

Resistin acutely impairs insulin-stimulated glucose transport in rodent muscle in the presence, but not absence, of palmitate

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Junkin KA, Dyck DJ, Mullen KL, Chabowski A, Thrush AB. Resistin acutely impairs insulin-stimulated glucose transport in rodent muscle in the presence, but not absence, of palmitate. *Am J Physiol Regul Integr Comp Physiol* 296: R944–R951, 2009. First published February 4, 2009; doi:10.1152/ajpregu.90971.2008.—Resistin is a cytokine implicated in the development of insulin resistance. However, there has been little investigation of the effects of resistin on fatty acid (FA) metabolism and insulin response in skeletal muscle, a key tissue for glucose disposal. The purpose of the present study was to examine the role of altered FA metabolism as a cause of resistin's inhibition of insulin-stimulated glucose transport in muscle. Isolated rat soleus muscles were incubated acutely (2 h) in the presence or absence of 600 ng/ml resistin, with or without 2 mM palmitate. Resistin acutely impaired insulin-stimulated glucose transport and Akt phosphorylation, but only in the presence of palmitate, implicating a role for altered FA metabolism. This impairment of glucose transport induced by resistin plus palmitate could be pharmacologically rescued by the inclusion of amidazole carboxamide ribonucleotide, a stimulator of AMP-activated protein kinase and FA oxidation, as well as inhibitors of ceramide synthesis (myriocin, fumonisin). However, to our surprise, resistin actually blunted the palmitate-induced increase in muscle ceramide content; as expected, ceramide content was significantly lowered by fumonisin. In summary, the acute impairment of insulin response by resistin was manifested only in the presence of high palmitate and was alleviated when FA metabolism was manipulated (increased oxidation, inhibited ceramide synthesis). Resistin's acute impairment of insulin response does not appear to require an absolute increase in ceramide content; however, reducing ceramide content alleviated the impairment in glucose transport and insulin signaling.

fatty acid metabolism; adipokines; amidazole carboxamide ribonucleotide; myriocin; fumonisin; diacylglycerol; ceramide

THE LINK BETWEEN EXCESS STORES of body fat and the development of skeletal muscle insulin resistance is complex. Several adipose-derived cytokines (adipokines) have been implicated in the regulation of insulin sensitivity. Resistin is an adipokine (11, 13, 28) implicated in the development of insulin resistance based on initial observations in rodents that 1) circulating resistin is increased in genetic and diet-induced models of obesity; 2) resistin administration impairs whole body glucose homeostasis and insulin sensitivity; 3) neutralization of resistin improves these parameters in diet-induced obesity; and 4) resistin expression is markedly reduced by antidiabetic drugs, such as thiazolidinediones (27). The role of resistin in human obesity and diabetes is less clear, as its circulating concentration does not correlate well with the presence of insulin resistance (10) and its expression in adipocytes is very low (12, 21). However,

resistin is produced by human mononuclear cells and macrophages (14), which can infiltrate adipocytes surrounding muscle or situated between muscle fibers and may exert a paracrine effect. Thus better understanding the mechanisms underlying the potential role of resistin in diabetes and insulin resistance remains important.

Resistin impairs insulin signaling [insulin receptor substrate (IRS)-1, IRS-2, Akt] and glucose transport in cultured myotubes (15, 17) and mature rodent skeletal muscle (19). However, until very recently, the effects of resistin on muscle fatty acid (FA) metabolism were not studied. In particular, the accumulation of reactive lipid species, such as ceramide (6), has been implicated in the development of insulin resistance in muscle. Adipokines such as leptin and adiponectin increase FA oxidation and decrease muscle triacylglycerol (TAG) content (26, 36), which is associated with improved insulin sensitivity in rodents (37, 38). Resistin was recently demonstrated to impair AMP-activated protein kinase (AMPK) activation and FA oxidation in L₆ muscle cells and ultimately increase intracellular lipid content within 24 h (18). To our knowledge, there are no reports examining 1) the direct effects of resistin on FA metabolism in mature skeletal muscle; 2) whether the presence of FA, which are typically elevated in obesity, are necessary for resistin to impair insulin sensitivity; or 3) whether resistin increases reactive lipid species such as ceramide or diacylglycerol (DAG), implicated in insulin resistance.

The purpose of this study was to 1) determine whether the acute exposure of isolated rat soleus muscle to resistin results in impairment of insulin-stimulated glucose transport and signaling; and 2) whether this is dependent on the presence of the saturated FA, palmitate, in the incubation medium. Next, we explored the possibility that by either 3) stimulating FA oxidation with the well-known pharmacological AMP analog, amidazole carboxamide ribonucleotide (AICAR), or blocking ceramide synthesis (myriocin, fumonisin), the inhibition of insulin-stimulated glucose transport in the presence of resistin might be prevented. Finally, 4) we attempted to specifically assess the role of ceramide accumulation as a mechanism by which resistin might impair insulin-stimulated glucose transport.

METHODS

Animals

Female Sprague-Dawley rats (150–180 g; Charles River, Saint Constant, Quebec) were used for all experiments. Rats were housed in a controlled environment on a 12:12-h reverse light-dark cycle and

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allowed ad libitum access to standard chow and water. Ethical consent for all experimental procedures was obtained from the Animal Care Committee at the University of Guelph.

Experimental Procedures

Preparation of skeletal muscle strips. Before all surgical procedures, rats were anesthetized with 6 mg/g body wt of pentobarbital sodium. Soleus muscle strips (20–25 mg) were carefully procured for subsequent incubation in gassed (95% O₂-5% CO₂) modified Krebs-Heinseleit buffer (KHB; pH 7.4) containing 4% FA-free BSA and 5.5 mM glucose. Incubation conditions were defined by the absence or presence of 1) 2 mM palmitate [high fat (HF)] and 2) 600 ng/ml recombinant murine resistin (R) (Peprtech, Rocky Hill, NJ). Several series of experiments were conducted to perform all necessary measurements, as outlined below. All incubations were performed at 30°C.

Experiments to determine the acute effect of resistin on glucose transport, insulin signaling, and FA metabolism. GLUCOSE TRANSPORT. Basal and insulin-stimulated (10 mU/ml) 3-*O*-[³H]methyl-D-glucose transport in isolated soleus strips was measured following 2 h of incubation under one of the four experimental conditions: 1) fat free (FF) without R (FF - R), 2) FF plus R (FF + R), 3) HF without R (HF - R), and 4) HF plus R (HF + R). Rats were categorized according to the palmitate concentration (0, 2 mM), and, within each rat, each leg was designated for the resistin condition, with or without. Within each leg, one soleus strip was used to determine basal glucose transport, and the other for insulin stimulated glucose transport.

Following the 2-h incubation, muscles were transferred into KHB-0.1% BSA containing 8 mM glucose and 32 mM mannitol, in the presence or absence of insulin (10 mU/ml, maintained in all subsequent incubations) for 20 min. Soleus strips were then washed twice (10 min each) in glucose-free KHB (4 mM pyruvate, 30 mM mannitol) before finally being incubated for either 20 min (insulin) or 40 min (basal) in KHB {4 mM pyruvate, 8 mM 3-*O*-[³H]methyl-D-glucose (800 μCi/mmol), 28 mM [¹⁴C]mannitol (60 μCi/mmol)}. The presence or absence of resistin was maintained constant throughout all phases of the glucose transport determination, as in the initial 2-h incubation. Muscle strips were blotted and weighed and digested in glass tubes containing 1 ml of 1 M NaOH at 95°C for 10 min. Two hundred microliters were sampled in duplicate for liquid scintillation counting.

INSULIN SIGNALING. A separate experiment was conducted for the Western blot analyses of phosphorylation of Akt and Akt substrate 160 (AS160), and total protein content of IRS-1, p85 subunit of phosphatidylinositol 3-kinase (p85-PI3-kinase), and Akt of the insulin transduction pathway. Soleus muscle strips were incubated for 2 h under the conditions previously described (FF - R, FF + R, HF - R, HF + R). Muscles were then transferred into freshly gassed buffer containing 10 mU/ml insulin for 10 min, previously shown to capture the transient phosphorylation of insulin signaling proteins in rodent skeletal muscle (25). The strips were then weighed, frozen in liquid N₂, and stored at -80°C until analyzed.

FA METABOLISM. In a separate series of experiments, palmitate oxidation and esterification into TAG were measured during the final hours of the 2-h incubation. Soleus strips were incubated for 1 h and then transferred to freshly gassed buffer containing [1-¹⁴C]palmitate, (0.5 μCi/ml buffer). FA metabolism was only assessed during HF conditions. Palmitate oxidation and incorporation into TAG was determined by capturing ¹⁴CO₂ and [¹⁴C]-label incorporated into TAG, as our laboratory has previously outlined in more detail (7).

Experiments to rescue the deleterious effects of resistin on glucose transport: implications for altered FA metabolism. GLUCOSE TRANSPORT AND INSULIN SIGNALING. Results from the above experiments demonstrated that resistin acutely impaired insulin-stimulated glucose transport in soleus muscle, but only in the HF condition (see RESULTS). Accordingly, we tested whether the impaired insulin-stimulated glucose transport induced by the combination of HF + R could be

prevented by including in the incubation medium 1) AICAR, 2 mM (Toronto Research Chemicals, North York, Ontario, Canada), a stimulator of AMPK and FA oxidation; or 2) inhibitors of ceramide synthesis [myriocin, serine palmitoyltransferase inhibitor (100 μM, Sigma, St Louis, MO) or fumonisins B₁, ceramide synthase inhibitor (50 μM; Sigma)]. We also examined whether insulin-stimulated Akt phosphorylation was altered in the presence of the myriocin or fumonisins, as ceramides are known to specifically inhibit this site (6). For these experiments, soleus strips from each hindlimb were paired as HF + R and HF + R + test compound (AICAR, myriocin, fumonisins).

CERAMIDE AND DAG LIPID ACCUMULATION. Following 2 h of incubation in either control (FF), HF, HF + R, and HF + R + fumonisins, muscle samples were immediately frozen in liquid N₂ for subsequent analysis of ceramide and DAG content. Briefly, 30 mg of soleus muscle were freeze dried, cleaned of any visible adipose tissue and blood, and extracted using the Folch et al. (8) method of extraction modified according to van der Vusse et al. (34). Muscle lipids were separated using thin-layer chromatography on silica gel plates (0.22 mm Kieselgel 60; Merk, Darmstadt, Germany) and detected using gas liquid chromatography (3, 24).

Western blot analyses. Frozen muscles were homogenized [1% Triton X-100, 50 mM Tris·HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 10 mM sodium β-glycerol phosphate, 5 mM sodium pyrophosphate, 2 mM DTT, 1 mM orthovanadate, 1 mM PMSF, and 10 μg/μl each of aprotinin, leupeptin, and pepstatin] on ice for the extraction of cytosolic proteins and centrifuged, the supernatant collected, and protein concentration determined using a BCA protein assay.

Samples were solubilized in 4× Laemmli's buffer (glycerol, SDS, 0.5 M Tris·HCl, 1% bromophenol blue, 31 mg/500 μl DTT) to achieve a final protein content of 40 μg/μl. Samples were then boiled, resolved by SDS-PAGE, and wet transferred to polyvinylidene difluoride membranes. Membranes were blocked for 60 min and incubated with primary antibodies specific for the following: Ser473-phosphorylated Akt, Thr308-phosphorylated Akt, total Akt (Santa Cruz Biotechnology, Santa Cruz, CA), IRS-1 and p85-PI3-kinase (Upstate Cell Signaling Solutions, Millipore, MA), Thr642-phosphorylated AS160 (Medicorps, Quebec, Canada), Thr172-phosphorylated AMPK, and Ser79-phosphorylated acetyl-CoA carboxylase (Cell Signaling, Danvers, MA). Following incubation with secondary goat anti-rabbit antibodies for 1 h, membranes were washed, and proteins were detected using enhanced chemiluminescence (Syngene Chemigenius 1, Perkin Elmer, Waltham, MA). Equal loading was confirmed using nonspecific protein staining with Ponceau-S stain (Sigma Aldrich, Oakville, Ontario, Canada).

Calculations and Statistics

Palmitate oxidation and incorporation into lipid pools (nmol·g⁻¹·wt⁻¹) were calculated based on the specific activity of the incubation medium (dpm radiolabeled palmitate/nmol total palmitate). Total palmitate oxidation was calculated by summing all ¹⁴C-label trapped in CO₂ as well as in the acid-soluble phase via isotopic fixation. Glucose transport was calculated by correcting the total 3-*O*-[³H]methyl-D-glucose analog accumulation for the extracellular contamination ([¹⁴C]mannitol). Results are expressed as means ± SE. Data were analyzed with appropriate ANOVA tests. A Student-Newman-Keuls post hoc test was used to test for significant differences revealed by the ANOVA. Significance was accepted at *P* ≤ 0.05.

RESULTS

Experiments to Determine the Acute Effect of Resistin on Glucose Transport, Insulin Signaling, and FA Metabolism

Glucose transport. Insulin increased glucose transport (Fig. 1) approximately two- to threefold, regardless of the presence or

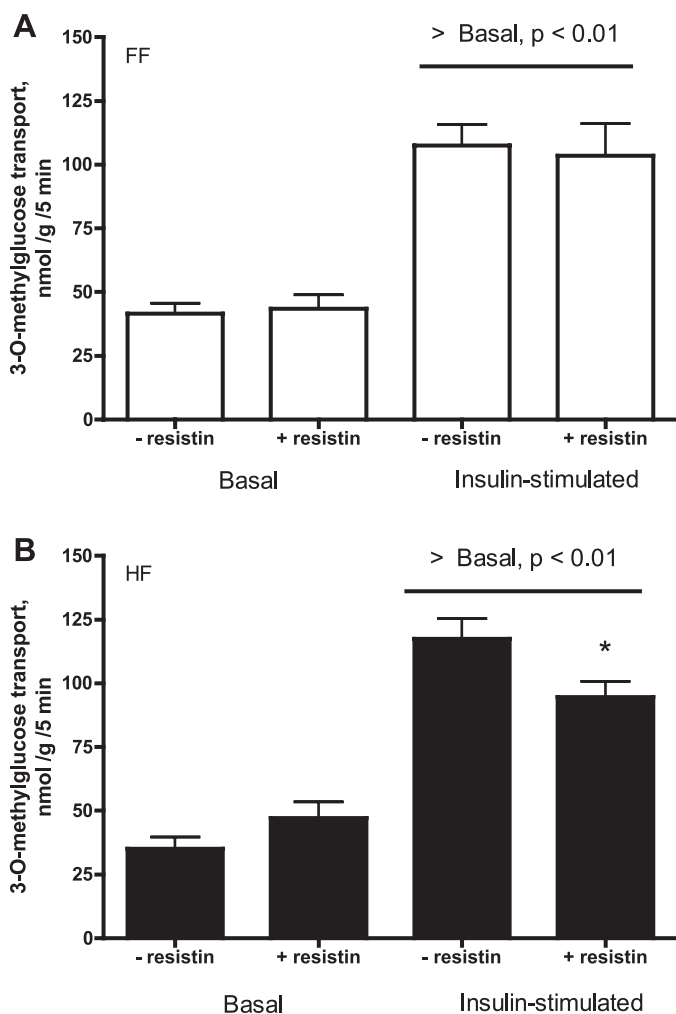


Fig. 1. Effect of resistin on basal and insulin-stimulated glucose transport in isolated soleus muscle in fat-free (FF; A) and high-fat (HF; B) conditions. Bars represent means \pm SE; sample size is 10–12. *Significantly different from paired condition, $P < 0.05$. FF – R, fat free without resistin; FF + R, fat free plus resistin; HF – R, high fat without resistin; HF + R, high fat plus resistin.

absence of palmitate ($P < 0.01$). Glucose transport rates, under both basal and insulin-stimulated conditions, were similar, regardless of the presence or absence of palmitate. Exposure to resistin for 2 h did not affect insulin-stimulated glucose transport when palmitate was absent from the incubation medium (Fig. 1A), but significantly reduced insulin-stimulated glucose transport (-20% , $P < 0.05$) in the presence of 2 mM palmitate (Fig. 1B). Resistin had no effect on basal glucose transport, regardless of the presence of HF.

Insulin signaling, akt and as160 phosphorylation. Under insulin-stimulated conditions, Akt phosphorylation was increased approximately two- to threefold (Fig. 2), both in the presence and absence of palmitate ($P < 0.01$). Resistin did not alter the phosphorylation state of Akt, either at Ser473 or Thr308, in the absence of palmitate (Fig. 2A). However, in the presence of palmitate, resistin blunted the phosphorylation of Akt at Ser473 (-27% , $P = 0.05$) and Thr308 (-22% , $P < 0.05$) under insulin-stimulated conditions. In the presence of insulin, phosphorylation of AS160 Thr642 increased ($P < 0.05$) $\sim 50\%$ (Fig. 3). Resistin had no effect on the phosphor-

ylation of AS160, regardless of the presence or absence of palmitate, during insulin-stimulated conditions.

Akt and AS160 phosphorylation were unaltered by the presence of resistin under basal (noninsulin stimulated) conditions.

IRS-1, AKT AND P85-PI3-KINASE PROTEIN CONTENT. Regardless of the presence or absence of 2 mM palmitate, resistin and insulin had no significant effect on the protein content of IRS-1, p85-PI3-kinase, or Akt following 2 h of incubation (Table 1).

FA metabolism. Resistin had no acute effect on the total palmitate uptake or its oxidation and incorporation into TAG (Table 2).

Experiments to Correct the Effects of Resistin on Glucose Transport: Implications for Altered FA Metabolism

The addition of 2 mM AICAR, a pharmacological stimulator of AMPK and FA oxidation, to the medium containing palmitate and resistin significantly increased insulin-stimulated glucose transport (HF + R, 94 ± 15 nmol \cdot g $^{-1}$ \cdot 5 min $^{-1}$ vs. HF + R + AICAR, 135 ± 22 nmol \cdot g $^{-1}$ \cdot 5 min $^{-1}$; $P < 0.001$). Phosphorylation of AMPK and acetyl-CoA carboxylase did not differ following the 2-h incubation in the presence or absence of AICAR, immediately before the measurement of glucose transport (data not shown). Thus improved glucose transport was not a function of a continuous AMPK activation.

The addition of either myriocin (serine palmitoyltransferase inhibitor) or fumonisins (ceramide synthase inhibitor) significantly increased insulin-stimulated glucose transport ($\sim 50\%$) in the presence of high palmitate and resistin, compared with resistin and high palmitate alone (Fig. 4). Phosphorylation of Akt Ser473 and Thr308 were also increased $\sim 50\%$ ($P < 0.05$) by these pharmacological agents (Fig. 5).

Soleus ceramide and DAG content increased following 2 h of incubation with 2 mM palmitate, compared with the FF control (Fig. 6). To our surprise, neither ceramide nor DAG were significantly greater than the control condition when resistin was included with 2 mM palmitate. The presence of fumonisins completely prevented the palmitate-induced increase in ceramide content. However, ceramide content following palmitate plus resistin exposure was also not significantly different from palmitate alone, suggesting that resistin did not totally prevent ceramide accumulation. Neither resistin nor fumonisins altered soleus ceramide or DAG content within 2 h in the absence of palmitate (data not shown).

DISCUSSION

In the present study, the novel observation made was that resistin impairs insulin-stimulated glucose transport in isolated skeletal muscle, but only when accompanied by high-palmitate availability. Palmitate alone did not impair glucose transport within a 2-h period, although it is likely that this would have occurred with a longer exposure, as our laboratory has previously demonstrated in isolated human muscle (33). This implies that resistin either accelerates or is additive to the insulin-desensitizing effects of palmitate. Accordingly, pharmacological stimulation of FA oxidation (AICAR) and inhibition of ceramide synthesis (myriocin, fumonisins) rescued insulin-stimulated glucose transport in the presence of resistin and palmitate. Therefore, it was somewhat surprising that we did not find evidence of altered FA uptake, oxidation, or incorporation into TAG with resistin. Perhaps most unexpected was our finding

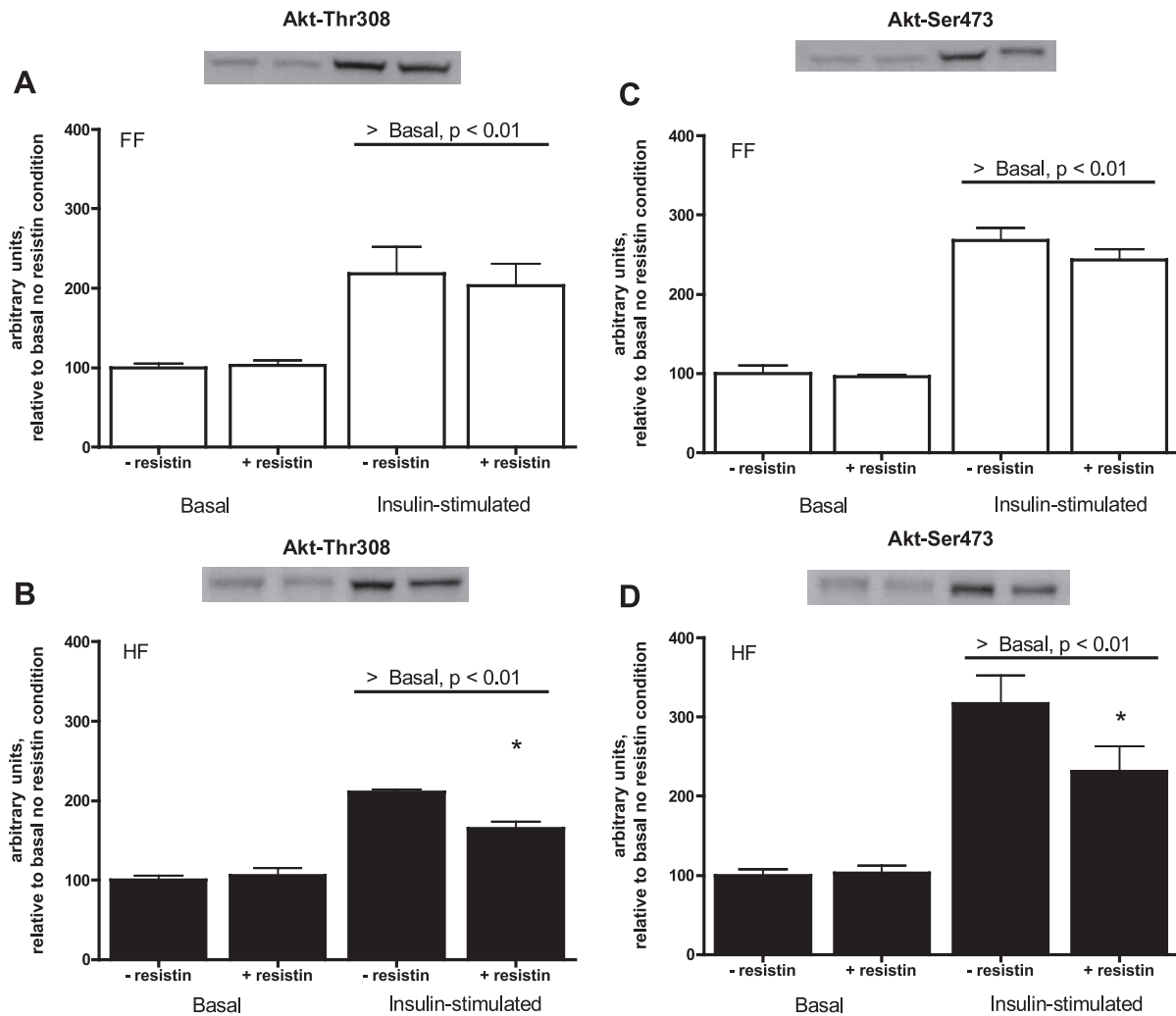


Fig. 2. Effect of resistin on basal and insulin-stimulated Akt Thr308 and Ser473 phosphorylation in isolated soleus muscle in FF (A and C) and HF (B and D) conditions. Bars represent means \pm SE; sample size is 10–12. *Significantly different from paired condition, $P < 0.05$.

that resistin actually blunted the palmitate-induced increase in ceramide and DAG content. Thus aberrations in muscle FA metabolism, i.e., impaired oxidation, increased lipid formation, do not appear to be a direct mechanism by which resistin acutely accelerates, or is additive to palmitate-induced insulin resistance. Regardless, improving FA metabolism i.e., increased oxidation, decreased ceramide formation clearly can rescue this situation.

Resistin Impairs Insulin-stimulated Glucose Transport and Akt Phosphorylation

Resistin has previously been shown to decrease IRS-1 and Akt protein content and phosphorylation in muscle following exposure ranging from 24 h to 7 days (17, 20). Acutely, i.e., within 30 min, resistin can impair insulin-stimulated glucose transport in L_6 muscle cells in the absence of any changes in insulin signaling or GLUT-4 translocation (15). In the present study, we demonstrate that the acute inhibitory effects of resistin on insulin-stimulated glucose transport required the presence of high (2 mM) palmitate. This impairment coincided with a decrease in Akt phosphorylation at both Ser473 and Thr308 sites. AS160 Thr642 phosphorylation was increased by

50% in response to insulin, which was unaffected by resistin. Our assessment of insulin signaling at 10 min was based on a previous study investigating the time course of insulin signaling response in muscles of different fiber types (25). A more recent study indicates that peak transient phosphorylation of AS160 occurs at a later time point than Akt, i.e., 15 vs. 5 min (4). In hindsight, it is likely that we assessed insulin signaling too early to catch the peak phosphorylation of AS160 and, therefore, cannot accurately comment on the ability of resistin to inhibit its phosphorylation.

Altered FA Metabolism as a Potential Mechanism of Resistin's Effects

Resistin's effect on palmitate oxidation and deposition. Palmitate has been shown to acutely increase reactive lipid species, particularly ceramide, and induce skeletal muscle insulin resistance (6, 16, 22, 32, 39). Although the accumulation of DAG and ceramide have each been associated with the development of skeletal muscle lipid-induced insulin resistance, recent evidence (6, 29) has implicated ceramide as potentially the more important species. Specifically, this appears to be mediated through the inhibition of Akt activation.

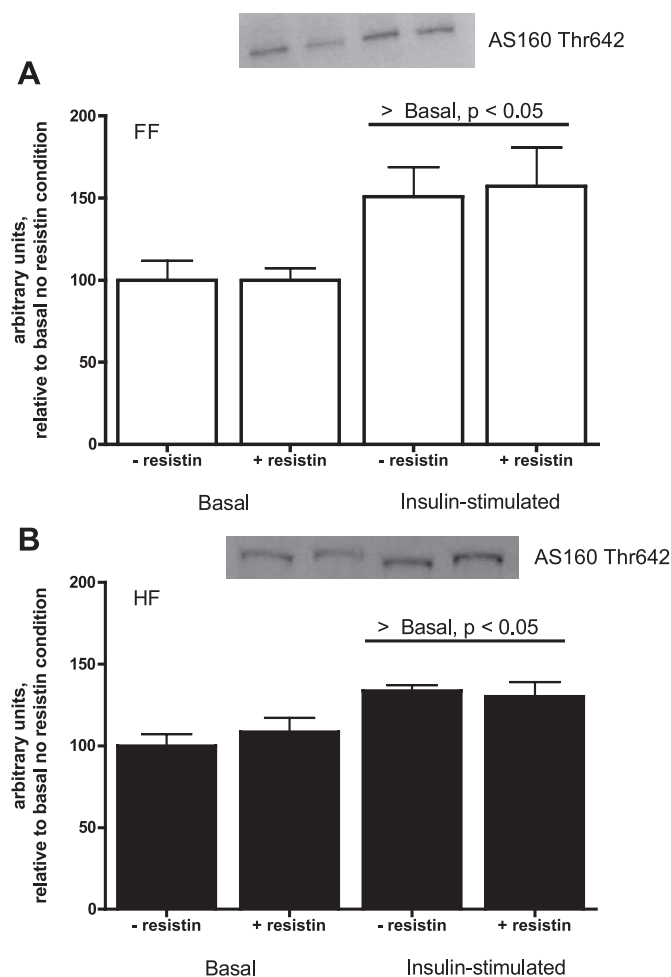


Fig. 3. Effect of resistin on basal and insulin-stimulated Akt substrate 160 (AS160) Thr642 phosphorylation in isolated soleus muscle in FF (A) and HF (B) conditions. Bars represent means \pm SE; sample size is 10–12.

In the present study, palmitate alone did not induce skeletal muscle insulin resistance during the 2-h incubation period but would likely have done so with a longer duration of exposure, as shown by ourselves (33) and others (1, 2).

No prior study has considered whether the presence of FA is necessary for resistin to exert its inhibitory effect on insulin-stimulated glucose transport. Given that plasma FA and other

Table 1. Protein content of IRS-1, Akt, and p85 PI3-kinase under basal and insulin stimulated conditions, with and without 2 mM palmitate

	Basal	Basal + Resistin	Insulin Stimulated	Insulin Stimulated + Resistin
Fat free				
IRS-1	100 \pm 10	98 \pm 4	120 \pm 15	119 \pm 21
Akt	100 \pm 13	101 \pm 12	105 \pm 16	108 \pm 18
p85 PI3-kinase	100 \pm 8	98 \pm 4	96 \pm 6	99 \pm 10
High fat				
IRS-1	100 \pm 11	110 \pm 18	106 \pm 9	108 \pm 11
Akt	100 \pm 12	109 \pm 10	106 \pm 8	107 \pm 10
p85 PI3-kinase	100 \pm 7	110 \pm 5	93 \pm 9	107 \pm 6

Values are means \pm SE in arbitrary density units, relative to basal condition (100%); n = sample size of 10–12 muscles/condition. IRS-1, insulin receptor substrate-1; PI3-kinase, phosphatidylinositol 3-kinase.

Table 2. Effect of resistin on the fate of palmitate in incubated soleus muscle

Fate of Palmitate	2 mM Palmitate	2 mM Palmitate + Resistin
Palmitate incorporation into triacylglycerol, nmol \cdot g wet wt ⁻¹ \cdot h ⁻¹	179.1 \pm 27.6	175.8 \pm 20.7
Palmitate oxidation, nmol \cdot g wet wt ⁻¹ \cdot h ⁻¹	40.7 \pm 6.1	44.0 \pm 5.8
Total FA uptake, nmol \cdot g wet wt ⁻¹ \cdot h ⁻¹	236.9 \pm 26.6	234.4 \pm 23.1
Oxidation-to-TAG storage ratio	0.3 \pm 0.1	0.3 \pm 0.1

Values are means \pm SE; n = sample size of 10–12 muscles. FA, fatty acid; TAG, triacylglycerol.

lipids are generally increased in the obese/insulin resistant state, this would seem an important consideration. Our findings clearly demonstrate that the presence of palmitate is necessary for resistin to acutely exert its inhibitory effect on insulin-stimulated glucose transport, at least in the short term. Surprising, we did not observe any direct effect of resistin on labeled palmitate oxidation or incorporation into TAG. This is in contrast to the results of Palanivel and Sweeney (18), the only other study to directly examine the effect of resistin on muscle FA metabolism. In that study (18), 24-h exposure of L₆ muscle cells to resistin caused a decrease in oleate oxidation and an increase in total lipid content. Possible reasons for the different results between the Palanivel and Sweeney study and the current research may be the use of a different FA (palmitate vs. oleate), duration of exposure to resistin (2 vs. 24 h), and different muscle models (mature skeletal muscle vs. L₆ cells).

Attenuating resistin's effects: inhibiting ceramide synthesis and stimulating AMPK. Ceramides have been implicated by others in the development of insulin resistance in rodents, specifically by acting directly at the level of Akt activation (6, 30, 31). Given our observation that insulin-stimulated glucose transport and Akt phosphorylation (a ceramide target) were impaired only in the combined presence of palmitate and resistin, it seemed reasonable to speculate that resistin might further stimulate the palmitate-induced formation of ceramide. We subsequently tested and found that two inhibitors of ceramide biosynthesis, myriocin (inhibitor of serine palmitoyl transferase) and fumonisins (inhibitor of ceramide synthase), were able to counter the palmitate plus resistin-induced inhi-

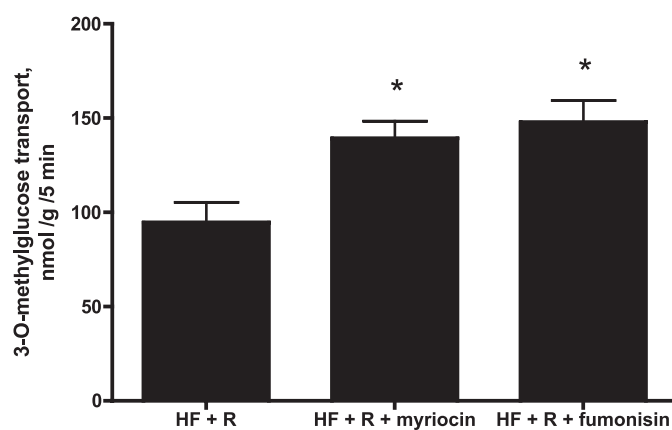


Fig. 4. Effect of ceramide synthesis inhibitors, myriocin (100 μ M) and fumonisins B₁ (50 μ M), on insulin-stimulated glucose transport in the presence of both high palmitate and resistin. Bars represent means \pm SE; sample size is 10–12. *Significantly different from HF + R, P < 0.05.

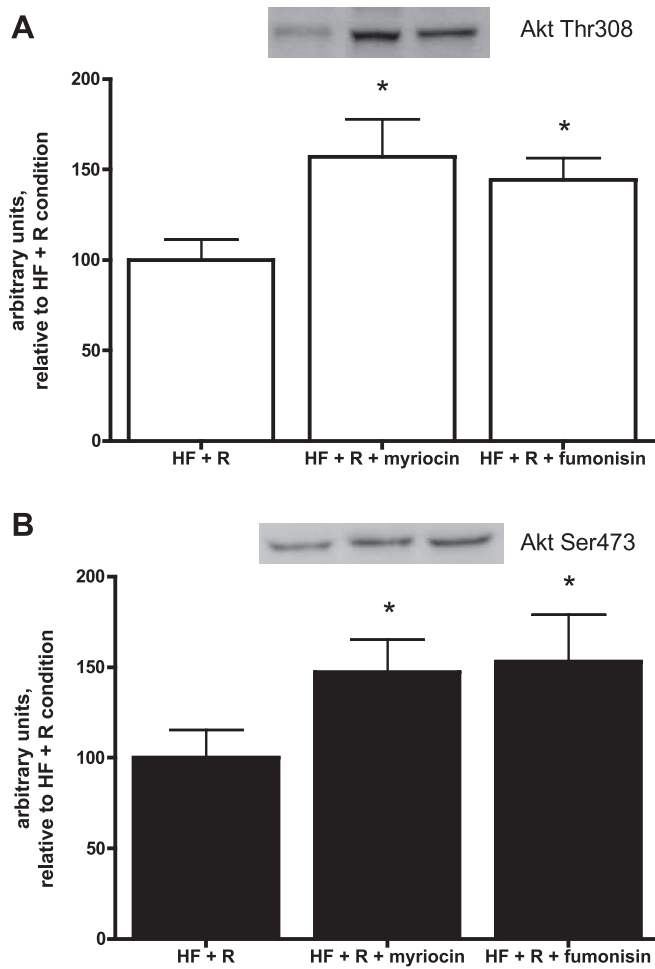


Fig. 5. Effect of ceramide synthesis inhibitors, myriocin (100 μ M) and fumonisin B₁ (50 μ M), on insulin-stimulated Akt Thr473 (A) and Ser308 (B) phosphorylation in the presence of both high palmitate and resistin. Bars represent means \pm SE; sample size is 10–12. *Significantly different from HF + R, $P < 0.05$.

bition of insulin-stimulated glucose transport. However, the results of our final set of experiments revealed that muscle ceramide (and DAG) content were clearly not further increased when resistin was included in the high-palmitate medium (HF + R) relative to the high-palmitate only (HF) condition; in fact, resistin actually attenuated the palmitate-induced increase in ceramide. The highest ceramide and DAG concentrations were observed following the high-palmitate incubation, which did not lead to an impaired insulin response within 2 h. It is possible that a longer duration may be required for increased ceramide/DAG content to impair insulin signaling. The inclusion of fumonisin resulted in a significant reduction in ceramide content relative to the high-palmitate plus resistin condition, which coincided with improved insulin-stimulated glucose transport and Akt phosphorylation. Collectively, our data suggest that, while a reduction in ceramide content can improve insulin response, resistin may be working through a mechanism independent of ceramide accumulation. It should also be acknowledged that changes in ceramide in specific cellular locations (i.e., to directly interact with Akt) may have been altered in our experimental paradigms that were not reflected in the whole muscle determinations.

In the present study, the AMPK activator, AICAR, also increased insulin-stimulated glucose transport in the presence of palmitate and resistin. AMPK is involved in the contraction-induced increase in GLUT-4 translocation and glucose transport (5, 9, 35), as well as FA oxidation (23), in rodent muscle. Our results are consistent with the previous finding that resistin impairs AMPK phosphorylation in L₆ muscle cells (18). It seems paradoxical that AICAR, a known stimulator of FA oxidation, can reverse the effects of resistin and palmitate on glucose transport when we were unable to initially detect any impairment in FA oxidation by resistin. However, this does not discount the possibility that further stimulation of FA oxidation during the 2-h incubation is of benefit, regardless of whether this was initially impaired by resistin or not. We did not measure muscle ceramide and DAG content in the AICAR experiments; however, it is interesting to note that, in a recent study by Alkathheeb et al. (1), the development of palmitate-induced insulin resistance in isolated rodent soleus following 6 h of incubation was associated with continued impairment of FA oxidation, but not further increases in ceramide or DAG content. Furthermore, insulin sensitivity can be rescued in this model, i.e., following the induction of insulin resistance by palmitate (1), by the stimulation of FA oxidation in the absence of any decrease in ceramide or DAG (A. Bonen, personal

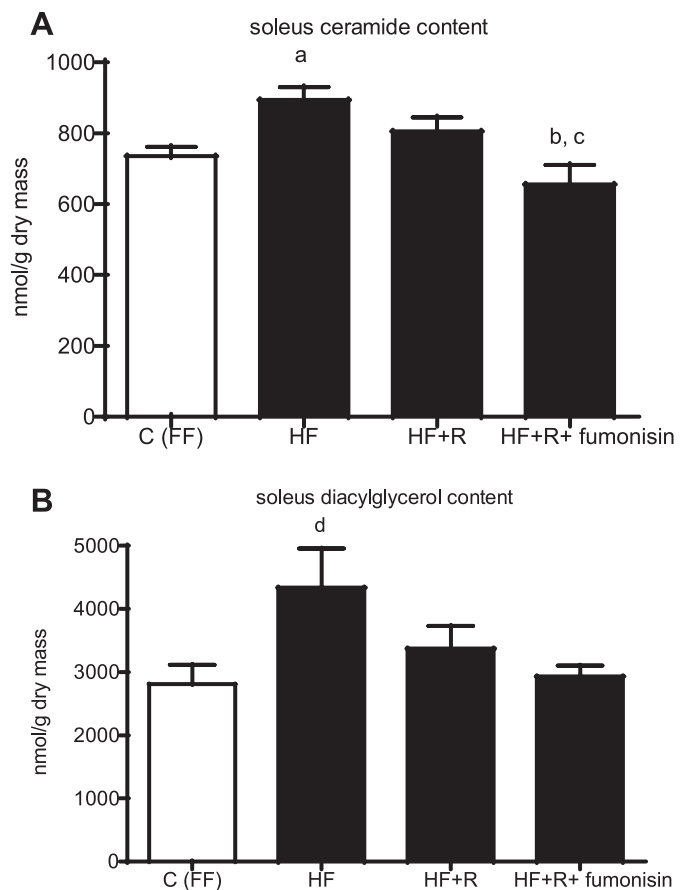


Fig. 6. Effect of HF, resistin, and resistin plus fumonisin B₁ (50 μ M) on muscle ceramide and diacylglycerol content. Bars represent means \pm SE; sample size is 9–12. ^aSignificantly different from control [C (FF)] and HF + R + fumonisin, $P < 0.05$. ^bSignificantly different from HF, $P < 0.001$. ^cSignificantly different from HF + R, $P < 0.05$. ^dSignificantly greater than all other conditions.

communication). Thus the relative roles of FA oxidation and the formation of reactive lipids such as ceramide and DAG in the impairment, as well as the rescue, of insulin response are still far from clear.

Perspectives and Significance

In summary, resistin impaired skeletal muscle Akt phosphorylation and insulin-stimulated glucose transport within a brief, 2-h exposure. This impairment was only observed in the presence of a high concentration of palmitate and could be blunted by the addition of 1) AICAR, a stimulator of AMPK and FA oxidation, or 2) fumonisin and myriocin, inhibitors of ceramide synthesis. Interestingly, muscle FA oxidation was not impaired, nor was muscle ceramide or DAG content further increased in the presence of resistin. While it is clear that "improving" muscle FA metabolism, i.e., increasing oxidation, decreasing ceramide content, can counter the deleterious effects of palmitate and resistin on insulin response, it is not clear exactly how resistin contributes to the acute development of insulin resistance. However, this does not appear to involve an increase in total ceramide content. Finally, it should be acknowledged that these results are limited to the oxidative soleus muscle; whether the same results would have occurred in other fiber types remains to be answered. Overall, our findings reveal a complex role of resistin as a regulator of muscle insulin sensitivity, as well as to further question the specific role of reactive lipids, such as ceramide, as mediators of insulin resistance.

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