Plasma Resistin Concentrations Measured by Enzyme-Linked Immunosorbent Assay Using a Newly Developed Monoclonal Antibody Are Elevated in Individuals with Type 2 Diabetes Mellitus

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Resistin is an adipocyte-derived peptide that might play a role in obesity and insulin resistance. However, its role in humans is largely unclear. Although many studies have measured the expression of human resistin in tissues, the circulating concentrations of resistin and its relation to metabolic parameters in humans are unknown. We developed an ELISA for human resistin and measured plasma concentrations in aged individuals with or without type 2 diabetes mellitus. To validate the results of plasma resistin concentrations in our subjects, plasma adiponectin concentrations were also determined, which were higher in nondiabetic subjects than in type 2 diabetic patients and correlated with the homeostasis model assessment for insulin resistance (HOMA-IR). Log-transformed plasma resistin concentrations (log-resistin) were higher in diabetic patients compared with normal individuals (0.50 ± 0.39 vs. 0.28 ± 0.51 ng/ml; P < 0.001), and this difference was significant after controlling for gender and body mass index. Log-resistin did not show a significant correlation with HOMA-IR, waist circumference, body mass index, blood pressure, or total cholesterol. The plasma glucose concentration was an independent factor associated with log-resistin. In conclusion, plasma resistin concentrations are elevated in patients with type 2 diabetes, but are not associated with insulin resistance or obesity. (J Clin Endocrinol Metab 89: 150–156, 2004)

Obesity is a well known risk factor of type 2 diabetes mellitus and is strongly associated with insulin resistance. Traditionally, adipose tissue was thought to be an inert tissue that stores excess energy and insulates the body from temperature and trauma. It is now evident that it is an active endocrine organ secreting many kinds of adipokines, such as leptin, TNFα, IL-6, and adiponectin (Acrp30/adiponectin), that may affect insulin action in other tissues (1–4). Among them, adiponectin is one of the most abundant serum proteins, and its role in insulin resistance has been extensively studied. Plasma levels of adiponectin are significantly lower in insulin-resistant states, including type 2 diabetes (5), and can be increased upon administration of the insulin-sensitizing thiazolidinedione class of compounds (6). The association of low adiponectin levels with obesity, insulin resistance, coronary artery disease, and dyslipidemia indicates that this protein may be an important new marker of the metabolic syndrome (7). Recently, adiponectin was reported to exert its metabolic action by increasing AMP-activated protein kinase, peroxisome proliferator-activated receptor α ligand activity, fatty acid oxidation, and glucose uptake through receptor-mediated mechanism (8).

Resistin (FIZZ3/ADSF) is another adipocyte-derived peptide first identified during a search for targets of thiazolidinediones. Stepan et al. (9) reported that serum concentrations of resistin are markedly increased in obese mice and are decreased by treatment with thiazolidinediones. They also found that administration of an antiresistin antibody increases insulin-stimulated glucose uptake in obese mice and that treatment of normal mice with recombinant resistin impairs insulin action. Thus, resistin might link obesity with insulin resistance and diabetes in mice models. However, subsequent studies in rodent models (10–12) have produced disparate findings on the role of resistin in obesity and insulin resistance. In humans, the expression of resistin in adipocytes is very low compared with that in rodents, but resistin mRNA is readily detectable in circulating mononuclear cells, which suggests that human resistin may be regulated by a different mechanism or has a different role from that in rodents (13). Furthermore, the expression of resistin in adipocytes does not differ among normal, insulin-resistant, and type 2 diabetic individuals (13–15). However, some genetic case-control studies have demonstrated that genetic variations in the resistin gene are associated with insulin

Abbreviations: BMI, Body mass index; CV, coefficient of variation; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; log-resistin, log-transformed plasma resistin concentration; MAb, monoclonal antibody; PBS, PBS containing 1% BSA and 0.05% Tween 20; RELM, resistin-like molecule.

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resistance and obesity in human (16, 17). Therefore, it is still possible that resistin might link obesity with insulin resistance or diabetes in humans as well as mice. As resistin is a secretory protein (9), it would be important to determine the circulating resistin concentrations and examine their relations with systemic metabolic effects in human. This would provide a clue to the role of resistin in human.

In this study we developed an ELISA method to measure human resistin using a newly developed monoclonal antibody (MAb), determined the plasma concentrations of resistin in type 2 diabetic patients and nondiabetic controls, and then examined the relationship between circulating resistin concentrations and metabolic parameters. Although the physiological roles of resistin are far from clear, those of adiponectin have been replicated in many independent studies. Therefore, we measured the plasma adiponectin concentrations as well to validate the results of plasma resistin concentrations in our study subjects.

### Subjects and Methods

#### Subjects

We studied 199 unrelated patients with type 2 diabetes and 185 nondiabetic control individuals, aged 60–75 yr, from the Diabetes Clinic of Seoul National University Hospital. Type 2 diabetes was diagnosed according to WHO criteria (18). None of the patients had typical presentations of type 1 diabetes, such as acute symptoms with ketosis or a history of diabetic ketoacidosis. All patients had negative tests for antitriglutamic acid decarboxylase antibodies. Patients treated with thiazolidinedione were excluded. To select the nondiabetic control individuals, the following criteria were used: no diabetes in their first degree relatives, fasting plasma glucose concentration less than 6.1 mmol/liter, and hemoglobin A1c concentration less than 5.8%. The clinical characteristics of the individuals are shown in Table 1. The institutional review board of Seoul National University Hospital approved the study protocol according to the Declaration of Helsinki, and written informed consent was obtained from each subject. Blood pressure, height, weight, and circumferences of waist and hips were measured. The body mass index (BMI) was calculated for each individual. Fasting plasma glucose, total cholesterol, triglycerides, and high density lipoprotein (HDL) cholesterol concentrations were measured enzymatically using an autoanalyzer (Hitachi 747, Hitachi, Ltd., Tokyo, Japan). Hemoglobin A1c was measured by affinity chromatography. Plasma insulin concentrations were measured by RIA (BioSource S.A., Nivelles, Belgium). The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as previously described (19).

#### Expression of recombinant proteins

The genes encoding human resistin and adiponectin were amplified from the HL-60 cDNA library and the human adipocyte cDNA library (Clontech, Palo Alto, CA), respectively, by PCR. For making His-tagged versions of the proteins, the portions of the respective genes encoding presumed mature polypeptides were amplified and cloned into PET21a (Novagen, Madison, WI). The proteins were induced and purified as either soluble forms or refolded forms through affinity chromatography according to a standard protocol. The FLAG-tagged human adiponectin globular domain was also expressed by means of CD5 leader sequence in 293 EBNA cells (Invitrogen, Carlsberg, CA). Conditioned medium containing the FLAG globular domain was harvested and passed through an anti-FLAG affinity column (Sigma-Aldrich Corp., St. Louis, MO). The eluted fraction was dialyzed against PBS and was kept frozen until use. The globular domain-lacking version of adiponectin, called headless protein, was a gift from Alexis Biochemicals (Lausen, Switzerland). For ELISA standards, recombinant human resistin and recombinant adiponectin expressed in mammalian cells were purchased from PeproTech, Inc. (Rocky Hill, NJ), and R&D Systems (Minneapolis, MN), respectively. Recombinant human resistin protein was also produced as a His-tagged form from 293 EBNA cells. The genes representing the open reading frame of human resistin excluding the translational termination codon was amplified with the following primer set: 1) forward primer, 5'-GAAAGATCTGGGACCTGTGCTCCATGGAAGAAGACCA-3'; and 2) reverse primer, 5'-CCGGCTCGAGTCAATGGTGATGGT-GATGGTGGGCGCTGCACTACAGCACGACGCACCGG-3'. The amplified fragments were digested with NdeI and Xhol, cloned into pCEP4 that had been digested with the enzyme pair, and transfected into 293 EBNA cells. The culture supernatant was employed for immunoprecipitation.

For producing Fc fusion proteins, the full-length genes, including signal peptides but excluding their own stop codons, were amplified and cloned in-frame into the human immunoglobulin heavy chain constant regions, CH2 and CH3, which had been placed in pCEP4, a mammalian expression vector (Invitrogen). The 293 EBNA cells were stably transfected with these expression vectors using Fugene transfection reagents (Roche, Indianapolis, IN). Serum-free DMEM was used to collect the secreted Fc fusion proteins from confluent cells for 2 d. The medium was then harvested and spun down to remove cells, and the pH was adjusted to 8.0 with 1 M Tris. The medium was filtered with a 0.4-μm pore size filter and loaded onto an antibody A column. The protein was then eluted with 100 mM glycine, pH 3.0, and was immediately neutralized with 0.1 vol 1 M Tris, pH 8.0.

#### Generation of MAb s against human adiponectin and resistin

BALB/c mice were repeatedly immunized with human resistin-Fc or adiponectin-Fc fusion protein until the polyclonal sera from the immunized mice exhibited strong immune responses. Splenocytes were isolated from the mice and fused to mouse myeloma Sp2/0. Screening and single cell cloning conformed to the established standard protocol. To

### TABLE 1. Clinical characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 185)</th>
<th>Type 2 diabetes (n = 195)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>65 ± 3</td>
<td>66 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>62:123</td>
<td>85:110</td>
<td>0.028</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>83.0 ± 8.3</td>
<td>88.3 ± 8.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>94.4 ± 6.0</td>
<td>97.1 ± 6.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood</td>
<td>144 ± 24</td>
<td>140 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood</td>
<td>84 ± 12</td>
<td>82 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.27 ± 0.82</td>
<td>5.15 ± 0.88</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mmol/liter)</td>
<td>1.58 ± 0.84</td>
<td>1.80 ± 1.11</td>
<td>0.027</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.19 ± 0.28</td>
<td>1.20 ± 0.30</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4 ± 3.0</td>
<td>24.4 ± 2.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.88 ± 0.05</td>
<td>0.91 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma</td>
<td>52.8 ± 22.8</td>
<td>79.2 ± 60.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.0 ± 0.9</td>
<td>5.9 ± 4.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
increase the number of positive clones and confirm specificity, the primary screen used the immunized Fc fusion protein as an antigen, whereas His-tagged adiponectin or resistin was used in the confirmatory screen for the positive hybridoma clones. Ascites were prepared from the immunocompromised BALB/c mice. Immunoglobulin fractions were prepared through protein G.

Western blot and immunoprecipitation

One microliter of serum was subjected to SDS-PAGE. Subsequently, protein bands were incubated with MAbs or polyclonal rabbit sera diluted 1:2000. Target protein bands were detected by chemiluminescence (Pierce Chemical Co., Rockford, IL). One milliliter of the serum-free supernatant from the HEK293 cells transfected with human resistin gene was harvested and mixed with 10 μg anti-His or HRES106 along with 1 μg protein A. Immunoreactive protein was pulled down and detected with polyclonal human resistin antibody.

ELISA

Resistin sandwich ELISA. Polyclonal human resistin antibody was biotinylated with a kit (Pierce Chemical Co.) according to standard protocol. Primary antibody, HRES106, was diluted to a concentration of 5 μg/ml, added to each well of microtiter plate, and incubated overnight at 4 C. The coated plate was then washed three times with PBS containing 1% BSA and 0.05% Tween 20 (PBST), blocked with 200 μl blocking buffer (1% BSA in PBST) at 37 C for 1 h, and washed three times with PBST. The secondary antibody reaction at a concentration of 3.5 μg/ml was performed at 37 C for 1 h and washed three times with PBST. For colorimetric reactions, horseradish peroxidase-conjugated streptavidin (Zymed Laboratories, South San Francisco, CA) diluted 1:1000 in PBS and 2.2'-azino-bis(2-ethylbenzothiazole-6-sulfonic acid) (Pierce Chemical Co.) as substrate were used. The OD was measured at 492 nm. Linearity in a range of 100 pg/ml to 1.5 ng/ml was consistently shown in multiple between- and within-run assays. Human adiponectin, human leptin, mouse resistin, and BSA were not detected by this ELISA system. To calculate the coefficient of variation (CV) within or between assays, five plasma samples from healthy subjects were subjected to ELISA in a total of five independent measurements with duplicate determinations. The CV (percentage) is defined as (sd/mean value) × 100%. Although within-assay analyses were conducted on a given single day, between-assay analyses were carried out on given consecutive days.

Adiponectin sandwich ELISA. Polyclonal human adiponectin antibody was biotinylated with a kit (Pierce Chemical Co.) according to a standard protocol. Primary antibody, ADI741, was diluted with coating buffer (0.1 M carbonate) to a concentration of 5 μg/ml, added to each well of a microtiter plate, and incubated overnight at 4 C. The coated plate was then washed three times with PBST, blocked with 200 μl blocking buffer (1% BSA in PBST) at 37 C for 1 h, and washed three times with PBST. The secondary antibody reaction at a concentration of 3.5 μg/ml was performed at 37 C for 1 h and washed three times with PBST. For colorimetric reactions, horseradish peroxidase-conjugated streptavidin (Zymed Laboratories, South San Francisco, CA) diluted 1:1000 in PBS and 2.2'-azino-bis(2-ethylbenzothiazole-6-sulfonic acid) (Pierce Chemical Co.) as substrate were used. The OD was measured at 492 nm. Linearity in a range of 100 pg/ml to 1.5 ng/ml was consistently shown in multiple between- and within-run assays. Human adiponectin, human leptin, mouse resistin, and BSA were not detected by this ELISA system. To calculate the coefficient of variation (CV) within or between assays, five plasma samples from healthy subjects were subjected to ELISA in a total of five independent measurements with duplicate determinations. The CV (percentage) is defined as (sd/mean value) × 100%. Although within-assay analyses were conducted on a given single day, between-assay analyses were carried out on given consecutive days.

Statistical analysis

All continuous variables are expressed as the mean ± sd unless otherwise specified. Pearson’s correlation analyses, t tests, and multiple linear regression analyses were performed using SPSS software (SPSS, Inc., Chicago, IL). As plasma resistin concentrations did not follow a normal distribution, we used log transformation before analysis. P < 0.05 was considered statistically significant.

Results

Generation of MAbs recognizing human resistin or adiponectin

To develop sandwich ELISA systems enabling measurement of the plasma concentrations of human resistin or adiponectin, an antiresistin MAb, HRES106, and two antiadiponectin MAbs, ADI 773 and ADI 741, were generated. To determine whether HRES106 can recognize the native form of human resistin, we expressed resistin in human embryonic kidney 293 cells (HEK293) in the His-tagged form at its carboxyl terminus and performed immunoprecipitation using anti-His antibody or HRES106. The precipitated proteins were subjected to Western blot and then detected with the use of antiresistin polyclonal antibody. As shown in Fig. 1A, both antibodies were able to immunoprecipitate the recombinant human resistin, suggesting that HRES106 can recognize the native form of human resistin.

ADI 773 and ADI 741 appear to recognize different epitopes. First, as shown in Fig. 1B, although ADI 773 recognized human serum adiponectin, recombinant human headless adiponectin, and full-length recombinant human adiponectin in Western blots, ADI 741 not only recognized all of these proteins, except the headless protein, but also recognized the recombinant FLAG-tagged globular domain. Second, as shown in Fig. 1C, whereas ADI 741 recognized serum adiponectins derived from multiple species, such as human, mouse, rat, cow, horse, and rabbit, ADI 773 exclusively recognized human adiponectin, with bovine adiponectin marginally detected. This supports the observation that the globular head domain is highly conserved between species (20). Thus, ADI 741 may be used as a versatile capture antibody for the development of cross-species ELISA systems for measuring serum or plasma concentrations of adiponectin. These characteristics of ADI 741 and HRES106 enabled us to measure the plasma concentrations of these important adipokines, which are responsible for modulating insulin resistance.

Optimization of sandwich ELISA for human resistin or adiponectin

By using human recombinant resistin or adiponectin, standard curves were established in which each linearity was shown (Fig. 2, A and D). Each standard curve yielded a consistent r² value greater than 0.99. Each ELISA system was able to detect as little as 100 pg/ml of the respective cytokine. For measuring plasma resistin concentrations, plasma was diluted 1:10. Although dilution at 1:5 resulted in similar plasma concentrations of resistin as those diluted at 1:10 using the present system, dilution over 1:20 led to concentrations below the detection limit compared with standard for some plasma samples. Underscoring the fidelity of each ELISA system, multiple within- and between-assay analyses were performed such that the CV of each assay was calculated. The data suggest that these assays were stably performed within 11% of the maximal range of CV.
Plasma adiponectin concentrations

Plasma adiponectin concentrations were higher in nondiabetic subjects than in type 2 diabetic patients (6.7 ± 3.7 vs. 5.9 ± 3.5 μg/ml; P = 0.024) and correlated with HOMA-IR (r = -0.154; P = 0.024).

Plasma resistin concentrations

To normalize the distribution of plasma resistin concentrations, we used log transformation. Log-transformed plasma resistin concentrations (log-resistin) were higher in type 2 diabetic patients than in normal individuals (0.50 ± 0.39 vs. 0.28 ± 0.51 ng/ml; P < 0.001), and this difference was significant in each gender group (Fig. 3). As it has been reported that the obese subjects showed higher mRNA expression of resistin in adipose tissue than the lean subjects (21), we divided our subjects into two groups according to BMI to control the potential effect from different BMI between type 2 diabetic and nondiabetic subjects. In each BMI group, log-resistin levels were higher in type 2 diabetic patients than in nondiabetic controls. (Fig. 4). Unlike the plasma adiponectin concentrations, those for plasma resistin did not differ between males and females.

Log-resistin did not show a significant correlation with HOMA-IR, waist circumference, BMI, blood pressure, or total cholesterol. However, serum triglyceride (r = 0.131; P = 0.013) and fasting plasma glucose concentrations (r = 0.18; P < 0.001) were significantly correlated with log-resistin. The fasting plasma glucose concentration was an independent factor associated with log-resistin by multivariate analysis including age, sex, total cholesterol, triglyceride, HDL cholesterol, and BMI (P = 0.011).

Discussion

Adiponectin and resistin are believed to play important roles in regulating insulin resistance (9, 20). It has been documented that serum adiponectin levels significantly correlate to the onset of obesity-related diseases such as type 2 diabetes and atherosclerosis (22, 23). Moreover, a recent study suggests that plasma adiponectin levels in healthy individuals can be used to predict type 2 diabetes (24). Although the implication of the plasma resistin levels in human type 2 diabetes remained unclear before this study, as this cytokine is preferentially expressed by white adipose tissues in mice and seems to be involved in hepatic insulin resistance along with resistin-like molecule (RELM)/

![Image](image.png)

FIG. 1. Biochemical properties of a human anti-resistin MAb, HRES106, and two distinct human anti-adiponectin MAbs, ADI 773 and ADI 741. A. Immunoprecipitation of recombinant human resistin expressed by HEK293 cells, using MAb HRES106. His-tagged human resistin was expressed and then immunoprecipitated by either anti-His or HRES106. The resulting complex was electrophoresed and probed by an antihuman resistin polyclonal antibody. B. Serum proteins and different versions of human adiponectin were subjected to Western blot analysis with the use of MAbs ADI 773 and ADI 741. The presence of reacting proteins was detected by chemiluminescence. C. To check the cross-reactivity of ADI 773 and ADI 741, sera were prepared from multiple mammalian species as indicated and were subjected to Western blot analysis along with recombinant full-length adiponectin.
immune response can be mounted (26). As a result, we were able to raise ADI 741 as an autoantibody from the immunized mice that strongly recognized both its own serum adiponectin and multiple species' globular domains, suggesting that immunological tolerance was broken. We believe that this kind of approach is very useful for generating MAbs against other adipokines, such as RELMα, RELMβ, or other soluble factors derived from adipocytes. To our best knowledge, ADI 741 is the only MAb recognizing the adiponectin globular head domain, and HRES106 is also the only available MAb recognizing human resistin. These two antibodies would be very useful reagents to measure the levels of human adiponectin and resistin, respectively, at normal or pathological stages of obesity-related diseases involving type 2 diabetes.

We demonstrate here for the first time that plasma resistin and adiponectin ELISA systems. A–C, Human resistin standard curve was made with the use of five different concentrations (100, 250, 500, 1, and 2 ng/ml) of recombinant resistin in which linearity was seen. HRES106 was used as the capture antibody, whereas biotinylated resistin polyclonal antibody was used as the detector antibody. For within- and between-assay analyses, five different plasma samples were subjected to ELISA with duplicate determinations. CVs were calculated as described in Subjects and Methods. D–F, A human adiponectin standard curve was made with the use of five different concentrations (500 pg/ml, 1 ng/ml, 2 ng/ml, 4 ng/ml, and 8 ng/ml) of recombinant adiponectin in which linearity was seen. ADI741 was used as the capture antibody, whereas biotinylated adiponectin polyclonal antibody was used as the detector antibody. For within- and between-assay analyses, four different plasma samples were subjected to ELISA with duplicate determinations. CVs were calculated as well.

**FIG. 2.** Validation of human resistin and adiponectin ELISA systems. A–C, Human resistin standard curve was made with the use of five different concentrations (100, 250, 500, 1, and 2 ng/ml) of recombinant resistin in which linearity was seen. HRES106 was used as the capture antibody, whereas biotinylated resistin polyclonal antibody was used as the detector antibody. For within- and between-assay analyses, five different plasma samples were subjected to ELISA with duplicate determinations. CVs were calculated as described in Subjects and Methods. D–F, A human adiponectin standard curve was made with the use of five different concentrations (500 pg/ml, 1 ng/ml, 2 ng/ml, 4 ng/ml, and 8 ng/ml) of recombinant adiponectin in which linearity was seen. ADI741 was used as the capture antibody, whereas biotinylated adiponectin polyclonal antibody was used as the detector antibody. For within- and between-assay analyses, four different plasma samples were subjected to ELISA with duplicate determinations. CVs were calculated as well.

**FIG. 3.** Comparison of plasma resistin concentrations between type 2 diabetes (T2DM) and control individuals. Values are the mean ± SEM. *, P < 0.05; ***, P < 0.001 (compared with control).

**FIG. 4.** Comparison of plasma resistin concentrations between type 2 diabetes (T2DM) and control individuals according to BMI group. Values are the mean ± SEM. *, P < 0.05; ***, P < 0.001 (compared with control).
concentrations are elevated in patients with type 2 diabetes. There have been many contradictory observations since the first suggestion that resistin may link obesity with insulin resistance in rodent models (9). Apart from the controversies based on rodent models (10–12), the results in human studies still cannot explain the role of resistin in human diseases (13–15). Although many studies have measured the expression of resistin mRNA or the protein itself in tissues, until now no study has determined its circulating concentrations, which might help to explain its systemic effects. Using these newly developed ELISA methods, we measured plasma concentrations of resistin and adiponectin in aged type 2 diabetic and nondiabetic individuals. Our nondiabetic control individuals were expected to have a low chance of developing type 2 diabetes because they were old, did not have a family history of diabetes, and showed normal fasting glucose concentrations. Compared with these control individuals, we found that the log-transformed plasma resistin concentrations of type 2 diabetic patients were increased about 1.8 times in both men and women. The plasma resistin concentrations showed a positive correlation with serum triglyceride and fasting plasma glucose concentrations, but not with waist circumference, BMI, blood pressure, total cholesterol, or HOMA-IR.

In our study plasma adiponectin concentrations were decreased in patients with type 2 diabetes and correlated with waist circumference, BMI, triglyceride, HDL cholesterol, and HOMA-IR (data not shown). These results are quite consistent with the well established relation between adiponectin and systemic insulin sensitivity (20). In contrast to the association between plasma adiponectin and various metabolic parameters, plasma resistin concentrations did not show any relationship with indexes of obesity or HOMA-IR, although plasma resistin concentrations were unequivocally increased in type 2 diabetic patients. Therefore, we cannot conclude that plasma resistin concentrations are related to insulin resistance in human.

Why there are increased plasma resistin concentrations in patients with type 2 diabetes, but these have no significant association with indexes of obesity or insulin resistance, is unclear. It is possible that the increased plasma resistin concentrations found in these patients are merely the result of increased production of resistin in adipocytes in a hyperglycemic milieu. Although there are no data from human adipocytes, high concentrations of glucose significantly enhance resistin expression, whereas insulin suppresses its expression in murine adipocytes (27). We also found that plasma resistin concentrations showed a positive correlation with fasting plasma glucose concentrations, which turned out to be an independent factor by multivariate analysis. In addition, it is possible that insulin resistance, as occurs during the course of type 2 diabetes, reflects the diminished ability of insulin to suppress resistin expression by adipocytes (27). However, these results are still inconsistent with the observation that resistin expression in adipocytes is similar in normal and insulin-resistant individuals (13–15). Alternatively, it may be possible that plasma resistin concentrations in humans might be regulated by mechanisms different from those in rodents, because human resistin is predominantly expressed in mononuclear cells with low or absent expression in adipocytes (13). It was reported that resistin expression was higher in the adipose tissue of obese subjects than in that of lean subjects (13, 21), but these results could be confounded by the effects of cells other than adipocytes in adipose tissue (such as monocytes, macrophages, and lymphocytes) (13, 28). Indeed, human resistin mRNA was more readily detectable in the stromovascular fraction of white adipose tissue biopsies (13).

Recently, Rajala et al. (25) showed that administration of recombinant resistin impaired hepatic insulin sensitivity whereas it did not affect peripheral glucose disposal in rats. These findings suggest that resistin might be a marker of hepatic insulin resistance rather than peripheral insulin resistance. We did not measure hepatic and peripheral insulin sensitivity, which is a limitation of the current study.

In summary, we have demonstrated that plasma resistin concentrations are elevated in patients with type 2 diabetes. Further studies should be conducted to examine whether plasma concentrations are directly related to hepatic or peripheral insulin sensitivity.

Acknowledgments

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