Globular adiponectin resistance develops independently of impaired insulin-stimulated glucose transport in soleus muscle from high-fat-fed rats

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Mullen KL, Smith AC, Junkin KA, Dyck DJ. Globular adiponectin resistance develops independently of impaired insulin-stimulated glucose transport in soleus muscle from high-fat-fed rats. Am J Physiol Endocrinol Metab 293: E83–E90, 2007. First published March 13, 2007; doi:10.1152/ajpendo.00545.2006.—High-fat (HF) diets reduce insulin resistance and alter lipid metabolism, although controversy exists regarding the impact of saturated vs. polyunsaturated fats. Adiponectin (Ad) stimulates fatty acid (FA) oxidation and improves insulin sensitivity in humans and rodents, due in part to the activation of AMP-activated protein kinase (AMPK) and subsequent deactivation of acetyl coenzyme A carboxylase (ACC). In genetically obese, diabetic mice, this acute stimulatory effect on AMPK in muscle is lost. The ability of a HF diet to induce skeletal muscle Ad resistance has not been examined. The purpose of this study was to determine whether Ad’s effects on FA oxidation and AMPK/ACC would be reduced following different HF diets, and if this coincided with the development of impaired maximal insulin-stimulated glucose transport.

Rats were fed a control (10% kcal fat, CON), high unsaturated fat (60% kcal safflower oil, SAFF), or high saturated fat diet (60% kcal lard, LARD) for 4 wk. Following the dietary intervention, glucose transport, lipid metabolism, and AMPK/ACC phosphorylation were measured in the presence and absence of globular Ad (gAd, 2.5 µg/ml) in isolated soleus muscle. LARD rats showed reduced rates of maximal insulin-stimulated glucose transport compared with CON and SAFF (+68 vs. +172 and +184%, P ≤ 0.001). gAd increased pACC (+25%, P = 0.01) and FA oxidation (+28%, P ≤ 0.05) in CON rats, but not in either HF group. Thus 4 wk of HF feeding results in the loss of gAd stimulatory effect on ACC phosphorylation and muscle FA oxidation, and this can occur independently of impaired maximal insulin-stimulated glucose transport.

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glucose transport following a 4-wk feeding trial with diets of varying fat composition. Specifically, we aimed to determine 1) whether gAd-stimulated FA oxidation in skeletal muscle is impaired in response to HF feeding of either a high PUFA or high saturated fat diet; 2) whether gAd-stimulated phosphorylation of AMPK and ACC is impaired; 3) whether the development of impaired maximal insulin-stimulated glucose transport occurs concurrently with the loss of gAd’s effects; and 4) whether these responses differ, depending on the type of fat consumed. We hypothesized that the stimulatory effects of gAd on FA oxidation would be blunted in rats fed both HF diets, but that impaired insulin-stimulated glucose transport would only develop in animals fed a high saturated fat diet. Furthermore, since gAd may exert its acute stimulatory effects on FA oxidation by activating AMPK, we hypothesized that gAd will phosphorylate AMPK and ACC in control, but not HF-fed rats.

METHODS

Animals and Diets

Upon arrival, female Sprague-Dawley rats (140–145 g, Charles River, Quebec, Canada) were assigned to individual cages in a controlled environment with a reverse 12-h light-dark cycle and ad libitum access to Purina standard rodent chow and water. Following a 3-day acclimation period, rats were randomly assigned to one of three dietary interventions for 4 wk (Research Diets, New Brunswick, NJ): control diet (12% kcal from fat; CON), high unsaturated fat diet (60% kcal from safflower oil; SAFF), or high saturated fat diet (60% kcal from lard, LARD) (Table 1). HF-fed animals were pair-fed to CON rats (fed ad libitum) with respect to caloric intake on a daily basis, and body mass was recorded three times per week. After 4 wk on the diets, the animals were overnight fasted before experimental procedures. Ethical consent for all procedures used was obtained from the Animal Care Committee at the University of Guelph.

Table 1. Composition of experimental diets

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>SAFF</th>
<th>LARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
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<tr>
<td>Carbohydrate</td>
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<tr>
<td>Fat</td>
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</tr>
<tr>
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<td>100</td>
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<tr>
<td>kcal/g</td>
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Table 2. Ingredients

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<th>LARD</th>
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<td>200</td>
<td>200</td>
</tr>
<tr>
<td>L-Cystine</td>
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<td>12</td>
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<tr>
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<td>0</td>
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<tr>
<td>Maltodextrin 10</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Sucrose</td>
<td>69</td>
<td>275</td>
<td>69</td>
</tr>
<tr>
<td>Cellulose, BW200</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Lard</td>
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<td>0</td>
<td>270</td>
</tr>
<tr>
<td>Safflower Oil, USP</td>
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<tr>
<td>Mineral mix S10026</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
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<tr>
<td>Calcium carbonate</td>
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<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Potassium citrate, H2O</td>
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</tr>
<tr>
<td>Vitamin mix V10001</td>
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</tr>
<tr>
<td>Choline bitartrate</td>
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<td>2</td>
</tr>
<tr>
<td>Total</td>
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<td>4,057</td>
<td>774</td>
</tr>
</tbody>
</table>

Blood Collection and Analyses

One-milliliter blood samples were collected from the tail artery of each animal under anesthetic with isofluorane inhalant, before the dietary intervention was started. A terminal blood collection was also made at the completion of the treatment via cardiac puncture after first excising skeletal muscles for incubation. A glucometer reading (Bayer Elite XL, Toronto, ON, Canada) of whole blood glucose was made at each blood collection. All blood samples were collected after an overnight fast in heparinized tubes, centrifuged at 9,300 g for 5 min at 4°C, and the plasma was removed for analyses of insulin (sensitive rat RIA kit, Linco, St. Charles, MO) and Ad (mouse RIA kit, Linco).

Muscle Incubations

Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body mass), and the soleus (Sol) muscle was carefully dissected into longitudinal strips from tendon to tendon using a 27-gauge needle. Two strips from each Sol were then incubated under various conditions for measurement of glucose transport, fat metabolism, or Western blot analysis [phosphorylated AMPK (pAMPK) and ACC (pACC)]. Due to the number of conditions in these experiments, one animal was used for determination of glucose transport (4 Sol strips), and another animal was used for measurement of both lipid metabolism (2 Sol strips) and Western blot analysis (2 Sol strips). Sol muscle was chosen due to its oxidative potential and capacity to oxidize FA and because our laboratory has used this muscle in our laboratory’s previous experiments demonstrating diet-induced leptin resistance (25).

Glucose Transport

Individual strips were randomly assigned to one of four conditions: 1) basal, 2) gAd (2.5 µg/ml; Peprotech, ON, Canada), 3) insulin (10 mU/ml), or 4) insulin + gAd. These conditions were maintained in all buffers for the duration of the experiments. Immediately after excision from the animal, Sol strips were preincubated in a shaking water bath at 30°C for 30 min in 2 ml pregassed (95% O2/5% CO2) Krebs-Henseleit buffer (KHB) containing 0.1% FA-free BSA, 8 mM glucose, and 32 mM mannitol. Then strips underwent two 10-min incubations in glucose-free KHB containing 4 mM pyruvate and 36 mM mannitol. Finally, strips were transferred into a third KHB-based buffer containing 8 mM 3-O-[14C]methyld-glucose (0.5 µCi/ml) and 32 mM [14C]mannitol (0.2 µCi/ml) and incubated for 20 min (insulin stimulated) or 40 min (basal). After incubation, muscles were blotted of excess fluid, trimmed of tendons, and weighed. Strips were digested in 1 ml of 1 M NaOH at 95°C for 10 min, and 200 µ were then sampled in duplicate for liquid scintillation counting. Glucose transport was analyzed as accumulation of intracellular 3-O-[14C]methyld-glucose, as described previously (31).

FA Metabolism and AMPK/ACC Signaling

Sol strips were placed in 20-ml glass scintillation vials containing 2 ml of warmed (30°C), pregassed (95% O2/5% CO2) KHB containing 4% FA-free BSA, 5 mM glucose, and 0.5 mM palmitate, with or without gAd (2.5 µg/ml). After a 30-min equilibration, muscles were transferred to a new vial containing 2 ml of the same medium, with the addition of 0.5 µCi/ml of [1-14C]palmitate (Amersham, Oakville, ON, Canada) for an additional 60-min incubation. This permitted the monitoring of exogenous palmitate oxidation and incorporation of palmitate into endogenous triacylglycerol (TAG) and diacylglycerol (DAG) lipid pools, as previously described (7).

After the incubations, muscles were blotted of excess liquid, trimmed of tendons, weighed, and placed in a 14-ml centrifuge tube containing 5 ml of ice-cold 2:1 chloroform methanol. Muscles were homogenized using a handheld polytron (PT1200, Brinkman Institute, Mississauga, ON, Canada) and centrifuged at 10,000 g for 10 min. The supernatant was transferred to a clean 14-ml centrifuge, and 2 ml...
of distilled water were added. Samples were shaken for 10 min and centrifuged as before to separate the aqueous and lipophilic phases. One milliliter of the aqueous phase was quantified by liquid scintillation counting to determine the amount of \( ^{14} \text{C} \)-labeled oxidation intermediates resulting from isotopic fixation. The chloroform phase, containing the total lipids extracted from the muscle, was gently evaporated under a stream of \( \text{N}_2 \) and re-dissolved in 100 \( \mu \text{L} \) of 2:1 chloroform-methanol containing small amounts of dipalmitin and tripalmitin to facilitate identification of lipid bands on the silica plates. Fifty microliters of each sample were spotted onto an oven-dried silica gel plate (Fisher Scientific Canada, Mississauga, ON, Canada) and placed into a sealed tank containing solvent (60:40:3 heptane-isopropl ether-acetic acid) for 50 min. Plates were allowed to dry and were sprayed with dichlorofluorescein dye (0.2\% wt/vol in ethanol) and visualized under long-wave ultraviolet light. The individual lipid bands were scraped into vials for liquid scintillation counting.

Gaseous \( ^{14} \text{CO}_2 \) produced during the incubation was trapped by 250 \( \mu \text{L} \) benzethonium hydroxide contained in a microcentrifuge tube, followed by relaxation and sulfuric acid to then be captured in benzethonium hydroxide. The \( ^{14} \text{CO}_2 \) remaining in the buffer placed in the sealed 20-ml scintillation vial for the course of the chase phase. In addition, \( ^{14} \text{CO}_2 \) remaining in the buffer following the incubation was released by immediately transferring 1 ml of buffer into a sealed flask and acidifying with 1 ml of 1 M sulfuric acid to then be captured in benzethonium hydroxide. The tubes containing benzethonium hydroxide and trapped \( ^{14} \text{CO}_2 \) were counted using standard liquid scintillation counting techniques.

The remaining two Sol strips were incubated in the presence or absence of gAd (2.5 \( \mu \text{g} / \text{ml} \)) for 30 min in KHB containing 4\% FA-free BSA, 5 mM glucose, and 0.5 mM palmitate for the determination of pAMPK and pACC protein content. These measurements were repeated in Sol strips from additional CON animals at a 10-min time point. After incubation, the strips were immediately frozen and stored in liquid nitrogen until subsequent analyses.

**Western Blot Analysis**

Sol muscles (~50 mg) were homogenized (5,000 \( \mu \text{L} / \text{g tissue} \), 1:5 dilution) in ice-cold buffer suitable for protein extraction and preserving phosphorylation states of proteins, containing 50 mM Tris (pH 7.5), 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 5 mM sodium pyrophosphate, 10\% (vol/vol) glycerol, 1\% (vol/vol) Triton X-100, 2 mg/ml leupeptin, 2 mg/ml aprotin, 2 mg/ml pepstatin, 1 mM dihydrothreitol, and 1 mM phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 20,000 \( \times \text{g} \) for 20 min at 4\°C, and the supernatant was removed, and protein content was determined using BSA as standards. Fifty micrograms of this whole tissue lysate protein were solubilized in 4\times Laemmli’s buffer and boiled at 95\°C for 5 min, resolved by SDS-PAGE, and wet transferred to PVDF membranes (1.5 h, 100 V for pAMPK, 8 h, 30 V for pACC). The membranes were blocked for 1 h and then incubated with the specific primary antibodies for Thr172 pAMPK (Cell Signaling, Danvers, MA) or Ser79 pACC (Cell Signaling). After incubation with the appropriate secondary antibody and final wash, the immune complexes were detected using the enhanced chemiluminescence method, and immunoreactive bands were quantified with densiometry. Equal loading was confirmed by nonspecific protein staining with Ponso-S.

**Calculations and Statistics**

All data are reported as means ± SE. Results were analyzed using a randomized block design two-way ANOVA, and a Student Newman Keuls post hoc test was used to test significant differences revealed by the ANOVA. One-way ANOVA was used for body mass and blood measurements. Significance was accepted at \( P \leq 0.05 \).

The quantity of palmitate esterified and oxidized was calculated from the specific activity of labeled palmitate in the incubation medium (i.e., ratio of labeled to total palmitate in the incubation medium). Total palmitate uptake was calculated by summing the incorporation of labeled palmitate into lipid pools plus oxidation.

**RESULTS**

**Body Composition and Blood Measurements**

There was no significant difference between diet groups with respect to pretrial or terminal body mass (Table 2). Diet groups did not differ with respect to any measured blood parameter before commencing the experimental diets (data not shown). There was no significant difference in fasting blood glucose or insulin between groups following the 4-wk trial. Plasma Ad was significantly reduced in both HF-fed groups compared with CON by 4 wk \( (P \leq 0.05) \);

**Skeletal Muscle Glucose Transport**

Basal glucose transport was not different among the three dietary groups (Fig. 1A). However, the LARD rats showed significantly reduced rates of insulin-stimulated glucose transport (Fig. 1B) compared with CON and SAFF \((+68 \% \text{ vs. } +172 \%, P \leq 0.001)\). That is, only rats fed a high saturated fat diet developed skeletal muscle IR, while rats fed a high unsaturated fat or control low-fat diet did not. Glucose transport was unaltered by the addition of gAd to the incubation medium in basal \((CON, 0.11 \pm 0.01; \text{SAFF, 0.12 } \pm 0.01; \text{LARD, 0.10 } \pm 0.01 \mu \text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1})\) and insulin-stimulated conditions \((CON, 0.26 \pm 0.02; \text{SAFF, 0.25 } \pm 0.03; \text{LARD, 0.20 } \pm 0.02 \mu \text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1})\), regardless of the dietary treatment.

**Skeletal Muscle Lipid Metabolism**

**FA oxidation.** Basal FA oxidation rate was significantly higher in SAFF \((49.8 \pm 3.5 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1})\) and LARD \((46.4 \pm 3.4 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1})\) groups compared with CON \((34.4 \pm 2.8 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}, P \leq 0.05)\), suggesting a diet-induced adaptation by the SAFF and LARD rats to the higher fat availability. Adiponectin was significantly increased in Sol from CON rats (+28\%, \(P \leq 0.05\)), but had no stimulatory effect on FA oxidation in either HF group (Fig. 2A).

**FA esterification.** FA esterification into TAG was greater in both HF groups compared with CON \((67.9 \pm 5.8 \text{ SAFF, 66.8 } \pm 3.4 \text{ LARD vs. } 47.6 \pm 3.3 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}, P \leq 0.05)\), but gAd did not have a significant effect within dietary treatments (Fig. 2B). FA esterification into DAG was not different between the three dietary treatments and was unaffected by gAd (Fig. 2C).

**Oxidation-to-TAG esterification ratio.** There was no difference in the ratio of palmitate oxidized to esterified between dietary groups under basal conditions. However, the ratio was significantly greater in the CON group \((0.93 \pm 0.1)\) compared

**Table 2. Body mass and blood measurements**

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>SAFF</th>
<th>LARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass pretrial, g</td>
<td>144±2</td>
<td>145±2</td>
<td>143±1</td>
</tr>
<tr>
<td>Body mass posttrial, g</td>
<td>242±4</td>
<td>240±3</td>
<td>246±4</td>
</tr>
<tr>
<td>Body mass gain, g</td>
<td>98±4</td>
<td>95±3</td>
<td>103±3</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>6.6±0.2</td>
<td>6.8±0.2</td>
<td>6.8±0.2</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>2.7±0.3</td>
<td>2.1±0.5</td>
<td>3.2±0.7</td>
</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>4.5±0.5</td>
<td>2.6±0.2*</td>
<td>3.5±0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 12 \) animals. *Significantly different from control: \( P \leq 0.05 \).
liver FA and glucose metabolism. However, low circulating levels of Ad characterize most cases of obesity (1), and, recently, suggestions of an impaired peripheral response to this adipokine have been reported in genetically obese mouse

with both SAFF and LARD (0.56 ± 0.05 and 0.68 ± 0.07, P ≤ 0.05) following exposure to gAd.

**Total palmitate uptake.** Basal rates of total palmitate uptake tended to be greater in both HF groups (131 ± 10 nmol/g SAFF, 120 ± 7 nmol/g LARD) compared with CON (101 ± 7 nmol/g, P = 0.058). Treatment with gAd did not change total FA uptake in any group (107 ± 4 nmol/g CON; 126 ± 9 nmol/g SAFF; 120 ± 13 nmol/g LARD).

**Influence of Ad on pAMPK and pACC**

Basal pAMPK and pACC (Fig. 3) were similar across all three diet groups. There was no significant effect of acute gAd exposure (30 min, Fig. 3A; 10 min, Fig. 4A) on pAMPK in any group. Phosphorylation of ACC was significantly elevated after 30-min gAd exposure (Fig. 3B) in CON rats only (+25%, P ≤ 0.01). There was no effect of gAd on pACC after 10 min (Fig. 4B).

**DISCUSSION**

Ad is an adipocyte-derived cytokine with antidiabetic properties, mediated predominantly by its effects on muscle and
models (29) and in obese humans (3). In the present study, we used isolated Sol muscle to determine the direct effects of gAd on maximal insulin-stimulated glucose transport and FA metabolism in skeletal muscle from rats following 4 wk of HF feeding. More specifically, our primary goal was to determine whether the consumption of a diet high in saturated or unsaturated fat led to the loss of gAd’s stimulation of FA oxidation in skeletal muscle, and if this coincided with the development of impaired insulin-stimulated glucose transport. Furthermore, we wanted to determine whether consumption of a HF diet would interfere with the acute stimulatory effect of gAd on the AMPK-ACC axis. Several novel observations were made in the present study: 1) both HF diets resulted in the loss of gAd’s ability to stimulate FA oxidation; 2) the lack of stimulation of FA oxidation in response to gAd is supported by the lack of ACC phosphorylation in these dietary groups; 3) the development of gAd resistance did not necessarily coincide with the development of impaired insulin-stimulated glucose transport, as both conditions were manifested only in the LARD group; and 4) we confirmed previous findings in rodent muscle that gAd results in the phosphorylation of ACC independently of AMPK.

**Effect of Diet on Blood Parameters**

As expected for a brief dietary intervention, plasma glucose levels did not differ between groups. However, somewhat surprisingly, there was also no change in insulin levels. It is possible that 4 wk are not long enough to see whole body changes in insulin levels, even though impairment of insulin-stimulated glucose uptake in Sol muscle was evident with the LARD diet. However, the insulin response assessed in Sol may not be representative of other muscle types; additionally, blood samples were taken from fasted animals. As such, it is possible that postprandial insulin responses may differ between groups. Lastly, both HF groups had significantly decreased plasma total Ad concentrations compared with CON, consistent with previous findings in obese models (1). The physiological significance of gAd, which was utilized in this study, is unclear. It has been reported that the globular form may represent ~1% of the total circulating Ad (8), while other reports have failed to detect it (21). However, even as little as 1% of the total

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**Fig. 3.** The effect of 30-min gAd exposure on phosphorylated AMP-activated protein kinase (pAMPK; A) and phosphorylated acetyl coenzyme A carboxylase (pACC; B). Solid bars, basal; open bars, gAd stimulated. Values are means ± SE; n = 12. *Significantly different from basal, P < 0.01.

**Fig. 4.** The effect of 10-min gAd exposure on pAMPK (A) and pACC (B) in control rats. Solid bars, basal; open bars, gAd stimulated. Values are means ± SE; n = 10.
circulating Ad may be significant, as Ad is present in the circulation in much higher concentrations than other adipokines. Teleologically, it is also difficult to reconcile the presence of the Ad R1 receptor in skeletal muscle, which specifically binds the gAd form, if there is no gAd present in the circulation. Regardless of this controversy, it seems clear that the globular form does have significant effects on muscle FA metabolism and insulin sensitivity and might be of important therapeutic value.

**Effect of Diet and gAd on Skeletal Muscle Lipid Metabolism**

**Basal adaptations to increased fat availability.** Animals in both HF groups demonstrated several adaptations to the increased fat availability in their diet. Despite differences in the metabolic handling of saturated FA vs. PUFA, both HF-fed groups displayed similar compensatory increases in basal palmitate oxidation and esterification into TAG, similar to previous HF feeding trials (6, 24, 25).

**gAd-stimulated changes in fat metabolism.** Of greater interest to the present study are the differences between dietary groups with respect to lipid metabolism under gAd-stimulated conditions. As expected, gAd stimulated FA oxidation by ~30% in CON rats. This is in agreement with our previous findings in human muscle (3), and the findings of others using rodents (8, 28), which show increases in FA oxidation ranging from 10 to 60% in isolated muscle strips incubated with gAd. However, to the best of our knowledge, we are the first group to demonstrate that gAd does not stimulate FA oxidation in Sol muscle following the consumption of a HF diet, indicating the development of gAd resistance. Similar to our laboratory’s previous observations in rodents (25), baseline FA oxidation rates were elevated in HF-fed rats, potentially complicating the interpretation of a lack of further stimulation by gAd in these animals, i.e., FA had already reached maximal capacity. However, this seems quite unlikely, given that only 0.5 mM palmitate was present in the incubation buffer. Nevertheless, in light of this finding, we confirmed the ability of gAd to further stimulate FA oxidation when Sol muscles from CON-fed animals were incubated at a higher concentration of palmitate (0.75 mM), which elicits a basal rate of oxidation similar to that observed in the HF-fed rats. Under these conditions, gAd still stimulated FA oxidation (76.6 ± 4.1 vs. 65.6 ± 3.4 nmol·g⁻¹·h⁻¹; P < 0.05). Thus we interpret the inability of gAd to further stimulate palmitate oxidation above basal levels in HF rats as an indicator of resistance to the effect of Ad. Finally, the inability of gAd to stimulate FA oxidation in the HF-fed rats is also supported by a lack of ACC phosphorylation.

In addition to increasing the amount of palmitate oxidized, gAd has been previously shown to decrease intramuscular lipid accumulation in murine muscle (34). Contrary to our expectations, treatment with gAd did not acutely decrease lipid accumulation in either DAG or TAG lipid pools in any dietary group. However, the ratio of oxidation to TAG esterification was significantly greater in muscle from CON rats when incubated with gAd. This suggests a protective repartitioning of FA toward oxidation and away from storage as potentially active lipid moieties. While not immediately apparent in a short-term resting muscle preparation, over time this beneficial repartitioning by gAd may reduce the lipid content in muscle, a possible mechanism by which Ad improves insulin sensitivity. It is also possible that gAd may not have been able to decrease TAG and DAG in the absence of a stimulus that promotes esterification, such as insulin or TNF-α. This hypothesis is supported by the findings of a previous study by Bruce and Dyck (2), where leptin decreased DAG accumulation only in the presence of TNF-α, a known promoter of lipid storage. Furthermore, it is also possible that changes may have occurred in other lipid pools, such as ceramide or long chain fatty acyl CoA, which we did not measure.

**Effect of Diet and gAd on Skeletal Muscle Glucose Transport**

One of the major findings of the present study was that a diet high in saturated fat resulted in impaired insulin-stimulated glucose transport, but a diet containing 60% unsaturated fat did not. It should be noted that all animals were pair-fed to a control; thus changes in muscle responsiveness to insulin in LARD rats cannot be attributed to increases in body mass relative to the other two groups. Recently, there has been some controversy regarding the development of IR after consuming HF diets of varying composition. It has been previously shown that a diet high in PUFAs can induce liver and skeletal muscle IR, and partial substitution with n–3 FA is necessary for reversal (27, 32). However, more recently, studies have shown that, while a diet high in saturated fat leads to decreased whole body insulin sensitivity, a diet high in n–6 PUFA sustains insulin sensitivity (13). Direct examination of muscle cells in vitro indicate that, once taken up in skeletal muscle, saturated and unsaturated fats are metabolized differently. Unsaturated fats are more readily oxidized (9, 14) and are stored preferentially as TAG (9), whereas saturated fats are not readily oxidized and are stored as DAG (18) or ceramides (23), which can directly interfere with insulin signaling (5, 35).

Taken together with our laboratory’s previous findings that a high-SAFF diet can induce leptin resistance (25), it is noteworthy that this diet did not lead to a loss of maximal insulin-stimulated glucose uptake. However, it is important to note that, in our study, rodents were overnight fasted, which has previously been shown to ameliorate diet-induced muscle IR (20). Therefore, it is possible that our SAFF diet may have also led to impaired insulin-stimulated muscle glucose transport, which was reversed by the overnight fast. Nevertheless, the impairment in insulin-stimulated glucose transport clearly persisted in the LARD-fed animals, as did the resistance to gAd in both HF-fed groups and were not reversed by a brief fast. Whether prolonged HF feeding (i.e., >4 wk) would result in the eventual development of impaired Sol glucose transport in the high-SAFF group, persisting beyond an overnight fast, fast warrants further examination, as would future studies examining the time course of adipokine/IR in Sol muscle.

A recent study by Ceddia et al. (4) using L6 myotubes suggests that Ad exerts a stimulatory effect on skeletal muscle glucose transport by enhancing GLUT-4 translocation to the cell surface via AMPK activation. We were unable to show an effect of gAd on basal or insulin-stimulated glucose transport. This is also in contrast to our laboratory’s work in humans (3) in which glucose transport was stimulated by gAd in both lean and obese subjects. However, our results are in support of Tomas et al. (28), in which gAd-stimulated increases in glu-
cose transport occurred in the presence of AMPK activation (glycolytic extensor digitorum longus muscle), but not in its absence (oxidative Sol muscle). We, too, did not observe a gAd-stimulated activation of AMPK in Sol muscle. Thus it is possible that gAd stimulates glucose transport in a fiber-type-specific manner, exerting its effects through AMPK in fast-twitch glycolytic muscles, but having no effect in slow oxidative muscle where this acute phosphorylation is absent. However, it should be noted that Yamauchi et al. (33) do show an increase in AMPK activation in Sol muscle.

Effect of Diet and gAd on AMPK and ACC

Acutely, gAd has been shown to stimulate FA oxidation in muscle due, at least in part, to the phosphorylation (i.e., activation) of AMPK. Consequently, ACC is phosphorylated (deactivated), leading to decreased malonyl CoA content, relieving the inhibition on carnitine palmitoyltransferase I, and allowing for increased FA oxidation (4, 28, 33).

There is some controversy regarding AMPK activation by gAd in various muscle types. Stimulation of the AMPK-ACC axis has been shown in L6 myotubes (4) and glycolytic muscle (28). However, while Yamauchi et al. (33) have reported AMPK activation in oxidative muscle, Tomas et al. (28) have reported no change in pAMPK in oxidative Sol muscle. Still, Tomas et al. (28) did report an increase in pACC in oxidative muscle. The activation of AMPK by gAd has been shown to be transitory, with peak activation occurring at ~5 min and returning to baseline values by 60 min (4, 28, 33). In comparison, pACC levels have been shown to increase later and remain sustained longer (28). In agreement with Thomas et al. (28), our results showed that acute gAd exposure had no effect on Sol pAMPK in any dietary group, but did cause a significant increase in pACC in oxidative muscle. Since this observation was consistent at both 10 and 30 min of incubation, it is highly unlikely that the transitory increase in pACC was simply not detected. Therefore, it is possible that, in Sol muscle, gAd phosphorylates ACC, without acting through AMPK. As such, the phosphorylation of ACC in CON rats supports our findings that CON rats are sensitive to the stimulatory effects of gAd, while HF rats show signs of gAd resistance.

Summary

In conclusion, we suggest that 4 wk of HF feeding result in the development of skeletal muscle Ad resistance, and this occurs independent of impaired capacity for insulin-stimulated glucose transport. Moreover, this reduced response to gAd in HF-fed rats is likely due to a decreased phosphorylation of ACC. Thus it appears as though a general HF diet can induce Ad resistance, but the source of fat is important for the development of IR.

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