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Serum resistin is reduced by glucose and meal loading in healthy human subjects

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Abstract

Resistin is an adipokine that induces insulin resistance in mice; serum concentrations are decreased by fasting and increased by feeding. Adiponectin, another adipokine, improves insulin sensitivity. The aims of this study were to determine the effects of glucose and meal loading on serum resistin and total and high–molecular weight (HMW) adiponectin in humans and to explore potential determinants of fasting serum resistin and of changes in resistin. Serum resistin and total and HMW adiponectin were measured by enzyme-linked immunosorbent assay in young, lean, nondiabetic subjects during 75-g oral glucose tolerance test (OGTT) and meal tolerance test (MTT). Resistin single nucleotide polymorphism (SNP) −420 was typed. Serum resistin was decreased at 60 and 120 minutes during OGTT compared with baseline (n = 36, 1-way repeated-measures analysis of variance, $P < .0001$; Scheffe, $P = .0457$ and $P < .0001$, respectively). Serum resistin was also reduced at 240 minutes during MTT (n = 33, 1-way repeated measures analysis of variance, $P < .0001$; Scheffe, $P = .0002$). Multiple regression analysis adjusted for age, sex, and body mass index revealed that the reductions in serum resistin were dependent on baseline resistin levels. Subjects with greater baseline concentrations of resistin experienced more pronounced declines in resistin (OGTT, unstandardized regression coefficient (β) = −0.19, $P = .0005$; MTT, $\beta = -0.63$, $P < .0001$). Serum total and HMW adiponectin was unchanged. Fasting serum resistin was positively correlated with the G allele number of SNP −420 ($\beta = 7.70$, $P = .01$) and white blood cell count ($\beta = 0.007$, $P = .0001$) adjusted for age, sex, and body mass index. Therefore, in young, lean, nondiabetic humans, serum resistin was reduced by glucose and meal loading; the reduction in resistin was greater in subjects with higher fasting resistin. Fasting resistin was correlated with SNP −420 and white blood cell count.

1. Introduction

Resistin is a hormone secreted from adipocytes in mice [1,2]. It was identified as a gene whose expression is inhibited by an insulin-sensitizing drug, a peroxisome proliferators–activated receptor γ ligand [1]. In mice, liver-specific overexpression of the resistin gene induces insulin resistance [3], whereas knockout mice have reduced plasma glucose [4]. Therefore, resistin is an adipokine that plays a pathophysiological role in insulin resistance in mice.

Resistin is primarily expressed in monocytes or macrophages in humans, suggesting that the role of resistin in humans may differ from that in mice [5-7]. In mice, serum resistin is decreased by overnight fasting and increased by meal feeding or hyperglycemia [8]. In mice, clamp studies have shown that hyperinsulinemia or hyperglycemia increases resistin messenger RNA (mRNA) in adipocytes and serum resistin concentrations [8]. In humans, the role of resistin in insulin resistance remains controversial. Circulating resistin is increased or not affected in obesity or type 2 diabetes mellitus [2,9-13]. This controversy may have

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resulted from different responses of serum resistin to glucose or other nutrients among subjects. Therefore, whether the glucose or meal load affects serum resistin levels in humans merits further investigation.

We previously reported that the G/G genotype of resistin promoter single nucleotide polymorphism (SNP) −420 is associated with type 2 diabetes mellitus susceptibility [14]. Fasting serum resistin was highest in nondiabetic subjects and subjects with type 2 diabetes mellitus with the G/G genotype, followed by C/G and C/C [13,14]. In healthy volunteers, monocyte resistin mRNA was positively correlated with its simultaneous serum levels and was highest in subjects with the G/G genotype. Therefore, SNP −420 genotypes should be analyzed as a confounding factor when serum resistin levels are assessed.

Adiponectin is an adipocyte-specific secretory protein, which increases insulin sensitivity [15]. Adiponectin is present in serum as a trimer, a hexamer, or a high–molecular weight (HMW) form consisting of 12 to 18 subunits [15]. In humans, total adiponectin levels are reduced in obesity [16], type 2 diabetes mellitus [17], and coronary artery disease [18]. The ratio of HMW to total adiponectin is a better predictor of insulin sensitivity than total adiponectin [19,20]. An HMW adiponectin assay, using an antibody specific for the HMW form of adiponectin, has been recently described [21,22].

In view of this, the aims of this study were to determine the effects of glucose and meal loading on serum resistin and on total and HMW adiponectin levels in humans and to explore potential determinants of fasting serum resistin levels and of changes in resistin during the 75-g oral glucose tolerance test (OGTT) and meal tolerance test (MTT). The resistin SNP −420 was analyzed to adjust the effect of this SNP on serum resistin levels.

2. Subjects and methods

2.1. Participants' characteristics

Young, lean, healthy Japanese volunteers were recruited to undergo OGTT and MTT (Table 1). All participants had normal glucose tolerance, as assessed by OGTT, with no evidence of diabetes within first-degree relatives. Participants were also free of apparent diseases including infection or inflammation; results of their routine blood tests were within the reference ranges. Thirty-six subjects underwent OGTT. Of these, 33 subjects also participated in the MTT. Three subjects declined to undergo the MTT because of the relatively long duration of the test. The OGTT and MTT were performed in random order, and the interval between these 2 tests was 62.8 ± 43.0 (mean ± SD) days. There were no significant differences in clinical data between the OGTT and MTT groups. Written informed consent was obtained from all subjects. The protocol was approved by the Ethics Committee of Ehime University Graduate School of Medicine.

Table 1
Clinical characteristics of subjects

	OGTT	MTT
n (male/female)	36 (13/23)	33 (12/21)
Age (y)	24.3 ± 4.7	23.4 ± 2.6
BMI (kg/m^2)	20.4 ± 1.7	20.3 ± 1.6
HbA_{1c} (%)	4.8 ± 0.3	4.8 ± 0.2
FPG (mg/dL)	84.0 ± 7.6	83.9 ± 7.0
F-IRI (μU/mL)	7.7 ± 4.0	7.4 ± 4.2
HOMA-IR	1.6 ± 0.9	1.6 ± 1.0
F-resistin (ng/mL)	22.7 ± 15.7	20.6 ± 13.5
F-total adiponectin (μg/mL)	10.7 ± 4.8	10.2 ± 4.8
F-HMW adiponectin (μg/mL)	7.2 ± 4.4	5.8 ± 3.3

These healthy volunteers had normal glucose tolerance and no family history of type 2 diabetes mellitus. Data are mean ± SD. No significant differences were found between the OGTT and MTT groups (Student t test). The HOMA-IR was calculated by FPG (in milligrams per deciliter) × F-IRI (in microunits per milliliter)/405. F-resistin indicates fasting serum resistin; F-total adiponectin, fasting serum total adiponectin; F-HMW adiponectin, fasting HMW adiponectin.

2.2. Study design

The subjects underwent OGTT and MTT after an overnight fast. For the OGTT, subjects ingested a solution containing 75 g glucose. Blood samples were collected before and 30, 60, and 120 minutes after ingestion of the glucose. For the MTT, subjects ingested an isocaloric mixed meal (750 kcal; protein, 25 g; fat, 35 g; and carbohydrate, 80 g). Blood samples were collected before and 60, 120 180, and 240 minutes after the meal. Because serum resistin is correlated with white blood cell (WBC) counts [23], high-sensitivity C-reactive protein (hsCRP) [12,24], and renal function [25,26], WBC, hsCRP, and creatinine were analyzed in the preload samples.

2.3. Measurement of serum resistin and adiponectin levels

Serum resistin was measured using a human resistin enzyme-linked immunosorbent assay (ELISA) kit (Linco Research, St Charles, MO), following the manufacturer's protocol [14]. Serum adiponectin was measured using a human total adiponectin ELISA kit (Otsuka Pharmaceutical, Tokyo, Japan) [16] and a human HMW adiponectin ELISA Kit (Fuji Rebio, Tokyo, Japan) [21].

2.4. SNP typing

Polymerase chain reaction direct sequencing was performed as described previously [14,27]. To type SNP −420, sequences of minus strands were checked using the pr1R primer.

2.5. Statistical analysis

Potential regulators of serum resistin concentrations in the fasted state were examined using simple regression analysis. Fasting serum resistin level was included in the regression model as the dependent variable; and either age, sex, body mass index (BMI), fasting plasma glucose (FPG), glycated

hemoglobin (HbA_{1c}), fasting plasma immunoreactive insulin (F-IRI), homeostasis model assessment insulin resistance index (HOMA-IR), SNP −420, WBC count, hsCRP, or creatinine was entered as the independent variable. Multiple regression analysis was then performed involving fasting serum resistin level as a dependent variable and age, sex, BMI, and significant factors in simple regression analyses as independent variables. Simple regression analysis was also used to explore potential determinants of the reduction in serum resistin observed during the OGTT and MTT. The reduction in serum resistin was defined by serum resistin after loading (120 minutes for OGTT or 240 minutes for MTT) minus that before loading (in nanograms per milliliter, absolute value). Change in serum resistin from the preload condition was included as the dependent variable; and either age, sex, BMI, FPG, HbA_{1c}, F-IRI, HOMA-IR, SNP −420, or resistin before loading was entered as the independent variable. Multiple regression analysis was performed involving the reduction in serum resistin level during OGTT or MTT as a dependent variable and age, sex, BMI, and resistin before loading as independent variables. One-way repeated-measures analysis of variance (ANOVA) was used to assess significant differences in serum resistin and adiponectin during OGTT and MTT, and Scheffe test was used for post hoc analyses. Bonferroni correction was applied to simple regression analyses of the reduction in serum resistin (raw P value × 9) and those of fasting serum resistin (raw P value × 11). The correction was not applied to the subsequent multiple regression analyses using significant factors of these initial analyses. Student t test was used where indicated. Null hypotheses were rejected at $P < .05$. All analyses were performed with SAS 9.1 for Windows (SAS Institute, Cary, NC) except for 1-way repeated-measures ANOVA, which was performed with StatView version 5 (SAS).

3. Results

3.1. Serum resistin, but not adiponectin, was decreased at 60 and 120 minutes during the 75-g OGTT

An OGTT was used to determine whether glucose loading affects serum resistin or adiponectin levels in humans (Fig. 1). Serum resistin was significantly different among the time points assessed (1-way repeated-measures ANOVA, $F = 10.397$, $P < .0001$). Serum resistin was decreased at 60 and 120 minutes during the OGTT (absolute reduction values compared with that before loading, Scheffe test, −2.2 ng/mL at 60 minutes, $P = .0457$, and −4.3 ng/mL at 120 minutes, $P < .0001$). Serum total or HMW adiponectin

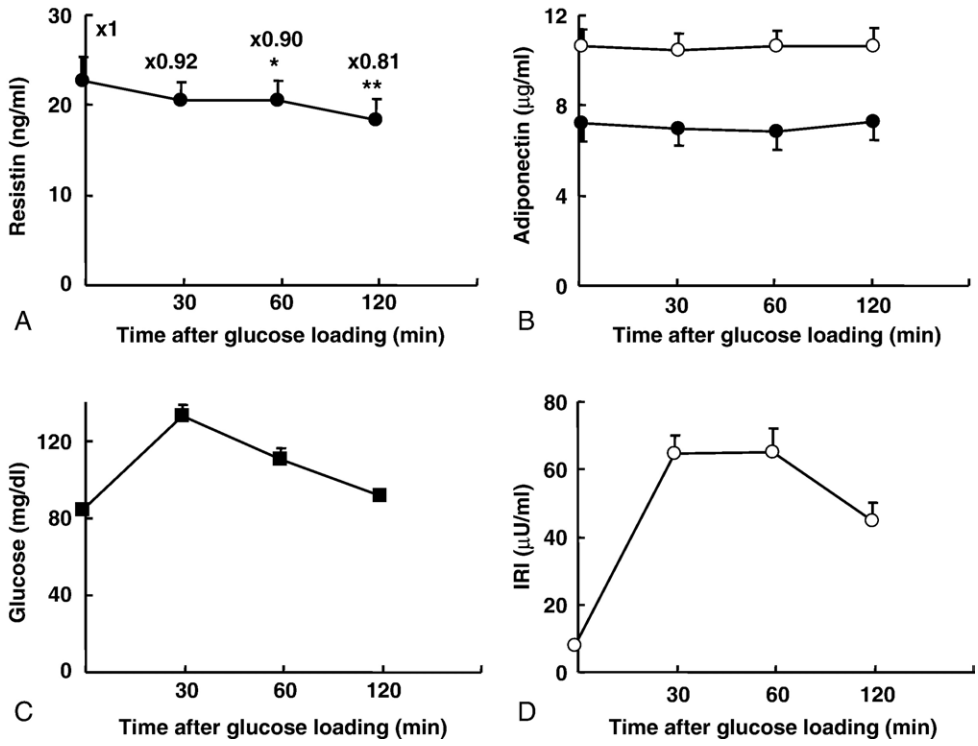

Fig. 1. Serum resistin was decreased during 75-g OGTT. The OGTT was performed as described in Subjects and methods (n = 36). Data are mean ± SE. A, Serum resistin. Relative values of serum resistin are also shown as fold induction (×) at each time point compared with 0 minute. One-way repeated-measures ANOVA: $F = 10.397$, $P < .0001$. Scheffe test: *$P = .0457$ at 60 minutes compared with 0 minute; **$P < .0001$ at 120 minutes compared with 0 minute. B, Serum total adiponectin (open circle) and HMW adiponectin (closed circle). No significant changes were found in serum adiponectin levels during OGTT. One-way repeated-measures ANOVA—total: $F = 1.264$, $P = .2905$; HMW: $F = 1.861$, $P = .1408$. C, Plasma glucose. D, Plasma immunoreactive insulin. IR indicates immunoreactive insulin.

levels were unchanged during the OGTT (total: $F = 1.264$, $P = .2905$; HMW: $F = 1.861$, $P = .1408$).

3.2. Serum resistin, but not adiponectin, was decreased at 240 minutes during MTT

The effects of a mixed meal on serum resistin and adiponectin were examined using an MTT (Fig. 2). Serum resistin was significantly different among the time points assessed (1-way repeated measures ANOVA, $F = 7.888$, $P < .0001$). Serum resistin was decreased at 240 minutes (absolute reduction values compared with that before loading, Scheffe test, −5.3 ng/mL at 240 minutes, $P < .0002$). Total or HMW adiponectin levels remained unchanged during the MTT (total: $F = 1.553$, $P = .1908$; HMW: $F = 0.216$, $P = .9290$).

3.3. The reduction in serum resistin was correlated with its levels before loading during the OGTT and MTT

Simple regression analysis was used to explore potential determinants of reduction in serum resistin during the OGTT and MTT (Supplementary Table 1). The reduction in serum resistin level (after loading at 120 minutes for OGTT or 240 minutes for MTT minus before loading) was correlated with baseline resistin concentration (OGTT: unstandardized regression coefficient (β) = −0.18, $P = .0007$, corrected $P = .0063$; MTT: $\beta = -0.63$, $P < .0001$, corrected $P < .0009$). The negative regression coefficients indicate that the reduction in serum resistin after loading was more pronounced in participants with greater fasting serum resistin concentrations. The relations between reduction in resistin and baseline resistin levels remained significant after adjusting for age, sex, and BMI (OGTT: $\beta = -0.19$, $P = .0005$; MTT: $\beta = -0.63$, $P < .0001$) (Table 2). Neither age, sex, BMI, FPG, HbA_{1c}, F-IRI, HOMA-IR, nor SNP −420 was associated with change in resistin.

3.4. Fasting serum resistin was positively correlated with the G allele number of SNP −420 and WBC count

Potential regulators of serum resistin concentrations in the fasted state were examined using simple regression analysis, which includes age, sex, BMI, FPG, HbA_{1c}, F-IRI, HOMA-IR, SNP −420, WBC count (mean ± SD, 5388 ± 1353; range, 3400-8900/μL), hsCRP (0.13 ± 0.33, 0.02-1.42 mg/dL), or creatinine (0.76 ± 0.14, 0.5-1.2 mg/dL) as the independent variable (Supplementary Table 2). Fasting resistin was associated with the number of G alleles of SNP −420 ($\beta = 9.92$, $P = .007$, corrected $P = .077$, suggesting at least tendency) and WBC count ($\beta = 0.007$, $P = .0001$, corrected

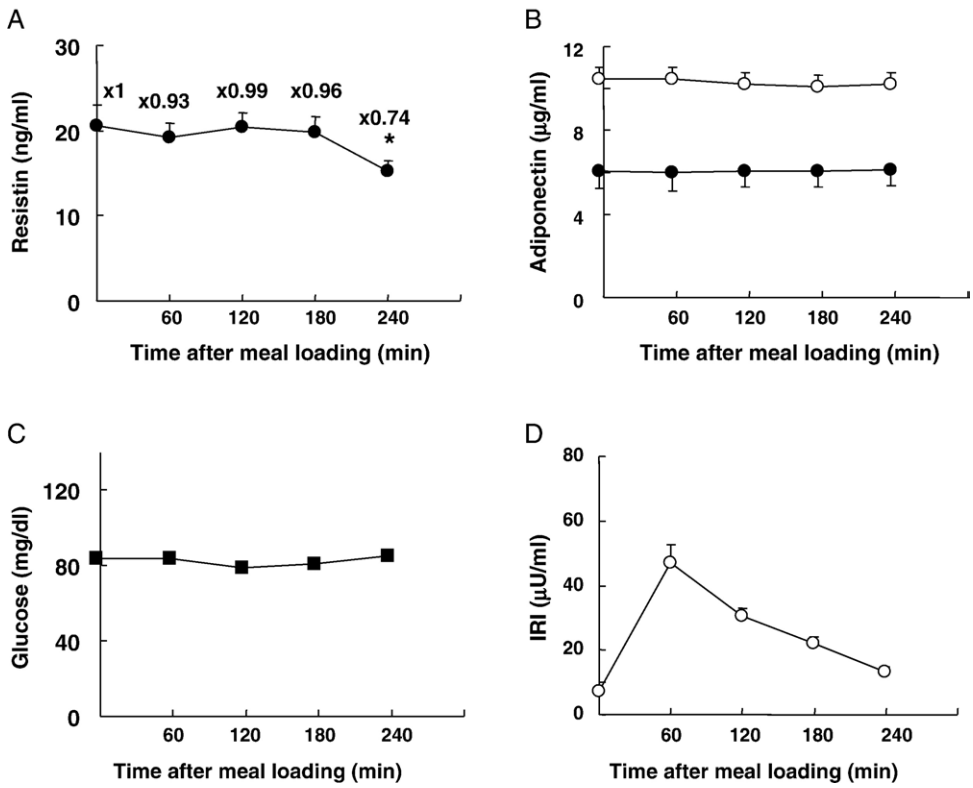

Fig. 2. Serum resistin was decreased during MTT. The MTT (750-kcal mixed meal) was performed as described in Subjects and methods (n = 33). Data are mean ± SE. A, Serum resistin. Relative values of serum resistin are also shown as fold induction (×) at each time point compared with 0 minute. One-way repeated-measures ANOVA: $F = 7.888$, $P < .0001$. Scheffe test: *$P = .0002$ at 240 minutes compared with 0 minute. B, Serum total adiponectin (open circle) and HMW adiponectin (closed circle). No significant changes were found in serum adiponectin levels during MTT. One-way repeated-measures ANOVA—total: $F = 1.553$, $P = .1908$; HMW: $F = 0.216$, $P = .9290$. C, Plasma glucose. D, Plasma immunoreactive insulin.

Table 2
The reduction in serum resistin was correlated with serum resistin levels before loading during OGTT and MTT in multiple regression analysis (dependent variable: the reduction in serum resistin [in nanograms per milliliter])

Independent variables	OGTT (120-0 min)				MTT (240-0 min)			
	Unstandardized regression coefficient	Standard error	Standardized regression coefficient	*P*	Unstandardized regression coefficient	Standard error	Standardized regression coefficient	*P*
Age (y)	−0.15	0.16	−0.13	.36	−0.4	0.30	−0.11	.19
Sex (female)	1.17	1.80	0.11	.52	4.36	1.90	0.22	.03
BMI	1.16	0.53	0.37	.04	0.50	0.59	0.08	.40
Resistin before loading (ng/mL)	−0.19	0.05	−0.55	.0005	−0.63	0.06	−0.88	<.0001

Multiple regression analysis was performed involving the reduction in serum resistin (serum resistin after loading at 120 minutes for OGTT or 240 minutes for MTT minus before loading) (in nanograms per milliliter) as a dependent variable and age, sex, BMI, or resistin before loading as independent variables as described in Subjects and methods. The reduction in serum resistin is defined by serum resistin after loading minus that before loading (in nanograms per milliliter, absolute value). The negative regression coefficients indicate that the reduction in serum resistin after loading was more pronounced in participants with greater fasting serum resistin concentrations. Sex: male = 1, female = 2.

$P = .0011$) (Fig. 3). Multiple regression analysis adjusted for age, sex, and BMI also showed that fasting serum resistin was positively correlated with the G allele number of SNP −420 ($\beta = 7.70$, $P = .01$) and WBC count ($\beta = 0.007$, $P = .0001$) (Table 3). From all these findings, serum resistin levels were decreased during the OGTT and MTT in humans. The reduction in serum resistin was correlated with fasting serum resistin levels associated with WBC count as well as SNP −420.

4. Discussion

The results of this study demonstrate that serum resistin, but not total or HMW adiponectin, levels were decreased during OGTT and MTT in healthy humans. Simple regression analysis revealed that the reduction in serum resistin level was correlated with the level before loading. Fasting serum resistin levels were positively correlated with WBC count as well as the G allele number of SNP −420.

The findings herein show that fasting serum resistin was associated with SNP −420 and WBC count. We previously reported that fasting serum resistin was highest in controls, subjects with type 2 diabetes mellitus, and the Japanese general population with the G/G genotype, followed by C/G and C/C [4,13,14,28]. Monocyte resistin mRNA was positively correlated with its simultaneous serum levels in healthy volunteers and was highest in individuals with the G/G genotype [13]. Kunnari et al [23] have shown that plasma resistin levels are positively associated with WBC count. Therefore, fasting serum resistin levels appear to be determined by at least 2 factors: genetics, that is, SNP −420 genotype, and the cellular source of resistin in humans, that is, WBC, including monocytes.

In this human study, serum resistin was decreased by OGTT and MTT. The opposite response of serum resistin to feeding has been reported in mice [8,29]. In mice, serum resistin is increased by meal feeding or hyperglycemia, whereas it is decreased by an overnight fast [8]. Serum resistin and resistin mRNA in adipose tissues are increased in response to hyperglycemia and/or hyperinsulinemia in clamp studies [8]. These findings suggest that the regulation of serum resistin levels by glucose and/or insulin differs between humans and rodents. The differential tissue distribution of resistin gene expression could account for

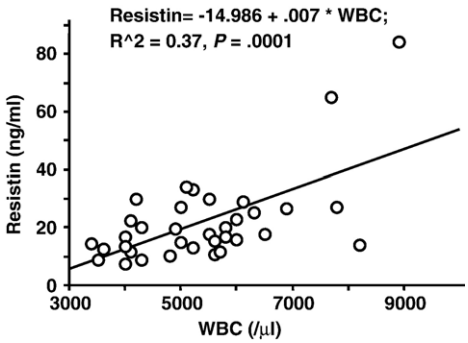

Fig. 3. Fasting serum resistin was positively correlated with its simultaneous WBC count. Fasting serum resistin levels were measured as described in Subjects and methods and compared with WBC count at the time of sampling. Simple regression analysis was used for statistical analysis. Fasting serum resistin level (in nanograms per milliliter) = −15.0 + 0.007 × WBC (per microliter), $R^2 = 0.37$, $P = .0001$.

Table 3
Fasting serum resistin was correlated with SNP −420 and WBC count in multiple regression analysis (dependent variable: fasting serum resistin [in nanograms per milliliter])

Independent variables	Unstandardized regression coefficient	Standard error	Standardized regression coefficient	*P*
Age (y)	0.005	0.42	0.001	.99
Sex (female)	6.88	5.04	0.22	.18
BMI	−0.32	1.42	−0.04	.82
SNP −420	7.70	3.0	0.35	.01
WBC count (/μL)	0.007	0.002	0.62	.0001

Multiple regression analysis was performed involving fasting serum resistin (in nanograms per milliliter) as a dependent variable and age, sex, BMI, SNP −420, or WBC count as independent variables as described in Subjects and methods. Sex: male = 1, female = 2; SNP −420: C/C = 1, C/G = 2, G/G = 3.

this. Resistin is most highly expressed in adipocytes in mice [1,2], whereas the main source of resistin appears to be monocytes and macrophages in humans [5-7].

To our knowledge, the effect of glucose loading on serum resistin in healthy human subjects has not been previously reported. In 19 female subjects with polycystic ovary syndrome (PCOS), serum resistin levels are not affected during 75-g OGTT [30]. Because PCOS patients have profound insulin resistance, they may respond differently from healthy volunteers. In contrast to the results of the present study, Gruendel et al [31] recently reported that serum resistin is increased at 1, 3, 4, and 5 but not 2 hours after the consumption of a standardized isocaloric liquid meal (600 kcal; protein, 24 g; fat, 23.2 g; carbohydrate, 73.6 g; no fiber) in 19 healthy subjects. The meal used in the present study had a greater energy, fat, and fiber content, which could account for the discrepant results. Body mass index could also affect serum resistin response because subjects reported by Gruendel et al had higher BMI (23.0) than those in the present study (20.4). In obesity, macrophages are known to infiltrate into adipose tissues [32], which could respond differently from circulating monocytes. In another report, serum resistin levels are unchanged after 48 hours of fasting in healthy subjects [9]. Changes in serum resistin by glucose and meal loading should be assessed over a prolonged time course and should also take energy, macronutrient content, and BMI into account.

A reduction in resistin secretion or resistin gene expression in WBC is one possible mechanism for the reduction in serum resistin induced by glucose and meal loading. Both glucose loading and meal loading alter blood glucose and insulin concentrations. Glucose loading is thought to have a stronger effect on both glucose and insulin levels than meal loading. In fact, in this study, serum resistin was decreased 2 hours earlier in the OGTT than in the MTT. In 3T3-L1 adipocytes, glucose increases resistin mRNA [33], which is in agreement with findings for rodents but not for humans. Conversely, insulin decreases resistin mRNA in these cells [33,34], which could explain findings for humans but not for rodents.

Another mechanism for the reduction in serum resistin by glucose and meal loading may involve WBC. Data related to changes in WBC count during glucose or meal loading are conflicting in healthy human subjects. White blood cell count has been reported to decrease during 75-g OGTT [35]. In contrast, neutrophil and lymphocyte count is increased by high-fat meal loading [36]. Neutrophil count is increased but lymphocyte count is decreased by a standardized high-protein mixed-meal load [37]. Although WBC count only was available before the glucose and meal loading in the present study, the time course for the WBC count and changes in specific WBC subpopulations such as neutrophils and lymphocytes merit further investigation.

Our findings show that subjects with higher fasting serum resistin levels had a greater reduction in resistin after glucose or meal loading. We recently found that circulating resistin was positively correlated with homeostasis model assessment insulin resistance index (HOMA-IR) in 2078 subjects in the Japanese general population [28]. We also reported that serum resistin was inversely correlated with insulin sensitivity index assessed by the minimal model in subjects with type 2 diabetes mellitus [38]. Thus, resistin may also reduce insulin sensitivity in humans. The decrease in resistin levels after a meal or glucose load may improve insulin sensitivity in the postprandial state. It should be noted that the change in serum resistin by glucose or meal load observed in healthy volunteers in the present study was small. This pathophysiological relevance should be further investigated in a larger number of subjects including those with impaired glucose tolerance or type 2 diabetes mellitus.

We found that neither total nor HMW adiponectin levels were affected by OGTT or MTT. Effects of meal or glucose loading on serum adiponectin levels appear to be dependent on a variety of factors in humans. An overall increase in plasma adiponectin, between 0 and 120 minutes after an OGTT, has been reported in subjects with PCOS [30]. It has been shown that postprandial adiponectin levels are increased in obese, but not in lean, subjects [39]. Peake et al [40] have reported that plasma adiponectin is not affected by a high-fat meal in either normal individuals or patients with type 2 diabetes mellitus. Differences in age, sex, BMI, disease, or the test meal among studies may account for the discrepant findings.

In summary, this study demonstrated that serum resistin, but not adiponectin, was decreased during OGTT and MTT. The reduction in serum resistin level was greater in participants with higher fasting resistin concentrations. Fasting resistin levels were positively correlated with the G allele number of SNP −420 and with WBC count. Why and how serum resistin levels are regulated by glucose and meal loading remain unknown. Further studies are required to clarify these points.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.metabol.2007.08.018.

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