Mouse and Human Resistins Impair Glucose Transport in Primary Mouse Cardiomyocytes, and Oligomerization Is Required for This Biological Action*

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The adipocytokine resistin impairs glucose tolerance and insulin sensitivity in rodents. Here, we examined the effect of resistin on glucose uptake in isolated adult mouse cardiomyocytes. Murine resistin reduced insulin-stimulated glucose uptake, establishing the heart as a resistin target tissue. Notably, human resistin also impaired insulin action in mouse cardiomyocytes, providing the first evidence that human and mouse resistin homologs have similar functions. Resistin is a cysteine-rich molecule that circulates as a multimer of a dimeric form dependent upon a single intermolecular disulfide bond, which, in the mouse, involves Cys26; mutation of this residue to alanine (C26A) produces a monomeric molecule that appears to be bioactive in the liver. Remarkably, unlike native resistin, monomeric C26A resistin had no effect on basal or insulin-stimulated glucose uptake in mouse cardiomyocytes. Resistin impairs glucose uptake in cardiomyocytes by mechanisms that involve altered vesicle trafficking. Thus, in cardiomyocytes, both mouse and human resistins directly impair glucose transport; and in contrast to effects on the liver, these actions of resistin require oligomerization.

The association between obesity, insulin resistance, and type 2 diabetes is well established, but the mechanisms linking increased adipose tissue mass with insulin resistance are partially understood. There is growing evidence that secreted products from adipocytes such as free fatty acids, tumor necrosis factor-α, leptin, and adiponectin can have significant effects on insulin sensitivity. Resistin is a recently described adipocytokine that was shown to impair glucose tolerance and insulin action in mice in vivo (1, 2). Its expression is negatively regulated by the anti-diabetic drugs thiazolidinediones, and serum levels are increased in mice and rats with diet-induced obesity as well as in genetically obese and insulin-resistant mice such as ob/ob and db/db mice, implying that resistin may represent another link between obesity and type 2 diabetes (1, 3, 4).

The nature of the in vivo targets of resistin is beginning to be elucidated. Acute administration of resistin in vivo impairs the ability of insulin to suppress hepatic glucose production without altering skeletal muscle glucose utilization (5). High fat feeding of rats leads to hyper-resistinemia and insulin resistance in skeletal muscle and liver. Treatment of these animals with a resistin antisense oligonucleotide normalizes hepatic insulin resistance without increasing skeletal muscle insulin sensitivity (4). Mice that are null for resistin exhibit reduced hepatic glucose production (6). Conversely, transgenic overexpression of resistin in mice increases hepatic glucose production without decreasing skeletal muscle glucose utilization (2). Taken together, these data suggest that the liver is a major target of resistin actions. In contrast, transgenic overexpression of resistin in the adipose tissue of SHR rats leads to dyslipidemia, glucose intolerance, and impaired insulin-stimulated glucose utilization in muscle (7). Similarly, adenovirus-mediated hyper-resistinemia in rats produces similar metabolic defects, which are associated with reduced insulin signaling in adipose tissue, skeletal muscle, and liver (8). It is not clear if the skeletal muscle phenotypes in these latter models represent direct effects of resistin or if they are secondary to the effects of resistin on the liver.

However, resistin has been shown to directly impair insulin-stimulated glycogen synthesis and glucose oxidation in isolated rat soleus muscle (7). Although the mechanisms were not elucidated, the previous study suggested that insulin action in skeletal muscle might be directly impaired by resistin in vitro. Studies in cultured cells have also suggested that adipocytes and skeletal muscle may be potential targets of resistin action. For example, exposure of 3T3L1 adipocytes to resistin impairs insulin-stimulated glucose uptake, whereas exposure to anti-resistin IgG augments glucose uptake (1). Resistin may impair insulin signaling in 3T3L1 adipocytes by mechanisms involving the up-regulation of SOCS-3 (suppressor of cytokine signaling 3) (9). Moreover, resistin was recently shown to acutely inhibit glucose uptake in L6 myotubes independent of any changes in proximal insulin signaling and GLUT4 trans-
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location (10). Mechanistic studies in primary muscle cells are currently lacking.

Resistin is a cysteine-rich protein that, when secreted, circulates as a hexamer composed of two trimers that are linked by intermolecular disulfide bonds (11). A single cysteine at position 26 has been demonstrated to be responsible for the covalent oligomerization of resistin because mutation of this cysteine to alanine (C26A) produces a molecule that exists only in the monomeric form (12). It should be noted that this “monomeric” mutant resistin likely circulates as a trimer that is maintained via protein-protein interactions, but is unable to form the disulfide bond that is necessary to produce the hexamer. In this study, monomeric resistin therefore refers to the C26A mutant that fails to form the disulfide-linked hexamers, and dimeric resistin refers to the wild-type molecule that circulates as the disulfide-linked hexamer. Recent studies have suggested that monomeric resistin is more potent than the hexameric protein in impairing hepatic insulin action (11). Whether or not this is the case for skeletal muscle and adipocytes is unknown.

A human homolog of the resistin gene exists, but its biological activity with regard to insulin action has not been characterized. Resistin is secreted from primary adipocytes isolated from subcutaneous and omental adipose tissue depots in humans, and the expression is 4-fold higher in abdominal adipose tissue versus adipose tissue obtained from the thigh (13, 14). Whether or not these regional differences in resistin expression contribute to the association between visceral obesity and insulin resistance is not known. There is also some debate in the literature as to whether or not resistin in humans is secreted primarily from adipocytes, preadipocytes, or other adipose tissue stromal cells (15), and some studies have failed to show any association between resistin expression in adipocytes and insulin sensitivity (16–18). Genetic studies that have examined the association between polymorphisms in the 3'- and 5'-flanking regions of the resistin gene have produced variable results with positive associations between resistin variants and insulin resistance and/or obesity found in some but not in other populations (19–23). More recently, data have emerged that hyper-resistinemia occurs in chronic inflammatory states that are associated with insulin resistance (24–26). Thus, determining the biological activity of human resistin may shed important insight into its potential function.

We have devised techniques for acutely dissociating viable mouse cardiomyocytes that are suitable for evaluation of insulin signaling and glucose transport (27–29). We (27–29) and others (30) have shown that isolated cardiomyocytes are insulin-responsive and share many characteristics of adipocytes and skeletal muscle in terms of insulin stimulation of glucose transport. Thus, we used this model system to determine whether resistin can modulate glucose transport and insulin signaling in primary murine muscle cells. The aims of this study were to determine the effect of murine and human resistin on glucose transport and to determine whether differences exist between the activity of the dimeric versus monomeric resistin (C26A mutant) in muscle cells.

MATERIALS AND METHODS

Antibodies and Reagents—Antibodies against insulin-regulated aminopeptidase (IRAP)1 were a kind gift of Paul F. Pilch (Boston University School of Medicine, Boston, MA). Antibodies against Akt and phospho-Akt (phosphorylated at Ser473) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and Cell Signaling Technology (Beverly, MA), respectively. Goat polyclonal antibodies against the GLUT4 glucose transporter were obtained from Chemicon International, Inc. (Temecula, CA). Alexa Fluor 488-conjugated donkey antigoat antibodies and Alexa Fluor 647-conjugated wheat germ agglutinin were obtained from Molecular Probes, Inc. (Eugene, OR). Recombinant FLAG, mouse monomeric and dimeric resistin-FLAG, and human dimeric resistin-FLAG were obtained using expression vector pZac2.1 and transfected into 293T cells as described previously (12). In some studies, we also used mouse recombinant resistin obtained using expression vector pFM1 and transfected into 293T cells (5).

Preparation of Isolated Cardiomyocytes—Cardiomyocytes were isolated from 10–15-week-old male C57BL/6 mice using our previously described protocol (28, 29).

2-Deoxyglucose Uptake in Isolated Cardiomyocytes—Preincubation and incubation of cells were done at 37 °C in humidified atmosphere of 95% O2 and 5% CO2. Preincubation (5–90 min) of the cells in the absence or presence of resistin was done in Dulbecco’s modified Eagle’s medium containing 5.0 mM glucose, 1 mM pyruvate, and 0.1% bovine serum albumin. Cells were then incubated for 40 min in the presence or absence of 0.1–10 nM insulin in glucose-free Dulbecco’s modified Eagle’s medium supplemented with 1 mM bovine serum albumin and 1 mM pyruvate. Glucose uptake was performed by adding 0.1 mM 2-deoxy-D-glucose and 3.33 nCi/ml 2-[1,2-3H]-deoxy-D-glucose for 30 min. Glucose transport experiments were terminated after 30 min by aspiration of the buffer, followed by two washes with ice-cold phosphate-buffered saline and then lysis in 1 N NaOH for 20 min. Nonspecific uptake was assessed in the presence of 10 μM cytochalasin B and subtracted from all of the measured values. Radioactivity was counted by liquid scintillation spectroscopy using a Beckman LS 5000 TD instrument and normalized to protein amount measured with a Micro BCA protein assay kit (Pierce).

IRAP Biotinylation Assay in Isolated Cardiomyocytes—Cells were incubated at 37 °C for 45 min with 15 μg/ml FLAG or resistin-FLAG prior to a 40-min incubation in the absence or presence of 10 nM insulin. Cells were then incubated for 40 min at 4 °C in ice-cold PBS containing sulfo-succinimidyl 2-(biotinamido)-ethyl-1,3-dithiopropionate (Pierce) at a concentration of 0.5 mg/ml. After three washes with ice-cold PBS, cells were lysed in ice-cold lysis buffer containing 10 mM HEPES, 5 mM EDTA, 250 mM sucrose, 10 mM aprotinin, 10 mM leupeptin, and 1 mM PMSF. An aliquot of the lysate was used for total expression of IRAP. One volume of 160 μl/ml UltraLinkTM Plus-immobilized streptavidin was added to the cell lysate and incubated overnight at 4 °C. After a brief centrifugation, the pellet was washed three times with ice-cold PBS and boiled for 5 min in Laemmli buffer supplemented with 10% β-mercaptoethanol. After a brief centrifugation, the supernatant was collected as the plasma membrane fraction. Total cell lysate and plasma membrane fractions were analyzed by immunoblotting with anti-IRAP antibodies (28).

Western Immunoblot Analysis—Cells were treated as described in the figure legends. Protein was separated on SDS-polyacrylamide gels, transferred to a polyvinylidene difluoride membrane (Fisher), and blotted for IRAP using the protocols described previously (28).

Glucose Translocation in Cultured Cardiomyocytes by Immunofluorescence—Cells plated on Lab-Tek chamber slides (Nalge Nunc International, Rochester, NY) were incubated at 37 °C for 45 min with 5 μg/ml resistin prior to a 40-min incubation in the absence or presence of 10 nM insulin. Cells were then fixed in 4% paraformaldehyde in PBS at room temperature for 15 min. After blocking with PBS containing 5% normal donkey serum and 0.5% saponin, cells were incubated overnight at 4 °C with primary antibodies against GLUT4. After washing, cells were incubated with secondary antibodies at room temperature. Cells were then fixed again in 4% paraformaldehyde in PBS for 30 min. After incubation with Alexa Fluor 647-conjugated wheat germ agglutinin, slides were washed and mounted using VECTASHIELD HardSet mounting medium (Vector Laboratories, Burlingame, CA). Immunofluorescence was analyzed by conventional confocal microscopy (Olympus America Inc., Melville, NY) using sequential image acquisition after excitation at 488 nm (Alexa 488; emission, 510–530 nm) for GLUT4 and at 633 nm (Alexa 647; emission, >660 nm) for wheat germ agglutinin. Images were recorded in optical slices that were <0.5 μm thick at a resolution of 0.115 μm/pixels. Settings were adjusted so that areas lacking cellular structures appeared essentially black, and the brightest pixels were not saturated. All images were recorded with identical settings. Individual cells were selected randomly based on wheat germ agglutinin staining. The images were recorded using a PlanApo 60×/1.4 oil infinity corrected/0.17 (Olympus America Inc.). To determine the relative distribution of GLUT4 in the plasma membrane, avoiding bias during analysis, the image series were coded and then batch-processed using the MetaMorph program (Universal Imaging Corp.,

1 The abbreviations used are: IRAP, insulin-regulated aminopeptidase; PBS, phosphate-buffered saline.
for an additional 40 min. We observed that the effect of resistin on impairment of basal (Fig. 2A) and insulin-stimulated (Fig. 2B) glucose uptake was maximal after 45 min of preincubation. We also observed that 10 and 5 min of resistin preincubation were sufficient to impair basal (Fig. 2A) and insulin-stimulated (Fig. 2B) glucose uptake, respectively.

Human Resistin Reduces Insulin-stimulated Glucose Uptake in Isolated Mouse Cardiomyocytes—We also examined the effect of the human homolog of resistin on glucose uptake in mouse cardiomyocytes. Similar to our observations with mouse resistin, human resistin inhibited insulin-stimulated glucose uptake. However, it did not change basal uptake (Fig. 3).

Resistin Down-regulates Insulin-stimulated Glucose Uptake at Physiological Concentrations—The serum concentration of resistin in wild-type mice and rats has been found to be in the range of 0.05–0.1 μg/ml (2, 4). We thus sought to determine whether resistin can impair insulin-stimulated glucose uptake at concentrations found in vivo. Indeed, resistin remained active at 0.02 μg/ml (Fig. 4).

Oligomerization Is Required for the Biological Effect of Resistin on Glucose Uptake—To investigate whether or not dimerization (i.e. disulfide bond linkage of resistin trimers to generate hexamers) is required for the full activity of murine resistin, we compared glucose uptake in cells incubated with either no resis-
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**Effect of human resistin on insulin-stimulated glucose uptake in cardiomyocytes.** Glucose uptake was measured in cardiomyocytes treated for 90 min with 20 μg/ml FLAG (white bars; no resistin) or resistin-FLAG (black bars) expressed in pZac2.1 prior to a 40-min incubation with (+) or without (−) 10 nM insulin. *, p = 0.02 versus cells incubated with insulin only (no resistin). Data are from seven independent experiments done in triplicate.

**Dose response of insulin-stimulated glucose uptake to resistin.** Glucose uptake was measured in cardiomyocytes for 40 min without (white bar) or with (black bars) 0.02–20 μg/ml recombinant resistin expressed in pFM1 prior to a 40-min incubation with insulin. Data are expressed as a percentage relative to transport rates observed in cells incubated without resistin (100%). *, p = 0.004 versus no resistin; †, p = 0.02 versus no resistin. Data are from 14 independent experiments done in triplicate.

Resistin or dimeric or monomeric (C26A) resistin. As shown in Fig. 5, monomeric resistin had no effect on basal or insulin-stimulated glucose uptake versus dimeric resistin. We further assessed whether monomeric resistin has an inhibitory effect on the ability of dimeric resistin to reduce glucose uptake by co-incubating the cells in the presence of both dimeric and monomeric resistins. As shown in Fig. 5, monomeric resistin had no inhibitory effect on the ability of dimeric resistin to reduce insulin-stimulated glucose uptake. Intriguingly, the monomer appeared to augment the effect of the dimer to reduce insulin-stimulated glucose uptake (p = 0.05 versus dimeric resistin).

**Resistin Inhibits the Translocation of GLUT4 to the Sarcolemmal Membrane of Isolated Cardiomyocytes—**To gain further insight into the mechanism(s) by which resistin impairs insulin-stimulated glucose uptake, we used two independent methods to determine whether murine resistin impairs GLUT4 translocation. Fig. 6 depicts the results of immunofluorescence analysis of resistin-treated cardiomyocytes. Exposure of cardiomyocytes to insulin resulted in a significant increase in sarcolemmal GLUT4 fluorescence (26% increase relative to the basal level; p < 0.0001). Interestingly, exposure of cardiomyocytes to resistin only resulted in a 13% increase in sarcolemmal GLUT4 fluorescence relative to untreated cells (p < 0.0001). Treatment of cells with insulin and resistin was not associated with any additional change in sarcolemmal GLUT4 fluorescence beyond the levels observed with resistin alone. Despite the modest increase in membrane fluorescence in resistin-treated cells, the degree of membrane fluorescence was significantly less than that observed with insulin alone (p < 0.001).

Immunofluorescence is unable to discern between GLUT4 molecules that may be docked or associated with the sarcolemma, but not fused or activated. Thus, we also adapted the IRAP biotinylation assay to measure insertion of GLUT4 vesicles into sarcolemmal membranes by exposing cells to non-cell-permeable biotin and determining the abundance of biotinylated IRAP in cells treated with insulin or resistin. IRAP is an integral component of GLUT4 vesicles that translocates along with GLUT4 in insulin-responsive cells and is more amenable to biotinylation than GLUT4. We obtained a 1.4-fold increase in surface IRAP appearance in insulin-stimulated cells (Fig. 7). However, in the resistin-treated cells, we did not detect any significant increase in insulin-stimulated IRAP (GLUT4) translocation. Taken together, these data indicate that resistin impairs insulin-mediated GLUT4 translocation. Resistin alone did not increase surface IRAP biotinylation (data not shown).

Thus, the observation that resistin caused a measurable association of GLUT4 vesicles with the sarcolemma in the absence of any increase in IRAP biotinylation suggests that resistin might also act to impair insertion of GLUT4 vesicles into sarcolemmal membranes.

**Resistin Does Not Impair Insulin-stimulated Akt Phosphorylation, but Impairs Endosomal Trafficking—**To examine whether or not the inhibition of insulin-stimulated GLUT4 translocation by resistin reflects impaired insulin signaling, we determined the effect of murine resistin on the phosphorylation of Akt. The abundance of total Akt was similar in resistin-treated and untreated groups. The -fold (insulin-stimulated) increase in Akt phosphorylation (normalized to total Akt) was identical in mouse cardiomyocytes treated with and without resistin (2 μg/ml) (Fig. 8). Insulin produced a 2-fold increase in the phosphorylation of glycogen synthase kinase-β in mouse cardiomyocytes that was also unaffected by resistin treatment (data not shown). Thus, resistin does not appear to inhibit the propagation of insulin signaling to Akt.

We then measured transferrin exocytosis rates to determine whether the impairment of glucose transport by resistin can...
occur on the basis of a defect in endosomal vesicle trafficking. Cardiomyocytes were incubated with biotinylated transferrin; and after equilibration, endosomal recycling was temporarily arrested by cooling the cells to 4 °C. Cells were then incubated in the presence or absence of resistin, and the rate of appearance of biotinylated transferrin in the media was determined. In control cells, there was progressive release of biotinylated transferrin into the media over 85 min (p < 0.02). In contrast, this pattern of transferrin release was not observed in resistin-treated cells. 5 min following exposure to resistin, biotinylated transferrin was present in the medium of resistin-treated cells and was similar in amount to that released by control cells at 5 min (p > 0.6). Subsequently, there was no significant additional accumulation of biotinylated transferrin in the medium obtained from resistin-
for each group. For each time point, are represented as -fold change relative to the values measured at 5 min released at 5 min in resistin-treated cells; thus, the data were equilibrated with biotin-labeled transferrin for 2 h at 37 °C prior to determining exocytosis. B, densitometric quantification of Western blots of biotin-labeled transferrin. Results were normalized to protein content at the end of the experiment in each culture plate. There was no difference in the amount of biotin-labeled transferrin released from cardiomyocytes into the culture medium in the absence or presence of 5 μg/ml resistin at the time points (in minutes) indicated. Cells were equilibrated with biotin-labeled transferrin for 2 h at 37 °C prior to determining exocytosis. B, densitometric quantification of Western blots of biotin-labeled transferrin. Results were normalized to protein content at the end of the experiment in each culture plate. There was no difference in the amount of biotin-labeled transferrin released at 5 min in resistin-treated versus control cells; thus, the data are represented as -fold change relative to the values measured at 5 min for each group. For each time point, n = 5. The accumulation of biotin-labeled transferrin above basal levels over the course of the experiment was statistically significant in control cells (p < 0.02), but not in resistin-treated cells (p = 0.6) as determined by the Kruskall-Wallis test. The area beneath the biotin-labeled transferrin curve for control cells was also significantly different from the area beneath the biotin-labeled transferrin curve for resistin-treated cells (p < 0.001) as determined by repeated-measures analysis of variance.

A

B

FIG. 9. Effect of resistin on transferrin exocytosis. A, representative Western blots of biotin-labeled transferrin that was cumulatively released from cardiomyocytes into the culture medium in the absence or presence of 5 μg/ml resistin at the time points (in minutes) indicated. B, densitometric quantification of Western blots of biotin-labeled transferrin. Results were normalized to protein content at the end of the experiment in each culture plate. There was no difference in the amount of biotin-labeled transferrin released at 5 min in resistin-treated versus control cells; thus, the data are represented as -fold change relative to the values measured at 5 min for each group. For each time point, n = 5. The accumulation of biotin-labeled transferrin above basal levels over the course of the experiment was statistically significant in control cells (p < 0.02), but not in resistin-treated cells (p = 0.6) as determined by the Kruskall-Wallis test. The area beneath the biotin-labeled transferrin curve for control cells was also significantly different from the area beneath the biotin-labeled transferrin curve for resistin-treated cells (p < 0.001) as determined by repeated-measures analysis of variance.

Discussion

This study has provided evidence that resistin can acutely impair cardiac muscle glucose utilization. Notably, we have also demonstrated that human resistin has properties similar to those of murine resistin and that dimeric resistin is more impotent than monomeric resistin in impairing glucose utilization in this system.

Recent in vivo experiments have suggested that resistin impairs glucose tolerance by inhibiting the ability of insulin to suppress hepatic glucose production (2, 4–6, 11) as well as by inhibiting glucose uptake in skeletal muscle (8). The results from our study indicate that resistin also alters glucose uptake in cardiac muscle. Cardiac muscle derives its energy primarily by oxidative metabolism; thus, the changes that we observed could potentially be extrapolated to predominantly oxidative muscles (31). In this regard, studies in isolated rat soleus (an oxidative muscle) demonstrated that resistin significantly impairs insulin-stimulated glycogenesis and glucose oxidation, but the contribution of impaired glucose uptake was not evaluated (7). Thus, it will be critical in future studies to determine whether resistin impairs glucose uptake specifically in skeletal muscles and to determine whether there are fiber type-specific differences in the response of various muscles to resistin.

The results from our study also highlight important differences between liver and muscle in the response to monomeric versus dimeric (hexameric) resistin. In vivo studies suggested that monomeric resistin more potently activated hepatic glucose production compared with hexameric resistin (11). In contrast, monomeric resistin had no impact on basal or insulin-stimulated glucose uptake in cardiomyocytes. Moreover, a combination of wild-type and monomeric resistins had a synergistic inhibitory effect on insulin-stimulated glucose uptake. The mechanism for the apparent synergistic effect of the monomeric and wild-type resistins on insulin-stimulated glucose uptake remains to be elucidated, but could involve noncovalent interaction between the monomer and dimer, as has been suggested by Chen et al. (32). Thus, fundamental differences may exist in signaling mechanisms that are responsible for the effects of resistin in muscle versus liver. The intriguing possibility is raised that, in vivo, resistin undergoes processing in the liver to generate monomers, which represent the active signaling moiety in hepatocytes. Analogous observations have been made for the adipocytokine adiponectin, where differences in the response of liver and muscle tissues may be partially explained by differences in the oligomeric structure of the molecule (33–35).

Another important finding of this study was the observation that human resistin caused a significant reduction in insulin-stimulated glucose uptake in cardiomyocytes. To the best of our knowledge, these are the first observations that demonstrate a direct link between human resistin and impaired insulin sensitivity. Genetic studies have indicated that human resistin is indeed the ortholog of its murine counterpart (36). However, there are important quantitative differences in the expression of resistin in mouse versus human adipocytes and relative differences in the expression of resistin in adipocytes versus macrophages in humans (15, 36, 37). Nevertheless, recent studies have indicated that polymorphisms of the resistin promoter that would increase resistin gene expression in humans are associated with increased circulating plasma levels of resistin and increased susceptibility to type 2 diabetes (38). Therefore, if human resistin impairs insulin action in insulin-responsive cells in vitro as we have demonstrated, then increased concentrations of resistin in humans could potentially impair insulin sensitivity in vivo and contribute to the association between hyper-resistinemia, glucose intolerance, and type 2 diabetes.

The effects of resistin on glucose transport are significant but modest, and there are limitations in the ability of available techniques to quantify glucose transporter translocation in cardiomyocytes and to distinguish between translocation and activation. For example, the methods may underestimate the translocation of GLUT4 to T-tubules. These technical limitations might account for the discrepancy in the 4-fold increase in insulin-stimulated glucose uptake that we obtained despite more modest increases in cell-surface IRAP biotinylation or sarcolemma-associated GLUT4. Nevertheless, both assays (biotinylation and immunofluorescence) independently produced concordant results, viz. that resistin impaired GLUT4 translocation and potentially insertion of GLUT4 vesicles into the sarcolemma.

An obvious potential mechanism for resistin-mediated impairment of insulin-stimulated glucose uptake is impaired insulin-stimulated activation of proximal signaling pathways leading to Akt phosphorylation. Although this has been demonstrated recently to be the case in 3T3L1 adipocytes (9), we did not observe any effect of resistin on impairment of the ability of insulin to phosphorylate Akt or glycogen synthase kinase-β. Thus, it is unlikely that proximal insulin signaling was impaired in our study. These results are also distinct from...
a report of impaired \textit{in vivo} insulin signaling in skeletal muscle from rats rendered hyper-resistinemic following injection of a resistin-expressing adenovirus; it is possible that the impairment of skeletal muscle insulin signaling in this model was secondary to hypertriglyceridemia and glucose intolerance that may have occurred because of increased hepatic glucose production (8).

The absence of a defect in proximal insulin signaling raised the possibility that resistin impairs glucose uptake by inhibiting distal events in glucose transporter vesicle trafficking such as translocation, docking, activation, and GLUT4 vesicle recycling. We began to address this issue by determining whether resistin treatment causes a generalized defect in endosomal trafficking, which could in turn impair GLUT4 vesicle exocytosis and/or recycling. Interestingly, we observed that resistin profoundly impaired transferrin receptor exocytosis in mouse cardiomyocytes, indicating that resistin impairs endosomal vesicle trafficking. GLUT4 vesicles traverse the endosomal vesicle recycling pathway before being sorted into the specific insulin-responsive vesicle compartment (28). Thus, impaired endosomal trafficking could alter the sorting of GLUT4 into the insulin-responsive vesicle. If this were the case, then this could account for some of the observations in this study such as (a) the time course for the maximal effect of resistin on impairment of glucose uptake (45 min), (b) the absence of a clear dose-response inhibition of glucose uptake by resistin, (c) the marked reduction in cell-surface IRAP biotinylation in insulin-treated cardiomyocytes, and possibly (d) the ability of resistin to impair basal glucose uptake.

In summary, this study has demonstrated that mouse resistin impairs glucose transport in primary cardiomyocytes and has demonstrated an orthologous function of human resistin. We propose that one potential mechanism for these effects is resistin-mediated impairment of endosomal vesicle trafficking. In contrast to the liver, disulfide-bond oligomerization is required for the biological actions of resistin in cardiac muscle cells. Thus, two adipocytokines with opposing effects on insulin action, resistin and adiponectin, have in common the intriguing property that different molecular forms exhibit major differences in their activities in muscle and liver.

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