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Effects of resistin expression on glucose metabolism and hepatic insulin resistance

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Abstract In order to observe the effect of increased serum resistin on glucose metabolism, insulin sensitivity, and hepatic insulin resistance (IR), mice were intravenously injected with recombinant adenovirus carrying the resistin gene (Adv-resistin-EGFP). Changes in hepatic glucose metabolism were observed using the Periodic Acid-Schiff method. Hepatic AMP-activated protein kinase (AMPK) activation was assessed by Western blot analysis, and glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression was determined using real-time RT-PCR. Although no effect on fasting blood glucose was detected, increased fasting insulin levels, decreased glucose tolerance and insulin sensitivity, and reduced hepatic glycogen levels and AMPK activation were seen in the Adv-resistin-EGFP mice. Finally, elevated G6Pase and PEPCK mRNA expression levels were detected upon overexpression of resistin. Resistin may inhibit hepatic AMPK activity, which results in elevated expression of gluconeogenic enzymes thereby affecting glucose metabolism and leading to decreased glycogen storage that contributes to the development of hepatic IR.

Keywords Adenovirus · Resistin · Insulin resistance · Hepatic insulin resistance · AMP-activated protein kinase · PEPCK · G6Pase

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Introduction

Resistin is a serum secretary cysteine-rich protein [1, 2] that is mainly expressed in white adipose tissue. Upon adipocyte differentiation, resistin expression is induced, and serum resistin levels increase in diet-induced or genetically obese mice [3, 4]. While administration of neutralizing antibodies improves insulin sensitivity, administration of recombinant resistin protein disrupts insulin-mediated glucose metabolism, as shown in both in vitro and in vivo experiments [3]. Therefore, initial studies reported a role for resistin in the formation of insulin resistance (IR). However, the most recent analysis indicates that the relationship between resistin and IR is far more complicated than previously predicted; both synergistic [3–6] and antagonistic [7–9] relationships have been reported. Furthermore, based on reports using various in vivo models [10–13] the effect of resistin on IR might be divergent and involve multiple insulin-sensitive target tissues, such as skeletal muscle, adipose, and liver. Alternatively, its major influence might be on the formation of hepatic IR (reviewed in [14]). Therefore, the molecular mechanisms governing resistin action in insulin-responsive target tissues have yet to be identified.

Because adenovirus-mediated gene transfer yields high levels of gene expression without recombining into the host's genome, thereby reducing DNA mutation carcinogenic events, this method was chosen to analyze the effects of resistin expression on glucose metabolism and insulin sensitivity in mice. Furthermore, adenovirus-mediated gene transfer is conducive for analyzing the effects of short-term protein expression.

In this study, changes in blood glucose levels and insulin sensitivity in response to increased resistin levels were examined in order to provide the basis for investigating the

effect of resistin on glucose metabolism. In addition, the influence of resistin on hepatic AMP-activated protein kinase (AMPK) activation, glycogenolysis, and gluconeogenesis was investigated to determine the possible mechanism underlying the effect of resistin on hepatic IR.

Materials and methods

Experimental animals

Seventy-one 12-week-old male 57BL/6 mice of clean grade were purchased from the Center of Animal Experiments, North Campus, Sun Yat-Sen University, and were randomized into three groups consisting of the normal control group (NC; $n = 24$), the adenoviral vector control group (Adv-EGFP; $n = 22$), and the experimental group (Adv-resistin-EGFP; $n = 25$). Resistin levels in mice were determined by enzyme-linked immunosorbent assays (ELISA; R&D Systems China Co. Ltd, Shanghai, China).

Preparation of the recombinant adenovirus construct, Adv-resistin-EGFP, and the adenoviral control, Adv-EGFP, construct

Bacterial strains carrying pGEM-T-resistin plasmid were used for packaging recombinant adenovirus. Bacteria stocks were verified by PCR analysis using the following primers: forward primer 5′-CGGAATTCATGAACCTTT-CATTTCC-3′ and reverse primer 5′-CTAGTCTAGATC AGGAAGCGACCTGCAGCT-3′. PCR thermal cycle parameters included an initial denaturation step at 96°C for 2 min followed by 30 cycles at 96°C for 30 s, 52°C for 1 min, and 72°C for 50 s with a final extension for 7 min at 72°C. The amplified product size (363 bp) was confirmed by agarose gel electrophoresis. PCR products were confirmed by sequence analysis of the resistin gene sequence before the plasmid and bacterial strains were sent for commercial adenovirus packaging (Adv-resistin-EGFP), concentration, and purification (Vector Gene Technology Company Limited (VGTC), Beijin, China). The successfully packaged recombinant adenovirus was then used to infect 293 cells to assess viral activity. Adenoviral control vector, a recombinant adenovirus carrying enhanced GFP (Adv-EGFP), was purchased from VGTC.

Construction of high-resistin mouse model

Mice were injected with Adv-resistin-EGFP or Adv-EGFP at a dosage of 1×10^9 PFU diluted to a final volume of 0.3 ml with PBS per mouse via the tail vein, and mice in the normal, control group were injected with an equal volume of PBS. All three groups of mice ate and drank ad libitum throughout the course of the experiments. Growth, weight change, and terminal fasting blood glucose were examined daily after the injection. On the fifth day after the injection, each group of mice were further divided into three subgroups that were subjected to differing analyses, including fasting blood glucose levels using glucose oxidase as well as fasting insulin levels (ELISA kit; Linco Research, Inc., St. Charles, MO, USA), the intraperitoneal insulin tolerance test (IPITT), and the intraperitoneal glucose tolerance test (IPGTT). Serum resistin levels were simultaneously monitored in each mouse.

Intraperitoneal insulin tolerance test

After 6 h of fasting, the mice were injected with insulin at a dosage of 0.75 U/kg. The blood glucose levels were examined with a blood glucose reader (Abbott, Abbott Park, IL, USA) using blood obtained from a cut tail tip at 0, 15, 30, 45, 60, and 90 min after insulin injection. The blood glucose level at 0 min was set as the reference basis, and the blood glucose levels at other time points were presented as a proportion of the reference basis. Curve fitting (least square) was carried out via the equation $C_t = C_0 \cdot e^{-K_I \cdot t}$,where K_I represents the percentage of blood glucose decrease every minute obtained, C_0 represents blood glucose levels before intraperitoneal insulin injection, C_t indicates blood glucose levels at different time points after intraperitoneal insulin injection, and t represents time after intraperitoneal insulin injection in minutes. K_I values were presented as percentage per minute. Thus, a decrease in K_I signified reduction in insulin sensitivity.

Intraperitoneal glucose tolerance test

After fasting for 14 h, mice of all three groups were injected with a 10% glucose solution at a dosage of 1.5 g/kg, and the blood glucose levels were examined with a blood glucose reader using blood obtained from a cut tail tip at 0, 5, 15, 30, 60, and 120 min after the injection.

Observation of glycogen storage

Liver samples were sliced and stained using the Periodic Acid-Schiff method (PAS). The specimens were observed under light microscopy. The amount of glycogen was evaluated and scored as 1 to 4 points, according to the proportion of glycogen in the field (1/4, 1/2, or 3/4). Five fields in each specimen were evaluated.

Western blot analysis

Liver tissue samples were homogenized in a total protein extract solution with protease inhibitors and separated by

centrifugation (14,000 rpm, 4°C for 30 min) after sufficient lysis was obtained. The supernatant was collected, and the protein concentration was examined using the BCA method. Proteins (30 μg) were separated using SDS-PAGE with a 12% polyacrylamide gel and then transferred onto a PVDF membrane. The membranes were blocked (1% skim milk powder in PBS) for 2 h before incubation with a rabbit-anti-mouse phospho-AMPK-α (Thr172) antibody at 4°C overnight. Then the membranes were incubated with an HRP-labeled secondary antibody at room temperature for 1 h and developed using ECL reagents. The membranes were then stripped, re-blocked, and incubated with a rabbit-anti-mouse AMPK-α antibody, which was used as a loading control. The films were scanned, and the density of the bands was analyzed by Quantity One software (Bio-Rad Laboratories, Hercules, CA).

Real-time RT-PCR analysis

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA), and RNA integrity and purity were determined prior to cDNA production. Real-time RT-PCR was carried out using an ABI PRISM 7000HT Real-Time PCR System and reaction conditions were according to the manufacturer's protocol. Relatively known concentrations of cDNA obtained through serial dilutions resulting from in vitro retrotranscription of high-concentration RNA extracted from normal control samples were subjected to PCR in order to provide a standard curve. Relative gene expression levels were calculated according to the standard curve and normalized with the β-actin internal control.

The primers used were as follows: phosphoenolpyruvate carboxykinase (PEPCK) sense 5′-AGCCTGCTCCAGCTTTGA-3′, PEPCK antisense 5′-CCCTAGCCTGTTCTCTGTGC-3′, glucose-6-phosphatase (G6Pase) sense 5′-TGCTGTGTCTGGTAGGCAAC-3′, G6Pase antisense 5′-AGAATCCTGGGTCTCCTTGC-3′, β-actin sense 5′-CCTGAGGCTCTTTTCCAGCC-3′, and β-actin antisense 5′-TAGAGGTCTTTACGGATGTCAACGT-3′.

Statistical analysis

Depending on the statistical distribution, continuous data were expressed either as means with standard deviations or as medians with ranges. Laboratory results were compared using analysis of variance (ANOVA) the Kruskal–Wallis test or *t*-test. Data were analyzed using SAS software version 9.0 (SAS Institute Inc., Cary, NC, USA); area under the curve (AUC) was calculated by Prism 4.0 (GraphPad Software, Inc., San Diego, CA, USA). a P-value < 0.05 indicated statistical significance.

Results

Adenovirus-mediated resistin expression in mice

As shown in Table 1, the resistin protein level was significantly increased (approximately 15 times larger) in the Adv-resistin-EGFP group compared to the normal control and Adv-EGFP vector control, indicating successful in vivo overexpression of resistin in mice ($P < 0.001$; Table 1).

Effect of resistin on fasting blood glucose

There was no significant difference in fasting blood glucose levels among the normal control, Adv-EGFP, and Adv-resistin-EGFP groups ($P = 0.331$; Table 1). The fasting blood glucose levels for each group were monitored

Table 1 Effects of adenovirus-mediated resistin expression on serum resistin protein levels (ng/ml), terminal fasting blood glucose levels (mmol/l), and fasting serum insulin levels (ng/ml)

	Normal control ($n = 24$)	Adv-EGFP ($n = 22$)	Adv-resistin-EGFP ($n = 25$)	P-value
Resistin (ng/ml)[a]	15.98 (4.30–28.36)	12.06 (7.03–33.76)	299.25 (43.37–300+)	<0.001*
Insulin (ng/ml)[a]	0.37 (0.05–1.01)	0.36 (0.10–0.95)	0.57 (0.25–1.27)	0.005*
Glucose (mmol/l)				
Day 0	7.86 ± 1.02	8.26 ± 1.30	7.79 ± 1.10	0.331
Day 1	7.94 ± 1.25	8.01 ± 1.32	7.44 ± 1.25	0.237
Day 2	7.97 ± 1.46	7.76 ± 1.33	7.26 ± 1.05	0.145
Day 3	7.95 ± 1.06	7.41 ± 1.09	7.36 ± 0.78	0.076
Day 4	7.69 ± 1.45	7.65 ± 1.27	7.26 ± 0.80	0.420
Day 5	7.68 ± 1.19	7.78 ± 1.10	7.70 ± 0.79	0.942

Data presented as median (range) or mean ± standard deviation

* $P < 0.05$ marked statistical significance, ANOVA test unless otherwise stated

[a] Kruskal–Wallis Test

continually for 5 days after the injection. However, no difference in fasting blood glucose levels among the three groups was observed (Table 1). Furthermore, glucose levels were also measured using the glucose oxidase method, and similar results were detected (data not shown).

Effect of resistin on fasting insulin levels

Fasting serum insulin levels in all three groups were determined on day 5 ($P = 0.002$). No difference was detected between the normal control and the Adv-EGFP groups (0.37 and 0.36, respectively). However, the serum insulin levels of mice in the Adv-resistin-EGFP group were significantly increased ($P = 0.005$; Table 1).

Increased resistin and insulin and glucose tolerance

As shown in Fig. 1, the effects of resistin expression on IPITT among three groups were analyzed. After 45, 60, and 90 min, a trend toward increased blood glucose levels was noted in the Adv-resistin-EGFP group. However, the difference was not significant ($P > 0.05$).

K_I values resulting from curve fitting were determined and compared between groups using the Kruskal–Wallis test. Ranks of the normal control, Adv-EGFP, and Adv-resistin-EGFP groups were 0.76, 0.76, and 0.55, respectively, indicating a significant decrease in K_I value as well as insulin sensitivity in the Adv-resistin-EGFP group ($P = 0.045$; Fig. 2).

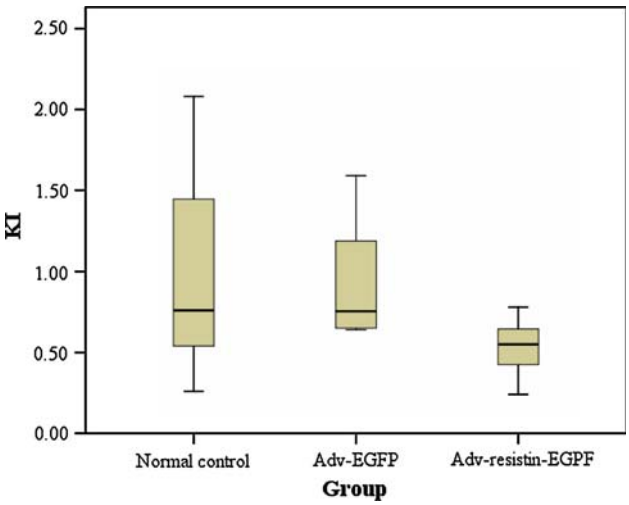

Fig. 2 Effects of resistin expression on K_I in IPITT. K_I values were presented as percentage per minute

During the IPGTT, no statistical difference in terminal fasting blood glucose levels among the normal control, Adv-EGFP, and Adv-resistin-EGFP groups was detected ($P = 0.207$; Fig. 3). At 30 min, blood glucose levels in the Adv-resistin-EGFP group were significantly higher than in the Adv-EGFP group ($P = 0.026$). At 60 and 120 min, there was a decreasing trend in the blood glucose of Adv-resistin group. In addition, AUC values were 1077, 1074,

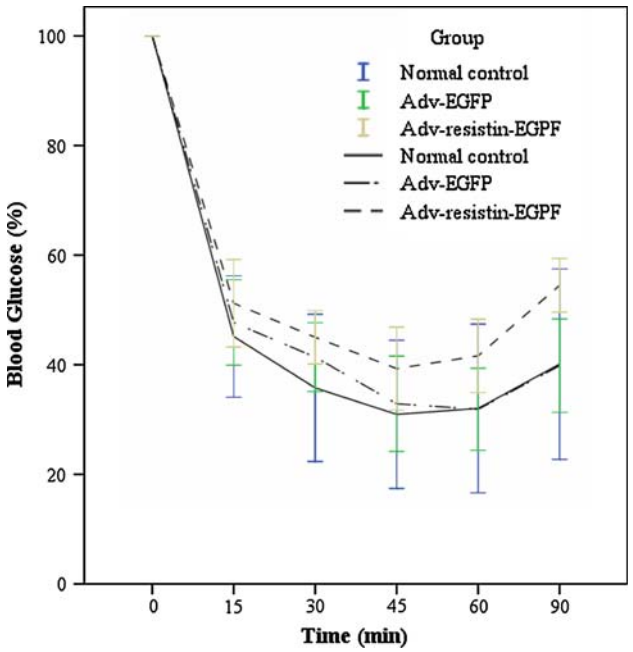

Fig. 1 Effects of resistin expression on IPITT. After 5 days post-tail vein injection of normal control, Adv-EGFP, and Adv-resistin-EGFP, fasting blood glucose levels of mice injected with 0.75 U/kg insulin (after 6 h fasting) was assessed for the three groups. Blood glucose at 0 min was used as the baseline reference

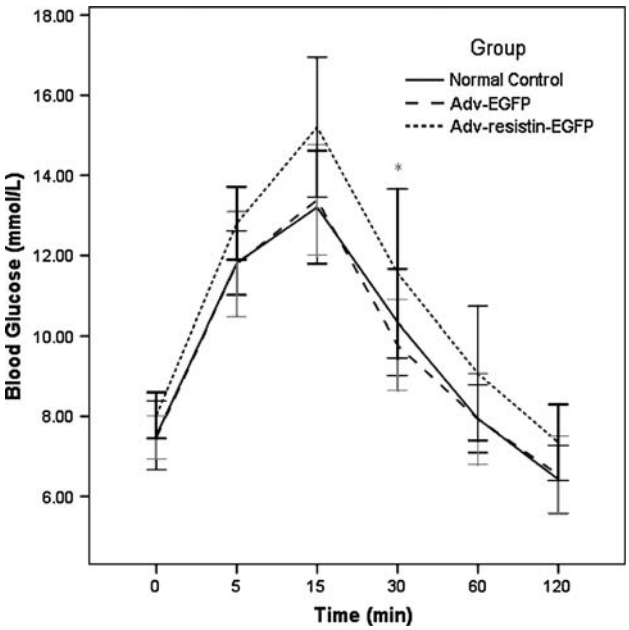

Fig. 3 Effects of resistin expression on IPGTT. After 5 days post-tail vein injection of normal control, Adv-EGFP, and Adv-resistin-EGFP, fasting blood glucose levels of mice given 1.5 g/kg of 10% glucose (after 14 h fasting) were assessed for the three groups. The AUC values were 861.8, 856.8, and 967.7 for the normal control, Adv-EGFP, and Adv-resistin-EGFP groups, respectively

and 1212 for the normal, Adv-EGFP, and Adv-resistin-EGFP groups, respectively.

Effect of resistin on glycogen storage

In order to determine the effects of resistin expression on hepatic glycogen, PAS staining was employed. In PAS staining, hepatic glycogen is detected as purple-red precipitated particles. Glycogen particles in hepatic cells of the normal control group were abundant, deeply stained, and presented a strongly positive reaction, suggesting normal hepatic cell metabolism (Fig. 4a, b). Although some particles were distributed unevenly, this polarization phenomenon is due to the fixation process. Glycogen particles of Adv-EGFP group were more lightly stained as compared with the normal control group; however, a positive reaction was detected (Fig. 4c, d). Conversely, in the Adv-resistin-EGFP group, fewer glycogen particles were detected, and those that were seen were more lightly stained as compared to the two control groups (Fig. 4e, f).

Further quantification of the glycogen content PAS staining was carried out using histological grading, and statistically significant differences in glycogen levels among the three groups were detected ($P = 0.001$; Table 2). These data indicate that resistin expression influences hepatic glycogen content.

Effect of resistin on liver AMPK activation

In order to determine the mechanism by which resistin expression influenced hepatic glycogen storage, AMPK activation was determined by Western blot analysis of hepatic tissue lysates from each group (Fig. 5). Phosphorylated AMPK was reduced in the Adv-resistin-EGFP group as compared to the normal control and Adv-EGFP groups. Further densitometric analysis was carried out to quantify

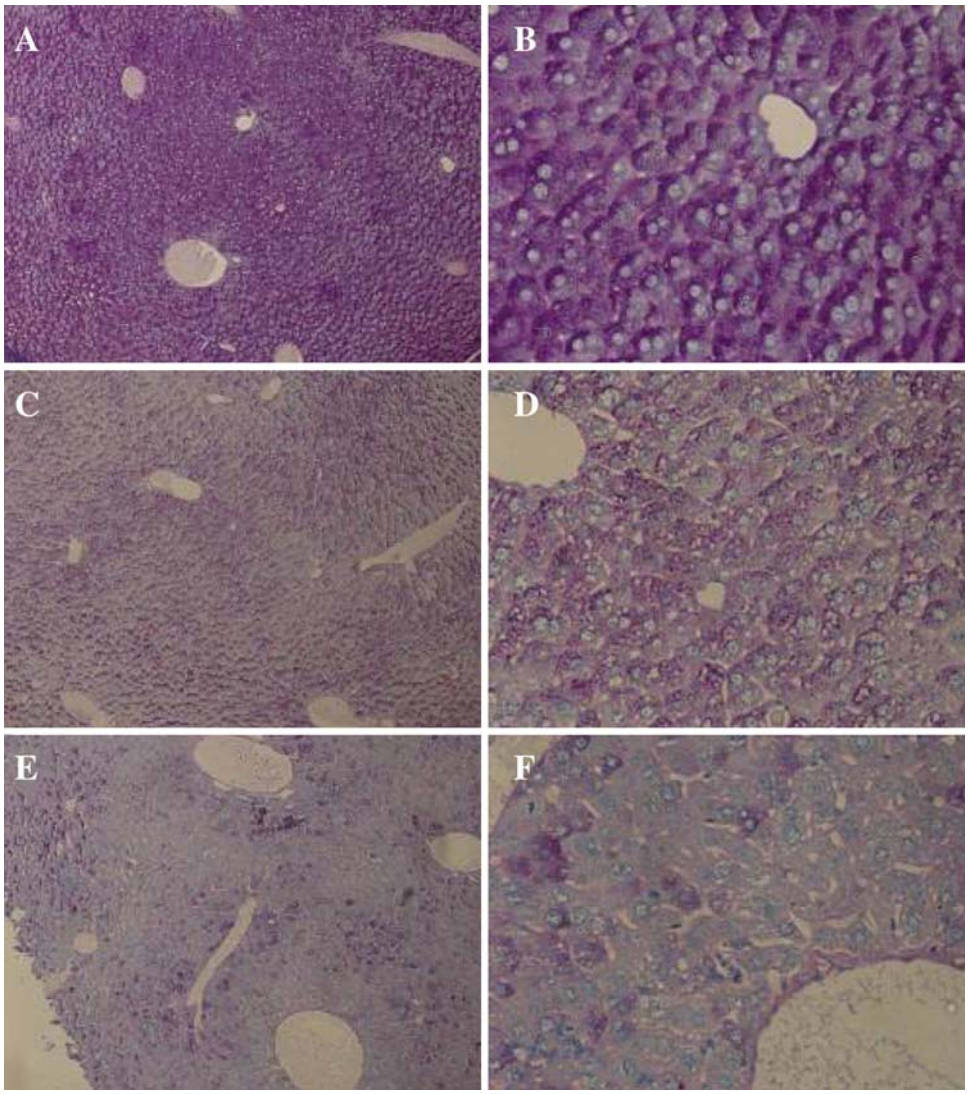

Fig. 4 PAS analysis of liver glycogen content. On day 5, liver tissues from the normal control (**a**, **b**), Adv-EGFP (**c**, **d**), and Adv-resistin-EGFP (**e**, **f**) groups were analyzed. Representative images are shown at ×100 and ×400 magnifications (left and right panels, respectively)

Table 2 Effects of adenovirus-mediated resistin expression on venous serum fasting glucose levels (mmol/l), serum resistin levels (ng/ml), hepatic glycogen content, and AMPK activation

	Normal control ($n = 8$)	Adv-EGFP ($n = 7$)	Adv-resistin-EGFP ($n = 8$)	P-value
Glucose (mmol/l)	6.02 ± 0.79	6.87 ± 1.43	6.89 ± 2.21	0.278
Resistin (ng/ml)[a]	25.07 (18.91–28.36)	21.88 (15.24–33.76)	271.5 (103.52–300.00+)	<0.001*
Glycogen[a]	3.0 (2.8–3.2)	3.0 (2.8–3.2)	2.5 (2.2–2.8)	0.001*
AMPK activation	0.93 ± 0.13	0.89 ± 0.05	0.78 ± 0.06	0.008*

Data presented as mean ± standard deviation or median (range)

* $P < 0.05$ marked statistical significance, ANOVA test unless otherwise stated

[a] Kruskal–Wallis test

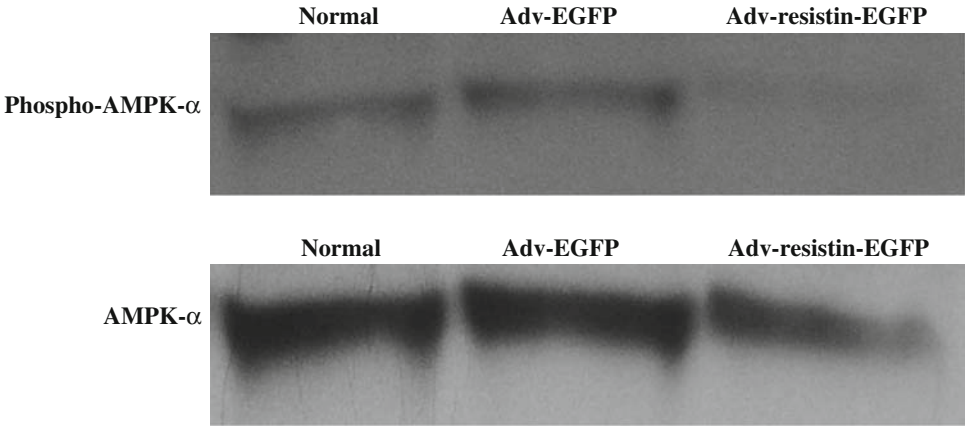

Fig. 5 Effects of adenovirus-mediated resistin expression on AMPK activation. Liver AMPK activation was determined by Western blot analysis using antibodies specific for phosphorylated AMPK as well as total AMPK in normal control, Adv-EGFP, and Adv-resistin mice

AMPK activation. As shown in Table 2, AMPK activation, as assessed by the proportion of phosphorylated AMPK divided by total AMPK per sample, was significantly reduced in the Adv-resistin-EGFP group as compared to the normal control and Adv-EGFP groups ($P = 0.008$), suggesting that resistin may inhibit AMPK activation.

Effect of resistin on PEPCK and G6Pase mRNA expression

Because resistin expression influenced AMPK activation, its effects on gluconeogenic enzymes, PEPCK, and G6Pase were next determined. After normalization with β-actin expression levels, increased PEPCK and G6Pase were detected in the Adv-resistin-EGFP group as compared to the Adv-EGFP group ($P = 0.043$ and 0.039, respectively; Table 3). Taken together, these data suggest resistin expression influences gluconeogenic enzyme expression, which in turn may affect hepatic glycogen content.

Table 3 Effects of adenovirus-mediated resistin expression on hepatic PEPCK and G6Pase mRNA expression

	Adv-EGFP ($n = 7$)	Adv-resistin-EGFP ($n = 8$)	P-value
PEPCK	2.63 ± 0.67	3.54 ± 0.90	0.043*
G6Pase	1.25 ± 0.78	2.14 ± 0.86	0.039*

Data presented as mean ± standard deviation or median (range)

*$P < 0.05$ marked statistical significance, t-test

Discussion

Increased resistin expression resulted in hyperinsulinemia without affecting fasting blood glucose

Using two methods to detect changes in fasting blood glucose, terminal fasting blood glucose using a blood glucose reader or as serum glucose level through glucose oxidase analysis, no alteration in fasting blood glucose was detected upon elevated resistin expression as compared to the control virus group. However, increased insulin levels were detected in the Adv-resistin-EGFP group. These results are similar to those previously reported by Satoh et al. [13] and Sato et al. [15], who also analyzed the effects of recombinant resistin-expressing adenovirus after 7 and 5 days, respectively.

Although studies using adenovirus-mediated resistin expression have revealed similar effects on fasting blood glucose and insulin levels, conflicting effects have been reported using other models. For example, increased fasting blood glucose was detected in resistin-expressing transgenic mice of normal body weight [16]. Furthermore,

the fasting blood glucose levels of resistin null mice (−/−) were significantly decreased by 20 to 30%, and re-administration of recombinant resistin restored the fasting blood glucose to levels found in the wild-type (+/+) group [11]. However, neither study detected differences in fasting insulin levels. Finally, administration of purified resistin protein in mice resulted in not only increased fasting blood glucose, but also elevated insulin levels [3, 17]. Hence, Steppan et al. [3, 17] speculated that changes in fasting blood glucose levels in response to resistin were perhaps related to IR.

The often opposing effects of resistin expression on fasting blood glucose and insulin levels may be partially explained by intrinsic differences in the models utilized. For example, various degrees of resistin overexpression may be attained within each model. Furthermore, in the transgenic or knockout mouse models, the influence of chronic hyperresistinemia or hyporesistinemia, respectively, was analyzed [11, 16] as compared to the acute resistin overexpression (5 days) analyzed in this study. Therefore, increasing the duration of resistin overexpression may possibly result in abnormal fasting blood glucose.

Resistin expression reduces glucose tolerance as well as insulin sensitivity

In this study, we demonstrated that resistin negatively affected glucose tolerance as well as insulin sensitivity, which was similar to other reports using in vivo rodent models [5, 10, 16]. Specifically, in a rat model, utilizing adenovirus-mediated resistin overexpression, IPGTT analysis revealed increased blood glucose levels 15 to 60 min after glucose loading as compared to the control group, and insulin levels followed a similar trend. However, IPITT revealed a significant reduction in the hypoglycemic activity of insulin at 30 min, which was sustained to 60 min. In a similar mouse model, IPITT analysis revealed no significant difference at 30 min, but insulin sensitivity was reduced for a prolonged time. Comparable results were obtained in resistin-expressing transgenic mice as well as animal models that received a subcutaneous injection of resistin overexpressing-3T3-L1 adipocytes [5, 16]. On the contrary, in resistin knockout or suppression rodent models, the results of the two tolerance tests were almost normal, and upon administration of recombinant resistin, abnormal glucose metabolism resumed [11, 12].

In this study, we not only compared the extent of blood glucose reduction at each time point, but also analyzed the K_I in order to evaluate insulin sensitivity. Since curve fitting was conducted, our results better reflected the vicissitudes of blood glucose during the whole experimental process.

Effects of resistin expression on glycogen storage

Resistin expression may result in glucose metabolism disturbance, hyperinsulinemia, glucose tolerance damage, and insulin insensitivity in mice. Furthermore, resistin expression might participate in the formation of IR in type II diabetes. However, studies analyzing the main insulin-sensitive target organs, such as liver, skeletal muscle, and adipose tissue, upon resistin overexpression are warranted to further investigate the pathological as well as physiological mechanisms by which resistin affects IR [18].

Recently it has been proposed that the liver is the key organ affected in type II diabetic IR, and that the early stage hepatic metabolic changes are closely linked to the development of IR, obesity, and type II diabetes (reviewed in [14]). In this study, the reduced glycogen staining in liver tissue indicated that resistin expression resulted in a reduction in glycogen storage, suggesting that long-term effects of resistin expression may result in increased glycolysis and hepatic glucose output as a result of decreased insulin-mediated hepatic glucose output inhibition under fasting condition as well as IR. These results conform to previous studies [4, 11, 12].

Using an extended hyperinsulinemic-euglycemic clamp technique in rats and sustained intravenous resistin injection, Rajala et al. [4] monitored glucose metabolism using [^{3}H]3-glucose and [U–^{14}C] lactic acid. Resistin inhibited glucose consumption by peripheral tissues while increasing hepatic glucose output. Hence, they proposed that resistin participated in the development of type II diabetes mainly through its affect on hepatic IR and subsequent glucose metabolism. In addition, although there was no difference in the glucose infusion rate between transgenic resistin-expressing mice and their wild-type counterparts, glucose production of the former increased 2.8 times [4], indicating increased hepatic glucose output. Hyperinsulinemic-euglycemic clamp experiments in mice subjected to a high fat diet for 3 weeks found that the glucose infusion rate dropped 59% compared with the control group due to decreased glucose disappearance and increased glucose production [12]. One week after administration with an antisense oligonucleotide specific to resistin mRNA, serum resistin returned to normal levels with no improvement in glucose disappearance while glucose production reduced to levels comparable to that of the control group [12]. However, upon acute reinjection of recombinant resistin protein, glucose production was again elevated. Thus, Muse et al. [12] speculated that the short-term effect of resistin expression resulted in increased glycogen production as well as hepatic IR, which was further supported by Banerjee et al. [11] in resistin null mice.

Studies using resistin null ob/ob mice reported a twofold increase in glucose infusion rate as compared to the ob/ob

mice, which was reversed by reinjection of resistin [19]. Nevertheless, no difference in hepatic glucose production was detected between the two groups, whereas the glucose disappearance increased in resistin null ob/ob mice. Qi et al. [19] speculated that increased glucose infusion rate might be associated with increased glucose intake in muscle, white fat, and brown fat tissues. However, in clamp experiments conducted after 4 weeks on a high fat diet, hepatic glucose output was reduced in the resistin null ob/ob mice as compared to those receiving reinjection of resistin, suggested that the impact of resistin on hepatic glucose metabolism remained [19].

Effect of resistin on hepatic AMPK activation and PEPCK and G6Pase expression

The mechanism by which resistin affects hepatic IR formation was analyzed in this study. AMPK signaling plays a critical role in hepatic glucose metabolism [20]. In intact cells, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) activates AMPK [21], and Bergeron et al. [22] reported that systemic injection of AICAR in both normal and obese rats with IR inhibited hepatic glucose output. Furthermore, adenovirus-mediated AMPKα2 expression in a diabetic mouse model indicated that short-term activation of hepatic AMPK could control hyperglycemia [23]. Therefore, the influence of resistin expression on AMPK activation was assessed in this study, and reduced AMPK activation was detected in response to resistin expression.

In hepatic cell cultures, AMPK signaling reduced PEPCK and G6Pase expression [24]. G6Pase is the key enzyme that catalyses the hydrolysis of glucose-6-phosphate to glucose, and its overexpression or hyperactivation results in increased blood glucose levels. PEPCK is the key enzyme that catalyses the first reaction of gluconeogenesis, and altered PEPCK expression or activation has crucial regulatory effects on gluconeogenesis. Therefore, in vivo reduction of PEPCK and G6Pase expression through AMPK activation or other means could reduce the intrinsic glucose production rate and alleviate IR status as well as diabetic hyperglycemia.

In this study, real-time RT-PCR analysis revealed that resistin expression increased PEPCK and G6Pase expression, which might result in elevated hepatic glucose output. Satoh et al. [13] also detected reduced hepatic AMPK activity as well as enhanced Akt phosphorylation and reduced tyrosine phosphorylation of insulin receptor substrate 2 in resistin-expressing rats, thereby suppressing the inhibitory effects of insulin on hepatic glucose output. In resistin null mice, expression of gluconeogenesis-related enzymes and hepatic glucose output decreased due to AMPK activation [11]. Finally, Muse et al. [12] found that increased hepatic AMPK phosphorylation and reversal of hepatic IR could be achieved by reducing serum resistin levels.

Study limitations

Although the adenovirus method of gene transfer has many advantages, it also has some disadvantages. In addition to increased resistin expression, other viral proteins were likely expressed once the adenovirus vector was introduced in vivo. This may likely elicit an immune response, which could affect the results obtained. Furthermore, liver damage was detected in both Adv-EGFP and Adv-resistin-EGFP groups, suggesting that adenovirus infection results in liver toxicity. However, it also indirectly indicated that the target gene, resistin, was expressed in the liver. Thus, the use of a group given a control virus carrying a reporter gene was necessary to detect resistin-specific differences among the groups and facilitated further functional assays.

Elevated PEPCK and G6Pase expression was detected in the Adv-resistin-EGFP group as compared to the control groups although not to statistical significance. This is likely due to subtle differences that may not be detected in the small sample size used for these experiments. However, glycogen storage was significantly reduced in the Adv-resistin-EGFP group, indirectly indicating that both PEPCK and G6Pase are affected by resistin expression, which is further supported by Rangwala et al. [16].

Although this study focused mainly on hepatic effects of resistin expression, possible effects involving other insulin-sensitive target tissues, such as skeletal muscle and adipose tissue, cannot be excluded [5, 13]. In addition, other mechanisms by which resistin could influence hepatic IR were not explored. For example, Singhal et al. [25] reported that the influence of resistin expression was mediated at least in part by hypothalamic expression of neuropeptide Y, suggesting that the effects of resistin may not be directly upon liver cells themselves, but upon hypothalamic neurons that innervate the liver. Thus, further studies are required to elucidate the full influence of resistin on IR.

Conclusions

Resistin might participate in hepatic IR formation through lowering hepatic AMPK activity, promoting expression of key gluconeogenesis enzymes, enhancing hepatic glycolysis, and subsequently increasing the hepatic glucose output.

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