, QB, Canada) were measured under insulin-stimulated conditions.

Following 4-h incubations with or without 2 mM palmitate (all groups), the following proteins were assessed: DGAT-1, glycerol-3-phosphate acyltransferase (mtGPAT), serine palmitoyltransferase...

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Lipid Analysis

Following the 4-h incubation with or without 2 mM palmitate (in SED, EX, SED + FB1 groups), samples were blotted and immediately frozen and stored in liquid nitrogen for further analysis of lipid content. Muscle samples were freeze-dried and cleaned of any visible adipose tissue and blood. Soleus muscles from the same animal were pooled to obtain a sufficient amount of tissue (−10 mg dry wt) to analyze triacylglycerol (TAG), diacylglycerol (DAG), and ceramide content, as previously described (7). Briefly, lipids were extracted using the Folch method of extraction (13) and modified according to van der Vusse et al. (33). Muscle samples were extracted in chloroform-methanol (2:1, vol/vol) with 0.01% butylated hydroxytoluene. Muscle DAG and TAG were separated using thin-layer chromatography (TLC) on silica gel plates (0.25 mm Kieselgel 60; Merck, Darmstadt, Germany) using heptane: isopropyl ether: acetic acid (10:90:1 vol, vol, vol). Plates were sprayed with 0.2% solution of 2% dichlorofluorescein in methanol, and the detected bands were scraped from the plate into vials and subsequently transmethylated in 1 ml of 14% boron fluoride in methanol at 100°C for 90 min, cooled, and extracted in 1 ml pentane and 0.5 ml water. Samples were dissolved in hexane and analyzed by gas liquid chromatography [Hewlett-Packard 5890 Series II, HP-INNOWax capillary column (50 m × 0.33 mm)].

FA Oxidation and Incorporation

FA oxidation and incorporation into DAG and TAG lipid pools and total uptake (sum of oxidation, DAG and TAG incorporation) were assessed during the final hour of the 4-h incubation, when the total uptake (sum of oxidation, DAG and TAG incorporation) were calculated as the sum of incorporation of labeled palmitate (25).

All data are expressed as means ± SE. A two-way repeated-measures ANOVA and a Student-Newman-Keuls post hoc test, to analyze significant differences revealed by the ANOVA, were used to evaluate basal and insulin-stimulated glucose transport and insulin-signaling protein expression and phosphorylation within groups (SED, EX, SED + FB1), and lipid accumulation and protein expression and phosphorylation between groups (SED vs. EX vs. SED + FB1). A one-way ANOVA was used to compare differences in insulin-stimulated glucose transport within a given condition (palmitate-free and 2 mM palmitate) and to assess differences in FA oxidation and incorporation.

RESULTS

Basal and Insulin-Stimulated Glucose Transport

Insulin significantly increased glucose transport above basal rates in the presence and absence of 2 mM palmitate conditions in all groups (SED, EX, SED + FB1, P < 0.001; Fig. 1A). Overall, 2 mM palmitate had no effect on basal or insulin-stimulated glucose transport in the EX or SED + FB1 groups. Insulin-stimulated glucose transport was significantly greater in the EX group compared with SED and SED + FB1. In the 2-mM palmitate condition, insulin-stimulated glucose transport was significantly reduced in the SED group (−28%; P < 0.01) compared with the palmitate-free condition. Within the 2-mM palmitate condition, insulin-stimulated glucose transport was significantly lower in the SED group compared with EX (P < 0.05), and there was a trend toward a difference compared with SED + FB1 (P = 0.06).

In support of the glucose transport data, exercise and FB1 prevented the palmitate-induced decrease in insulin-stimulated Akt and AS160 phosphorylation. A 4-h exposure to 2 mM palmitate significantly reduced Ser Akt, Thr Akt, and AS160 phosphorylation (−30%; P < 0.05) in the SED group. This impairment in Akt and AS160 phosphorylation was prevented with a prior bout of exercise. AS160 phosphorylation was also significantly lower in the SED group compared with SED + FB1. Total protein content of AS160 and Akt was not different between any group or condition (Fig. 1).

Muscle Ceramide, DAG, and TAG

Ceramide. Palmitate exposure (2 mM) significantly increased ceramide content in all groups (P ≤ 0.001), and ceramide accumulation was significantly different among the groups (SED > EX > SED + FB1, P ≤ 0.002). Following the palmitate-free incubation, muscle ceramide content was significantly reduced by a prior bout of exercise (EX) and exposure to FB1 (SED + FB1) compared with the SED group (P < 0.001; Fig. 2A). The net decrease in ceramide content (i.e., the difference between 2-mM palmitate and palmitate-free; Fig. 3A) was greater in both the EX and SED groups compared with SED + FB1 (P < 0.001); the increases in the EX and SED groups were not different.

DAG. Palmitate (2 mM) increased DAG content in all groups (P < 0.001; Fig. 2B). Following the palmitate-free incubation, DAG content was significantly lower in the EX group compared with SED and SED + FB1 (Fig. 2B; P ≤ 0.003). Although DAG content following 2-mM palmitate exposure was lowest in the EX group (P < 0.05), the net increase in DAG content was not different compared with the SED group, and this was significantly greater than SED + FB1 (Fig. 3B; P < 0.05).

TAG. Following 2-mM palmitate exposure, TAG content was significantly greater in the SED + FB1 group compared with SED (P ≤ 0.05). The net increase in TAG content was significantly greater in the EX compared with the SED, but not SED + FB1 group (P < 0.05, Fig. 3C).

Changes in Ceramide and DAG 16:10 and 18:0 Species

Ceramide and DAG 16:0 and 18:0 FA species are shown in Table 1. The 2-μM palmitate incubation increased ceramide 16:0 and 18:0 in all groups. A prior bout of EX significantly...
reduced 16:0 and 18:0 ceramide compared with SED in the palmitate-free condition. Only 16:0 ceramide was significantly reduced by a prior bout of EX following 2-mM palmitate incubation. Exposure to FB1 (SED+FB1) significantly reduced 16:0 and 18:0 ceramide following the palmitate-free and 2-mM palmitate incubation.

The 2-mM palmitate exposure significantly increased 16:0 and 18:0 DAG in SED rodents and 16:0 DAG in the EX group. A prior bout of exercise significantly reduced 16:0 and 18:0 DAG compared with SED in the palmitate-free and 2-mM palmitate incubation.

FA Oxidation and Incorporation

FA oxidation and incorporation into DAG and TAG lipids was measured only in the presence of 2 mM palmitate (Table 2). There was no difference in FA oxidation among the groups. FA incorporation into TAG and total palmitate uptake were greatest in the EX group. This further supports the lipid data, which demonstrated greater lipid accumulation in the EX group compared with SED and a redirection toward TAG in the SED+FB1 group. In accordance with this, the oxidation/total lipid esterification was significantly lower in the EX and SED+FB1 groups compared with SED, suggesting that a greater proportion of lipids were being directed toward lipid storage in these groups. Unexpectedly, FB1 significantly increased total palmitate uptake compared with SED. This was accounted for the greater palmitate incorporation into TAG. The incorporation of $^{14}$C palmitate into DAG was similar in all groups.

Enzyme Expression and Phosphorylation

Total protein content of DGAT-1, mtGPAT (Fig. 4) was not different between groups (SED, EX, SED+FB1) or conditions (palmitate-free vs. 2 mM palmitate). SPT, JNK, and phosphorylated JNK were not different between groups or conditions (data not shown).

DISCUSSION

We and others have previously speculated that storing excess lipid as TAG may provide protection against FA-induced insulin resistance, at least in part, by redirecting lipids away from ceramide and DAG accumulation (23, 26, 30). In the current study, we compared the ability of a single prior bout of exercise...
exercise (EX) to the pharmacological inhibition of ceramide synthesis (SED+FB1) to protect against palmitate-induced insulin resistance. The in vitro model used in this study allowed us to examine the effects of lipid exposure and potential insulin-sensitizing perturbations (EX, FB1) directly on muscle metabolism, independent of potential systemic effects. In the sedentary group, 4 h of palmitate incubation reduced insulin-stimulated Akt and AS160 phosphorylation and insulin-stimulated glucose transport by ~30%, and this was associated with the highest concentrations of ceramide and DAG measured. This is consistent with prior research demonstrating that longer-term incubation with palmitate impairs skeletal muscle insulin-stimulated glucose transport (2, 31). Both prior exercise and the pharmacological inhibition of ceramide synthesis (FB1) protected against palmitate-induced insulin resistance. Unexpectedly, the net increase in ceramide content during the 2-mM palmitate incubation was not different in the EX compared with the SED group, suggesting that in this experimental model, the protective effect of exercise from palmitate-induced insulin resistance may be independent of net ceramide accumulation. It is possible that the acute insulin-sensitizing effects of exercise (i.e., low glycogen, increased AMPK activity) are potent enough to override any desensitizing effect that a moderate increase in ceramide content would otherwise cause. Accordingly, it is quite possible that several hours postexercise, when these insulin-sensitizing signals are reduced, ceramides may play a more important role in regulating insulin sensitivity, and this should be further examined.

![Fig. 2. Muscle lipid content. Total ceramide (A), diacylglycerol (DAG; B) and triacylglycerol (TAG; C) content in soleus muscle of SED, EX, and SED+FB1-treated rats following 4-h incubation with (solid bars) or without (open bars) 2 mM palmitate. Data are expressed as means ± SE, using two-way repeated-measures ANOVA, significance was accepted at P ≤ 0.05. Main effect of 2 mM palmitate in all groups (SED, EX, SED+FB1). #P ≤ 0.001, main effect of palmitate within all groups (SED, EX, SED+FB1). *P ≤ 0.05 Within group (SED, EX, SED+FB1) significantly different compared with palmitate-free condition.](http://ajpregu.physiology.org/)

![Fig. 3. Delta lipid content. Delta ceramide (A), DAG (B), and TAG (C) in SED, EX, and SED+FB1-treated rats following 4-h incubation. Delta was calculated by subtracting palmitate-free from the 2-mM palmitate condition. Data are expressed as means ± SE, using one-way ANOVA; n = 12–13. *P ≤ 0.05, **P < 0.001.](http://ajpregu.physiology.org/)
It should also be noted that although prior exercise did not diminish net ceramide accumulation during the subsequent exposure to palmitate, exercise did reduce accumulation of the saturated C16:0 species, which may have contributed to the protection of insulin response. Thus, our data do not disprove that small changes in specific ceramide species can still mediate insulin response in the postexercise period. Finally, it should be acknowledged that our findings are limited to slow oxidative fibers, of which the soleus is primarily composed. Clearly, it is also important to establish whether our observations would be consistent in muscles of a more mixed, or glycolytic fiber composition.

In the current study, a prior bout of exercise reduced ceramide and DAG, but not TAG content compared with the SED group. This reduction in ceramide content is consistent with previous research using rodent skeletal muscle (11). The largest increase in TAG content occurred in the EX group. This increase in lipid accumulation was likely due to an exercise-induced increase in FA transport (4). Surprisingly, in the current study and in contrast to Schenk and Horowitz (26), the largest FA-induced increase in ceramide accumulation occurred in the EX group, despite demonstrating a fully recovered insulin response. This is in contrast to our hypothesis that exercise would cause a reduction in ceramide and DAG accumulation, but reflects previous research which demonstrated that the overexpression of DGAT-1 causes more FA to accumulate in TAG and protected against high fat diet-induced insulin resistance despite increases in ceramide and DAG content (23). Taken together, it appears that a prior bout of exercise causes a partial redistribution of cellular lipids toward TAG synthesis. A longer exposure time may have resulted in a different lipid distribution.

It is possible that a prior bout of exercise may have altered the activity of enzymes involved in ceramide synthesis, and thus the metabolic fate of ceramide. Our research demonstrated that a prior bout of exercise reduced the content of 16:0 and 18:0 ceramide species independent of palmitate exposure, and this may have contributed to the protective effects of the prior bout of exercise. These particular species have previously been shown to be increased in skeletal muscle of insulin-resistant (10) and obese humans (30). The cellular

Table 1. 16:0 and 18:0 ceramide and DAG

<table>
<thead>
<tr>
<th>2 mM Palmitate</th>
<th>SED</th>
<th>EX</th>
<th>SED-FB1</th>
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<tbody>
<tr>
<td>Ceramide, nmol/g</td>
<td>16 0</td>
<td>204.4 ± 8.8</td>
<td>150.7 ± 9.2b</td>
</tr>
<tr>
<td></td>
<td>18 0</td>
<td>182.5 ± 8.5</td>
<td>135.8 ± 13.5b</td>
</tr>
<tr>
<td>DAG, nmol/g</td>
<td>16 0</td>
<td>495.4 ± 114.4</td>
<td>63.5 ± 19.1b</td>
</tr>
<tr>
<td></td>
<td>18 0</td>
<td>666.9 ± 105.5</td>
<td>288.5 ± 16.5b</td>
</tr>
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Values are expressed as means ± SE; n = 10–14. Two-way repeated-measures ANOVA was used, and significance was accepted at P ≤ 0.05. Units are given as nanomoles per gram dry mass. Significant differences between 0 mM and 2 mM palmitate conditions (within a group) are indicated by b (P < 0.05). Significant differences between sedentary (SED) vs. exercised (EX), or sedentary + 50 μM fumonisin (SED+FB1) in the palmitate-free condition are indicated by c (P ≤ 0.05). Significant differences between SED vs. EX or SED+FB1 in the 2-mM palmitate condition are indicated by c (P ≤ 0.05).

Table 2. Fatty acid oxidation and incorporation

<table>
<thead>
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<th>SED</th>
<th>EX</th>
<th>SED+FB1</th>
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<tr>
<td>Palmitate oxidation, nmol/g</td>
<td>84.5 ± 6.1</td>
<td>74.4 ± 3.2</td>
<td>79.7 ± 4.2</td>
</tr>
<tr>
<td>DAG incorporation, nmol/g</td>
<td>40.0 ± 1.8</td>
<td>40.3 ± 2.9</td>
<td>36.2 ± 1.9</td>
</tr>
<tr>
<td>TAG incorporation, nmol/g</td>
<td>331.8 ± 19.5</td>
<td>394.7 ± 14.9</td>
<td>415.4 ± 30.8</td>
</tr>
<tr>
<td>Total uptake, nmol/g</td>
<td>443.4 ± 16.6</td>
<td>509.5 ± 15.8</td>
<td>516.3 ± 30.4</td>
</tr>
<tr>
<td>Oxidation/total lipid esterification</td>
<td>0.23 ± 0.02</td>
<td>0.17 ± 0.01*</td>
<td>0.18 ± 0.01*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; n = 9–11. Palmitate oxidation, and esterification in soleus muscle of sedentary (SED), exercised (EX), and SED + 50 μM fumonisin (SED+FB1)-treated rats during the last hour of 4-h exposure to 2 mM palmitate. *One-way ANOVA, significantly different compared to SED, P ≤ 0.05.

Fig. 4. Lipogenic protein expression. Diacylglycerol acyl transferase (DGAT-1) (A) and mitochondrial glycerolphosphate acyltransferase (mtGPAT) (B) protein content in SED, EX, and SED+FB1-treated rats following 4-h incubation with (solid bars) or without (open bars) 2 mM palmitate. Data are expressed as means ± SE; n = 12.
distribution of ceramide may also contribute to the metabolic effect of ceramide. Indeed, ceramide is found in lipid rafts and in the mitochondrial membrane, and it is likely that the proximity of ceramide to insulin-signaling proteins will contribute to its metabolic effect. Unfortunately, the assessment of the cellular distribution of ceramide content was beyond the scope of the current research and warrants further investigation.

Schenk and Horowitz (26) previously demonstrated that a single bout of exercise prior to an overnight lipid infusion completely prevents lipid-induced insulin resistance through a redirection of FA toward TAG and away from DAG and ceramide. This was associated with an increase in DGAT-1 and mtGPAT protein expression, suggesting that exercise increases skeletal muscle’s lipogenic capacity (26). In the current study, neither the 2-mM palmitate incubation nor a prior bout of exercise altered DGAT-1, mtGPAT, or SPT protein expression. It is possible that the perturbations in the current study did not cause a large enough (or long enough) stimulus to increase protein expression, or that more than 4 h may have been required to detect a measurable increase in protein expression, as observed in the Schenk and Horowitz study (26). It is also possible that differences between that study (26) and the current one simply reflect a species difference i.e., rat vs. human. Finally, we cannot discount the potential role of reduced glycogen content as a possible factor in the protection of insulin response postexercise, as the exercise perturbation used in this study caused a significant reduction in soleus glycogen content. However, classic work by Ruderman’s group has demonstrated that reduced glycogen content does not entirely explain improved insulin response postexercise (14); i.e., improved insulin response is maintained after glycogen is restored.

We acknowledge that a 4-h incubation with a single FA species (e.g., palmitate) at a high concentration is not reflective of a normal physiological condition. However, this perturbation has previously been demonstrated to acutely impair muscle insulin sensitivity (31), allowing us to examine potential protective effects of prior exercise and the underlying mechanisms by which this might occur. Furthermore, an ex vivo technique allows us to examine the specific effects of the FA exposure on skeletal muscle independent of other physiological stimuli (i.e., cytokine, blood flow, etc.).

### Inhibiting Ceramide Production Protects Against Palmitate-Induced Insulin Resistance

We demonstrated that inhibiting ceramide synthesis (FB1, ceramide synthase inhibitor) completely prevented palmitate-induced insulin resistance, and this was associated with a reduction in ceramide accumulation. This has previously been shown in C2C12 cells, and in rodents fed a high-fat diet (9, 18). It was surprising, nonetheless, to find that 2 mM palmitate still significantly increased ceramide accumulation in the SED + FB1 group as FB1 is a strong inhibitor of ceramide synthesis (36). The blunted ceramide accumulation in SED + FB1 compared with SED is similar to that observed in obese rodents fed myriocin, an inhibitor of serine palmitoyl transferase (18). Furthermore, FB1 inhibits ceramide synthase and thus de novo ceramide synthesis. FB1 does not interfere with sphingomyelinase, and therefore, the increase in ceramide content could have been attributed to this pathway (36).

In the current study, FB1 treatment caused a redistribution of lipids toward DAG and TAG, suggesting that inhibiting ceramide synthesis causes lipids to preferentially accumulate in other pools. It has previously been suggested that redirecting lipids toward TAG can protect against FA-induced insulin resistance (23, 26, 30), and the current research provides further support for this theory. It was interesting to find that the FA redirection was larger in the SED + FB1 compared with the EX group, since contraction is a large stimulus for FA transport, whereas ceramide content only accounts for <5% of lipid pools. This suggests that while reducing ceramide accumulation and redirecting lipids toward DAG and TAG can protect against the inhibitory effects of 2 mM palmitate, this is not required to maintain insulin sensitivity, as ceramide accumulation was markedly increased with EX. An unexpected finding was that FB1 increased FA uptake. Although this was accounted for by an increase in TAG synthesis, it is not clear why FA uptake was increased with FB1. We have no explanation for this observation, and are unaware of similar observations by others. At the very least, our finding does suggest that as with many other compounds, the effects may not be as specific as initially believed.

Some research has suggested that ceramide content is increased in obesity and impairs insulin signaling following saturated FA exposure (1, 7, 9, 18, 30, 32). However, not all studies support this relationship. For example, ceramide content is not reduced following 4 wk of exercise training and/or rosiglitazone treatment despite improvements in insulin-stimulated glucose transport (22). Paradoxically, rosiglitazone treatment increases muscle DAG and ceramide content in obese Zucker rats despite improvements in insulin sensitivity (22). We have recently demonstrated that resistin, a cytokine known to cause insulin resistance, alters insulin sensitivity through a ceramide-independent mechanism (20). Taken together with the current data, this suggests that although inhibiting ceramide accumulation certainly protects against lipid-induced insulin resistance (9, 18), ceramide accumulation may not be as strong of a negative regulator of in vivo skeletal muscle insulin sensitivity as previously believed or at least not under all situations. However, while net ceramide accumulation was increased in the postexercise condition, the accumulation of the saturated C16 was not; thus, it is possible that small changes in specific ceramide species can still influence insulin response during this period.

An alternative mechanism through which saturated FA may induce insulin resistance is through the activation of inflammatory pathways, such as JNK. JNK is up-regulated in skeletal muscle of obese rodents and is known to elicit a FA-induced inflammatory response in skeletal muscle (17). In the current study, JNK phosphorylation was increased by palmitate in the SED group, but this did not reach significance and may not have been a key modulator of FA-induced insulin resistance in this study (data not shown). It is possible that 4 h was not long enough to activate JNK signaling or that other inflammatory pathways may have been activated (i.e., nuclear factor-κB).

### Significance and Perspectives

In summary, the current research demonstrates that 4 h of lipid exposure induces skeletal muscle insulin resistance, and this is associated with ceramide and DAG accumulation. Importantly,
this can be completely prevented by a single prior bout of exercise; however, a single bout of exercise causes ceramide accumulation concurrent with completely recovered insulin response, suggesting that exercise protects against palmitate-induced insulin resistance through a ceramide-independent mechanism. This study adds to the growing body of research suggesting that although ceramide accumulation can impair insulin signaling and reducing ceramide content can restore this, it is possible that in certain situations, such as immediately postexercise, when several factors contribute to a heightened insulin sensitivity, any deleterious effects of ceramide may be overridden. Alternatively, small changes in specific ceramide species may also be functionally important in the postexercise period.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


