Skeletal muscle inflammation is not responsible for the rapid impairment in adiponectin response with high-fat feeding in rats

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Mullen KL, Tishinsky JM, Robinson LE, Dyck DJ. Skeletal muscle inflammation is not responsible for the rapid impairment in adiponectin response with high-fat feeding in rats. Am J Physiol Regul Integr Comp Physiol 299: R500–R508, 2010. First published June 16, 2010; doi:10.1152/ajpregu.00080.2010.—Adiponectin (Ad) is an insulin-sensitizing adipokine known to stimulate fatty acid (FA) oxidation in skeletal muscle. Skeletal muscle can become resistant to Ad very rapidly, after only 3 days of high saturated fat feeding in rats. Whether the same occurs following a high polyunsaturated fat diet is unknown. Obesity, insulin resistance, and hyperlipidemia are recognized as low-grade inflammatory diseases; therefore, we hypothesized that high-fat feeding induces inflammation, which interferes with Ad action at skeletal muscle. To this end, rats were placed into one of three dietary groups, control (CON, 10% kcal from fat), high saturated (SAT), or high polyunsaturated (PUFA) fat (60% kcal from fat) for 3 days to determine whether Ad resistance develops. Half of the animals from each group were further supplemented with aspirin, a common anti-inflammatory drug. Ad stimulated FA metabolism, Ad signaling intermediates [AdipoR1, APPL1, LKB1, AMPK, and acetyl-CoA carboxylase (ACC)], and inflammatory proteins [Toll-like receptor (TLR4), IkKα/β, IkBα, NF-κB, suppressor of cytokine signaling-3 (SOCS3), and JNK] were measured in soleus muscle. Three days of SAT feeding induced Ad resistance in soleus muscle, assessed as an inability of Ad to phosphorylate ACC and increase FA oxidation. In PUFA-fed animals, Ad-stimulated FA oxidation and ACC phosphorylation to the same degree as CON animals (FA oxidation: +35%, +41%; pACC +29%, +19%; CON, PUFA, P < 0.05). However, neither SAT nor PUFA feeding for 3 days induced skeletal muscle inflammation. Surprisingly, aspirin prevented Ad-stimulated increases in FA oxidation. In conclusion, FA type is critical in the development of Ad resistance, but this does not appear to be mediated by inflammation.

Saturated fatty acids; polyunsaturated fatty acid; aspirin; AdipoR1; APPL1; fatty acid oxidation; suppressor of cytokine signaling-3; NF-κB

Adiponectin (Ad) is an insulin-sensitizing adipokine. In skeletal muscle, the binding of Ad’s globular fragment (gAd) to its receptor, AdipoR1, triggers an intracellular signaling cascade, initiated by the association of APPL1 [adaptor protein containing pleckstrin homology domain, phosphotyrosine binding (PTB) domain and leucine zipper motif] with AdipoR1 and subsequent phosphorylation of acetyl-CoA carboxylase (ACC), resulting in the stimulation of fatty acid oxidation (11, 21, 42). The potential roles of other upstream kinases, (LKB1 and AMPK) in this pathway are somewhat controversial and may depend on fiber type (35, 42). This gAd-stimulated increase in skeletal muscle fatty acid oxidation may act to prevent increases in intramuscular lipid accumulation and thus maintain or improve skeletal muscle insulin sensitivity (11, 43). However, in cases of obesity, circulating Ad levels are decreased (1). Furthermore, we and others have shown evidence of Ad resistance in both obese animals and humans (6, 36), as demonstrated by a loss or blunting of the acute stimulatory effect of gAd on FA oxidation in skeletal muscle.

We have also shown that Ad resistance is inducible; that is, muscle from rats fed a diet high in either saturated (SAT) or polyunsaturated fat (PUFA) for 4 wk becomes completely resistant to gAd’s ability to stimulate FA oxidation (26). Of particular interest is our most recent finding that Ad resistance develops very rapidly, in as little as 3 days on a high saturated fat diet and prior to the derangement in muscle FA metabolism and onset of insulin resistance, which occurred between 2 and 4 wk of feeding (25). This suggests that the development of Ad resistance may be an initiating event in lipid-induced skeletal muscle insulin resistance. Whether the same rapid induction occurs following a diet high in PUFA has not yet been examined. Furthermore, the mechanism underlying the cause of lipid-induced Ad resistance is unknown.

Obesity is characterized by a state of chronic, low-grade inflammation (15, 31, 38). Conditions that induce insulin resistance by increasing lipid exposure stimulate inflammatory signaling (3, 16); conversely, blocking key components of the inflammatory cascade can protect against lipid-induced insulin resistance (28, 33, 46). Specifically, Toll-like receptor 4 (TLR4), a component of the innate immune response, has been identified on skeletal muscle and is activated by saturated fatty acids, causing phosphorylation of IKKβ (28), resulting in NF-κB translocation to the nucleus and the upregulation of proinflammatory gene expression (12, 28, 33). Triggering TLR4 can also induce suppressor of cytokine signaling-3 (SOCS-3) expression in innate immune cells and brain tissue (2, 5, 20), but it has yet to be demonstrated in skeletal muscle. There is strong evidence supporting the role of SOCS-3 in the development of skeletal muscle leptin resistance, as it interferes with leptin-stimulated phosphorylation of AMPK (34). Since leptin and Ad have similar mechanisms of action in muscle (23, 35), it is possible that SOCS-3 may also interfere with Ad signaling, although this is unknown. Recently, Yaspelkis et al. (44) reported increased SOCS-3 protein in skeletal muscle of rats following 12 wk of high-fat feeding, corresponding with decreased IRS-1 tyrosine phosphorylation. However, the role of SOCS-3 in the early adaptations to high-fat feeding is currently unknown. Furthermore, whether a short-term, high-fat diet will induce changes in skeletal muscle inflammatory signaling and whether this depends on the type of fatty acid ingested has not been examined.

Activation of the inflammatory signaling cascade can directly interfere with insulin signal transduction, as IKKβ phosphorylates IRS-1 at Ser307, thereby preventing further insulin...
signal propagation (45). Aspirin, (acetylsalicylic acid, ASA) a common anti-inflammatory drug, has been shown to be a weak inhibitor of IKKβ (45). In higher doses (i.e., grams/day), ASA has been shown to improve insulin sensitivity, decrease circulating FA levels, and decrease plasma glucose and insulin, effects that are at least partially attributable to its actions at IKKβ (17, 46). Despite its widespread usage, the role of ASA on skeletal muscle lipid metabolism and adipokine response is virtually unknown.

The objectives of the current study were to determine whether 1) Ad resistance develops in skeletal muscle in only 3 days on a high PUFA diet, similar to a high SAT diet; 2) inflammatory markers are altered after 3 days of HF feeding and are dependent on the type of fat consumed; and 3) blocking inflammation with ASA supplementation can maintain Ad response. It is hypothesized that 1) a diet high in PUFA will not cause Ad resistance in 3 days, 2) a high-fat diet will cause inflammatory changes in skeletal muscle as little as 3 days, and the changes will be more pronounced in SAT-fed rats, and 3) Ad sensitivity will be maintained in skeletal muscle of high-fat fed animals by using an anti-inflammatory drug (ASA).

METHODS

Animals, Diets, and Aspirin Supplementation

Upon arrival, female Sprague-Dawley rats (140–145 g, Charles River, Sainte-Anne-de-Bellevue, Quebec, Canada) were assigned to individual cages in a controlled environment with a reverse 12:12-h light-dark cycle with ad libitum access to a low-fat control diet and water.

Following the 3-day acclimation period, half of the rats began a 7-day ASA supplementation period, with 100 mg ASA/kg body mass mixed into their control diet daily. Following this 7-day priming period, both ASA-supplemented and nonsupplemented animals were further divided into one of 3 dietary groups for an additional 3 days, low-fat control (10% of Caloric intake on a daily basis, and body mass was recorded three times per week.

After the 3 days of dietary treatment, animals were overnight fasted before experimental procedures. Ethical consent for all procedures outlined previously (8).

Blood. Terminal blood collection was 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) of whole blood glucose was also made. All blood samples were collected in heparinized tubes after an overnight fast, centrifuged at 9,300 g for 5 min at 4°C, and the plasma was removed for analyses of ASA (salicylate enzyme assay; Cambridge Life Sciences, Cambridge, UK), total Ad (mouse RIA kit; Linco, St. Charles, MO), C-reactive protein (mouse/rat-singleplex; Millipore, Billerica, MA), insulin, leptin, TNF-α, MCP-1, and IL-6 (rat serum adipokine panel; Millipore, Billerica, MA).

Fatty acid metabolism. Soleus strips were equilibrated in 2 ml of pregassed (95% O2-5% CO2) Krebs Henseleit buffer (KHB; 4% BSA, 30°C), with 0.5 mM palmitate and 5 mM glucose for 30 min. Muscles were incubated for an additional 60 min with the addition of 0.5 μCi/ml [1-14C]palmitate (Amersham, Oakville, ON, Canada) and in the absence or presence of gAd (2.5 μg/ml) to determine palmitate oxidation and incorporation into endogenous triacylglycerol (TAG) and diacylglycerol (DAG) lipid pools, as outlined previously (8).

Briefly, after the incubations, muscles were blotted of excess liquid, trimmed of tendons, weighed and treated to separate the aqueous and lipophilic phases. One milliliter of the aqueous phase was quantified by liquid scintillation counting to determine the amount of 14C-labeled oxidation intermediates resulting from isotopic fixation. Muscle lipids were redissolved in 100 μl of 2:1 chloroform-methanol, spotted onto an oven-dried silica gel plate (Fisher Scientific Canada, Mississauga, ON, Canada), and placed into a sealed tank containing 60:40:3, heptane:isopropyl-ether: acetic acid for 45 min. The individual lipid bands were scraped into vials for liquid scintillation counting. 14CO2 accumulated in the buffer was released by transferring 1 ml of buffer into a sealed flask and acidifying with 1 ml of 1 M sulfuric acid and captured in benzothiazonium hydroxide. The trapped 14CO2 were counted using standard liquid scintillation counting techniques.

Total palmitate uptake was calculated by summing the incorporation of labeled palmitate into lipid pools plus oxidation.

Adiponectin-stimulated signaling proteins. Soleus strips were incubated in KHB containing 4% FA-free BSA, 5 mM glucose, and 0.5 mM palmitate for 30 min in the presence or absence of gAd (2.5 μg/ml) for the determination of total APPL1 and total and phosphorylated LKB1, AMPK, and ACC protein content. After incubation, the strips were immediately frozen and stored in liquid N2 until subsequent Western blot analyses.

Western Blot Analyses

Muscle tissue (~35 mg Sol) was homogenized in ice-cold buffer (1:9 wt/vol dilution) suitable for whole cell protein extraction and preserving phosphorylation states of proteins. Homogenates were sonicated for 5 s to ensure the nuclear membrane was completely broken, centrifuged at 1,500 g for 15 min at 4°C, and the supernatant was removed, and protein content was determined using BSA as standards.

Fifty micrograms of whole cell lysate protein (100 μg for pIKKβ and pSer536) were solubilized in 4X Laemmli’s buffer, boiled (95°C, 5 min), resolved by SDS-PAGE, and wet-transferred to polyvinylidene difluoride membranes [1,5 h, 100 V, total and Ser79 phosphorylated ACC; 8–15 h, 25–40 V, 4°C]. The membranes were blocked for 1 h and then incubated overnight at 4°C with the specific primary antibodies for AdipoR1 (Abcam, Cambridge, MA), APPL1, total and Ser428 phosphorylated LKB1, total and Thr172 phosphorylated AMPK, total and Ser79 phosphorylated ACC (pACC, Upstate, Billerica MA), TLR4, and total and Ser176/180 phosphorylated IKKα/β, total and Ser12 phosphorylated IκBα, total and Ser536 phospho-NF-κB p65, total and Thr183/185, Thr212/223 phosphorylated JNK (Millipore, Lake Placid, NY), and SOCS-3. All antibodies were purchased from AJP-Regul Integr Comp Physiol · VOL 299 · AUGUST 2010 · www.ajpregu.org
Differ between any groups. Acute gAd exposure significantly increased FA oxidation in CON (+41%, \( P \leq 0.05 \)) and PUFA (+35%, \( P \leq 0.05 \))-fed animals, but it failed to do so in SAT-fed animals. ASA supplementation resulted in a loss of the stimulatory effect of gAd on FA oxidation in all dietary groups (Fig. 1).

**Fatty acid esterification.** Fatty acid esterification into TAG and DAG was not different between dietary treatments and was unaffected by ASA supplementation or acute gAd exposure (range, TAG: 92.8 ± 3.8 to 116.9 ± 7.8 nmol/g/h; DAG: 13.3 ± 0.5 to 18.9 ± 1.4 nmol/g/h).

**Total uptake.** Total fatty acid uptake into skeletal muscle was unaffected by diet, ASA supplementation or acute gAd exposure (range: 142.8 ± 7.3 to 175.0 ± 11.8 nmol/g/h).

**Adiponectin-Stimulated Signaling**

**Total proteins.** There was no significant difference in total protein content of AdipoR1, APPL1, LKB1, or ACC between any treatment groups (Fig. 2). However, ASA supplementation significantly decreased total AMPK protein in all diet groups (Fig. 2). AdipoR1 and APPL1 content were also determined in glycolytic EDL muscle following the high-fat diet for comparison; these were unaffected (data not shown).

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### Table 1. Body mass and plasma measurements

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>CON + ASA</th>
<th>PUFA</th>
<th>PUFA + ASA</th>
<th>SAT</th>
<th>SAT + ASA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, g</td>
<td>239 ± 5</td>
<td>243 ± 8</td>
<td>241 ± 3</td>
<td>237 ± 8</td>
<td>237 ± 5</td>
<td>245 ± 9</td>
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<tr>
<td>Adiponectin, μg/ml</td>
<td>4.1 ± 0.7</td>
<td>6.2 ± 0.6*</td>
<td>2.5 ± 0.3†</td>
<td>4.2 ± 0.4*</td>
<td>2.8 ± 0.2* ( P = 0.06 )</td>
<td>4.6 ± 0.5*</td>
</tr>
<tr>
<td>Glucose, mmol</td>
<td>8.1 ± 0.8</td>
<td>7.6 ± 0.3</td>
<td>8.0 ± 0.5</td>
<td>8.1 ± 0.4</td>
<td>8.7 ± 0.6</td>
<td>8.0 ± 0.5</td>
</tr>
<tr>
<td>Insulin, pg/ml</td>
<td>1557 ± 191</td>
<td>1268 ± 329†</td>
<td>1124 ± 114</td>
<td>935 ± 150†</td>
<td>1624 ± 257</td>
<td>1153 ± 194†</td>
</tr>
<tr>
<td>Leptin, pg/ml</td>
<td>2868 ± 475</td>
<td>2427 ± 450*</td>
<td>2173 ± 410</td>
<td>1297 ± 105*</td>
<td>2863 ± 325</td>
<td>1507 ± 281*</td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>81 ± 21</td>
<td>83 ± 41</td>
<td>103 ± 15</td>
<td>101 ± 25</td>
<td>80 ± 10</td>
<td>57 ± 15</td>
</tr>
<tr>
<td>CRP, μg/ml</td>
<td>543 ± 35</td>
<td>484 ± 35</td>
<td>480 ± 36</td>
<td>497 ± 36</td>
<td>415 ± 31</td>
<td>501 ± 28</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; \( n = 7–12 \). PUFA, polyunsaturated fatty acids; SAT, high saturated (fatty acids). *Significantly different from non-acetyl-saliclyc (ASA)-supplemented \( P \leq 0.05 \). †Significantly different from non-ASA-supplemented \( P \leq 0.1 \). ‡Significantly different from control (CON) diet \( P \leq 0.05 \).
Phosphorylated proteins. Adiponectin significantly increased the phosphorylation of ACC in CON- and PUFA-fed animals, but not in SAT-fed animals (Fig. 3). Adiponectin, diet, and ASA had no significant effect on LKB1 or AMPK phosphorylation (Fig. 3).

Skeletal Muscle Inflammatory Signaling (Nonstimulated)

Total protein. There was no significant effect of diet or ASA treatment on total protein content of TLR4, IKKα/β, IkBα, NFκB, SOCS-3, and JNK in soleus (Fig. 4). Inflammatory markers were also unaffected in glycolytic EDL muscle (data not shown).

Phosphorylated proteins. Three days of high-fat feeding (SAT and PUFA) and 10 days of ASA supplementation did not significantly alter phosphorylation of any measured inflammatory signaling proteins in soleus (Fig. 5). The ratio of phosphorylated to total protein content did not change with diet or ASA (CON, 1.0 ± 0.04; CON + ASA, 0.9 ± 0.05; PUFA, 1.0 ± 0.04; PUFA + ASA, 0.9 ± 0.06; SAT, 1.0 ± 0.08; SAT + ASA, 0.9 ± 0.04). The effect of diet on phosphorylation of inflammatory proteins in EDL was unaffected (data not shown).

DISCUSSION

In the current study, we show that animals fed a high-PUFA diet remained Ad responsive, as evidenced by increased skeletal muscle ACC phosphorylation and FA oxidation in the presence of gAd, whereas SAT fed rats became resistant and did not respond to gAd. However, regardless of the type of dietary FA, 3 days of high-fat feeding did not alter muscle or blood markers of inflammation. Surprisingly, ASA supplementation decreased total AMPK protein and prevented gAd-stimulated increases in FA oxidation. Neither APPL1 nor LKB1 were affected by diet or ASA, suggesting that absolute changes in APPL1 and LKB1 content/phosphorylation are not required for altered Ad response.

Adiponectin Response Differs Depending on the Type of Fatty Acid Ingested

Saturated and polyunsaturated fatty acids are known to differentially affect insulin sensitivity and inflammation (19, 22, 24, 40). To the best of our knowledge, we are the first to study 1) the effects of PUFA vs. SAT feeding on adiponectin response in skeletal muscle and 2) the very early (i.e., 3 day)
adaptation of skeletal muscle to high-fat diets of differing FA composition. In support of our hypothesis, muscle from PUFA-fed animals remained Ad sensitive, as determined by the ability of gAd to acutely increase FA oxidation and pACC, while SAT-fed animals became Ad resistant. In our earlier study (26), we showed that although 4 wk of PUFA feeding did induce Ad resistance, these animals remained insulin sensitive. In comparison, SAT-fed animals, which demonstrate impaired Ad response in the soleus by 3 days of feeding, demonstrate reduced insulin-stimulated glucose transport between 2 and 4 wk (25). Thus, if Ad sensitivity can be maintained longer (as is the case when PUFA are the main source of the diet), then the development of insulin resistance appears to be delayed, supporting an initiating role of Ad resistance in the development of insulin resistance. Taken together, the findings of our current and previous (25, 26) studies indicate that a diet very high in PUFA (i.e., 60% kcal) will induce gAd, but not insulin resistance, by 4 wk, which appears to be less detrimental than a high SAT diet, which causes impairment in gAd response after only 3 days, and insulin resistance by 4 wk. However, we hypothesize that if the PUFA diet were administered longer (i.e., several months), eventually insulin resistance would occur in this group as well; thus, an extreme high-fat diet of any composition is likely to be detrimental.

Prior to commencing the current study, we hypothesized that a SAT diet would promote inflammation, which, in turn, would interfere with Ad signaling, while a PUFA diet would not cause inflammation and thus preserve Ad signaling. However, the results of the current study do not support this hypothesis. While the metabolic response to Ad was different between diets, there were no changes in muscle or blood markers of inflammation to explain this difference. Therefore, although it is clear that Ad response varies on the basis of the type of FA ingested, it remains unclear why these differences exist.

Three Days of High-Fat Feeding Is not Sufficient to Induce Inflammation

We hypothesized that a diet high in saturated fat would induce a state of elevated inflammation (i.e., increased muscle inflammation).
pIKK\(\beta\), pIkBo\(\alpha\), and pNF-\(\kappa\)B, and plasma CRP, TNF\(\alpha\), MCP-1) since SAT FA have been reported to trigger inflammation in several tissues and cell types (28, 30, 37, 44). Radin et al. (28) have shown that SAT FA stimulate TLR4 on the plasma membrane of skeletal muscle and trigger activation of the downstream IKK/NF-\(\kappa\)B signaling cascade, as indicated by an increased phosphorylation of IKK and IkBo\(\alpha\). Yaspelkis et al. (44) have recently shown that I\(\kappa\)B\(\alpha\) and IKK\(\beta\) phosphorylation is significantly increased in skeletal muscle following 12 wk of high-SAT feeding. In the current study, we did not show increased total protein content or phosphorylation state of any measured inflammatory protein following 3 days of high-fat feeding.

It is possible that the absolute amount or the phosphorylation state of individual proteins may not be the sole or best indicator of an altered inflammatory state. Cellular location or colocalization with other proteins may be equally important determinants of a protein’s action. In support of this, Yaspelkis et al. (44) reported increased SOCS-3 colocalization with IRS-1 following 12 wk of high-SAT feeding. In the current study, we did not show increased total protein content or phosphorylation state of any measured inflammatory protein following 3 days of high-fat feeding.

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It is also quite plausible that 3 days of HF feeding is simply not long enough to cause a detectable increase in the inflammatory markers measured in this study. Previous HF-feeding studies that report increased inflammatory signaling in skeletal muscle have ranged from 6 wk to several months in duration (3, 17, 28, 32, 44, 46). Although 3 days may be a short time period to observe inflammatory changes, there are clear and sustained changes in Ad-stimulated lipid metabolism after only 3 days of high-fat feeding. Furthermore, stimulation of TLR4 in macrophages can increase SOCS-3 mRNA in as little as 1 h (7), and 3 days of palmitate incubation can significantly up-regulate TLR4 protein content in human myotubes (30). Therefore, it is reasonable to presume that inflammatory changes might have occurred within 3 days, although the results of the current study do not support this.

It should be noted that in the current study, all animals were fasted for 12 h prior to the measurement of inflammatory markers in plasma and skeletal muscle. Therefore, it remains possible that an acute increase in inflammatory proteins occurred following ingestion of HF diet but subsided during the subsequent 12-h fast. Even if such is the case, the impaired response of skeletal muscle to Ad stimulation still remained following an overnight fast, in the absence of any alteration in inflammatory markers.

**Total AdipoR1 and APPL1 May not Predict Ad Response**

AdipoR1 protein content was not affected by diet, in agreement with our previous work (25), or by ASA, and thus does not explain the differences in Ad response between dietary and ASA treatments. Also, as expected on the basis of our previous findings (25, 26), changes in pACC mirrored changes in FA oxidation and thus Ad response. However, contrary to our hypothesis, there was no difference in

![Figure 4](http://ajpregu.physiology.org/Download.jpg)
APPL1 or total and phosphorylated LKB1 protein content between animals who were Ad responsive or resistant, suggesting that changes in the content of these Ad-signaling proteins are not necessarily required to alter Ad response.

Recently, Wang et al. (39) identified APPL2, a novel protein involved in the regulation of APPL1 association with AdipoR1. In a basal state, APPL2 sequesters APPL1 in the cytoplasm and also occupies the APPL1-AdipoR1 intracellular binding site. Upon binding of gAd to AdipoR1, APPL2 releases APPL1 and simultaneously dissociates from AdipoR1, thereby facilitating APPL1-AdipoR1 interaction. Thus, it is possible that while the total quantity of APPL1 and AdipoR1 did not change, a greater proportion of APPL1 remained sequestered in the cytoplasm and did not associate with the receptor upon gAd binding. Because of soleus muscle tissue limitations, we were unable to measure colocalization of APPL1 with AdipoR1. However, future studies should examine the potential role of APPL2 and APPL1 colocalization in the development of Ad resistance.

Aspirin Decreases Total AMPK Protein and Prevents Ad-Stimulated Fatty Acid Oxidation

A surprising finding of the current study was that ASA supplementation decreased total AMPK protein and prevented gAd stimulation of FA oxidation in soleus muscle. This does not necessarily mean that ASA interferes specifically with Ad signal transduction. We speculated that ASA might decrease mitochondrial content (oxidative capacity) of the muscle, rendering it incapable of responding to a stimulus of oxidation above basal levels. Very limited evidence in patients with Reyes Syndrome suggests that aspirin may inhibit palmitate oxidation in skin fibroblasts from both control and diseased patients (14). To this end, we measured two markers of mitochondrial content (citrate synthase, /H9252HAD protein content), but data did not show any decrease in their content in soleus muscle from ASA-supplemented animals (data not shown).

We are not the first group to show potentially undesirable metabolic responses to ASA supplementation. Recently, Xiao et al. (41) reported that 1 wk of ASA supplementation did not
prevent lipid-induced insulin resistance in overweight and obese nondiabetic men as assessed by the insulin sensitivity index. In fact, ASA supplementation decreased insulin sensitivity below that seen in nonsupplemented men (41). Additional conflicting results have been reported in humans following aspirin and salicylate derivative supplementation. Evidence of improved (10), unchanged (9, 18, 29), and decreased (2, 4, 13, 27) insulin sensitivity have all been reported following salicylate supplementation at doses (1–7 g/day in adult humans) and time periods (3 days to 4 wk), similar to the current study. In the current study, plasma ASA levels in supplemented animals averaged ~2 mM, consistent with previously reported levels in humans and animals (range 1–5 mM) and well below toxic levels (45, 46).

A potential limitation of the current study is that all measurements of FA metabolism and signaling proteins were made in soleus muscle, comprising predominantly type I fibers. The soleus muscle was chosen as we have previously established the time course of the development of Ad and insulin resistance in this muscle. Furthermore, its oxidative capacity allows for greater uptake and oxidation of FA compared with glycolytic muscle, and its fiber orientation allows for multiple strips to be made from a single muscle, thereby providing tight experimental control. We acknowledge that the findings of the current study may not necessarily reflect the development of Ad and insulin resistance in glycolytic or mixed-fiber muscles, although key signaling intermediates assessed in glycolytic EDL muscle did match the observations made in oxidative soleus muscle. However, there is evidence that oxidative muscle is more susceptible to the induction of insulin resistance by diet (40, 47). Thus, an examination of the development and significance of Ad resistance in glycolytic muscle and its relationship to insulin resistance is clearly needed.

**Perspectives and Significance**

Taken together, these results demonstrate that Ad resistance develops rapidly in skeletal muscle in response to high-fat feeding, but it is dependent on the type of dietary fatty acid. Saturated fatty acids induce Ad resistance, while PUFAs maintain Ad response, at least in the short term. Adiponectin’s stimulation of FA oxidation is not dependent on changes in total content or phosphorylation of other Ad signaling intermediates (AdipoR1, APPL1). In comparison, 3 days of high-fat feeding, whether PUFA or SAT, is insufficient to cause chronic changes in inflammatory proteins in the muscle or plasma. Therefore, we must conclude from the current findings that skeletal muscle inflammation is not responsible for the rapid diet-induced Ad resistance. Furthermore, the current results provide support for other recent findings demonstrating negative metabolic consequences to ASA administration.

**REFERENCES**


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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.
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INFLAMMATION DOES NOT CAUSE SKELETAL MUSCLE ADIPONECTIN RESISTANCE


