

## Associations of Adiponectin, Resistin, and Tumor Necrosis Factor- $\alpha$ with Insulin Resistance

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**Context:** Adipose tissue-derived adipokines may contribute to insulin resistance.

**Objective:** We tested the hypothesis that adipokines are associated with insulin resistance in a community-based cohort and that associations are maintained in people with and without the metabolic syndrome (high vs. low risk of diabetes).

**Design, Setting, and Participants:** We studied a cross-sectional sample of 2356 individuals attending the seventh examination (1998–2001) of the Framingham Offspring Study. We measured levels of glucose, insulin, adiponectin, resistin, and TNF $\alpha$  in fasting blood samples and defined metabolic syndrome by updated National Cholesterol Education Program criteria. We used ANOVA to test associations of adipokines with insulin resistance and multivariable logistic regression models to assess joint associations of adipokines and metabolic syndrome with insulin resistance.

**Main Outcome Measure:** Homeostasis model (HOMA-IR), with insulin resistance defined by HOMA-IR greater than the 75th percentile, was measured.

**Results:** Age- and sex-adjusted HOMA-IR levels were inversely related to adiponectin ( $r = -0.40$ ,  $P < 0.0001$ ) and positively related to resistin ( $r = 0.13$ ,  $P < 0.0001$ ) and TNF $\alpha$  ( $r = 0.12$ ,  $P < 0.0001$ ). The prevalence of insulin resistance increased with decreasing tertiles of adiponectin (from 10.9% in the third to 42.5% in the first tertile;  $P < 0.0001$ ) and increasing tertiles of resistin (from 19.3 to 30.9%;  $P < 0.0001$ ) and TNF $\alpha$  (from 18.8 to 32.0%;  $P < 0.0001$ ). Results were similar after adjustment for body mass index. These associations were present in individuals with or without the metabolic syndrome. In multivariable regression models, metabolic syndrome and adipokines individually and jointly were significantly associated with insulin resistance.

**Conclusion:** Adverse levels of adipokines are associated with insulin resistance in individuals at low or high diabetes risk. (*J Clin Endocrinol Metab* 93: 3165–3172, 2008)

Obesity and diabetes are epidemic worldwide (1). Adipose tissue is now recognized as an endocrine organ that contributes to the pathophysiology of type 2 diabetes. Adipokines, proteins produced by adipose tissue, have been identified as potential contributors to insulin resistance in humans (2). Over the

past few years, emerging evidence has shown that adipokines are produced both by adipocytes and macrophages in human adipose tissue and that diverse paracrine and autocrine pathways are involved in their regulation (2). To capture these interrelations, we selected a set of key adipokines representing these ad-

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Abbreviations: BMI, Body mass index; FPG, fasting plasma glucose; HOMA-IR, homeostasis model insulin resistance; IFG, impaired fasting glucose; NGT, normal glucose tolerance.

ipose tissue cell types, including adipokines produced primarily by adipocytes (adiponectin), macrophages (resistin), or both (TNF $\alpha$ ). Plasma levels of adiponectin, an antiinflammatory, antidiabetic hormone, are inversely related to central adiposity and insulin resistance (3, 4). Adiponectin is produced by differentiated adipocytes and circulates at high levels in the bloodstream (2). Low levels of circulating adiponectin levels are associated with a higher risk of future type 2 diabetes (5, 6). Resistin is a 12-Da polypeptide that was initially linked to insulin resistance in animal models (7). Results in human have been inconsistent, showing positive associations in some studies (8, 9) but not in others (10, 11). In human adipose tissue, resistin seems to be produced mainly by infiltrating macrophages (12). Circulating resistin is positively related to adiposity and may be implicated in proinflammatory signaling associated with excess adiposity (13). Within the adipose tissue, TNF $\alpha$  is a cytokine secreted by both adipocytes and macrophages (2). It may participate in the inflammatory reaction that links central adiposity to insulin resistance (14). Controversy about the role of TNF $\alpha$  in insulin resistance has been raised by inconsistent results in human studies, some finding no association (15, 16) whereas others have (17, 18).

Inconsistency in human data may be due in part to study of small samples of highly selected subjects and lack of assessment of several adipokines simultaneously. Therefore, the role and interrelations of adipokines in the pathophysiology of insulin resistance in community-based samples has not been fully characterized. It is unknown whether associations of adipokines with insulin resistance in humans are modified by the presence or absence of prediabetes defined by conditions such as metabolic syndrome or impaired fasting glucose (IFG). Comprehensive, simultaneous analysis of several adipokines among individuals at low or high risk for type 2 diabetes is needed to clarify the magnitude and significance of the role of adipokines in human insulin resistance.

Therefore, the aim of this study was to test in a large unselected sample the following hypotheses: 1) levels of adiponectin, resistin, and TNF $\alpha$  are associated with insulin resistance measured by the homeostasis model insulin resistance (HOMA-IR) without and with adjustment for body mass index (BMI); 2) adipokine-insulin resistance associations are maintained in individuals at low or high risk of developing type 2 diabetes (defined here by the absence or presence the metabolic syndrome or IFG); and 3) adipokine levels are associated with insulin resistance individually and simultaneously in multivariable models including prediabetes status.

## Subjects and Methods

### Study participants

The Framingham Offspring Study is a community-based study of cardiovascular disease risk factors (19). The study began in 1971 with the enrollment of 5124 people (mainly Caucasian) who were the children of the original Framingham Heart Study cohort, and the spouses of the children. During the seventh examination cycle (1998–2001;  $n = 3539$ ), participants provided fasting blood samples, and had a standardized medical examination. A total of 2356 subjects provided data for the present analysis, after exclusion of those with prevalent diabetes ( $n =$

389), missing values for the diagnosis of metabolic syndrome ( $n = 311$ ), or levels of adipokines ( $n = 483$ ). Individuals with *vs.* without missing values were similar in age, sex, and mean BMI distribution. The study protocol was approved by the Institutional Review Boards of the Boston University School of Medicine and the Massachusetts General Hospital; all the participants provided written informed consent.

### Exposure and outcome definitions

The primary dependent variable was insulin resistance, measured using the homeostasis model [HOMA-IR, calculated by (fasting glucose  $\times$  fasting insulin)/22.5] (20). We assessed insulin resistance both as a continuous and a categorical outcome, defining the state of insulin resistance for the individuals in the top quartile of the HOMA-IR distribution (21). The primary independent variables were fasting plasma levels of adiponectin, resistin, and TNF $\alpha$ . Total adiponectin, resistin, and high-sensitivity TNF $\alpha$  were measured by ELISA (R&D Systems, Minneapolis, MN). Laboratory methods for glucose, insulin, and lipid assays have been published (22). Fasting plasma glucose (FPG) was measured immediately with a hexokinase reagent kit (A-gent glucose test, Abbott, South Pasadena, CA), and other plasma samples were frozen at  $-80$  C until assay. Fasting plasma insulin was measured with a human-specific insulin assay having essentially no cross-reactivity to proinsulin or insulin split-products (Linco Inc., St. Louis, MO). Intraassay coefficients of variation were less than 3% for glucose, 6.1% for insulin, 5.8% for adiponectin, 9.0% for resistin, and 6.6% for TNF $\alpha$ .

Other covariates included standardized measurements cardiovascular risk factors. We measured height, weight, and waist circumference (at the umbilicus) with the subject standing. We calculated BMI as weight in kilograms divided by the square of height in meters. We used blood pressure as the mean of two measurements after the subject had been seated for at least 5 min. We classified people with prediabetes as having metabolic syndrome, or alternatively, with IFG. Metabolic syndrome was defined using the 2005 updated Third Report of the National Cholesterol Education Program's Adult Treatment Panel criteria, as any three or more of: FPG, 5.6–6.9 mmol/liter; waist circumference, 102 cm or greater (in men) or 88 cm or greater (in women); fasting triglycerides 1.7 mmol/liter or greater; high-density lipoprotein-cholesterol less than 1.0 mmol/liter (in men) or less than 1.3 mmol/liter (in women); and blood pressure 130/85 mm Hg or greater or treatment for hypertension (23). IFG was defined as FPG from 5.6 to 6.9 mmol/liter inclusively, and diabetes was defined by a FPG level above 7.0 mmol/liter or current use of hypoglycemic drugs (24).

### Statistical analysis

Means and SDs are presented for continuous percentages for categorical characteristics. The *t* tests and  $\chi^2$  tests were used to compare continuous and categorical baseline characteristics of individuals with and without metabolic syndrome. To compare adipokine levels with HOMA-IR as continuously distributed covariates, Spearman correlations, age-sex-adjusted linear regression, and scatter plot analysis were used. To test associations with prevalence of insulin resistance, we categorized participants into sex-specific tertiles of the distribution of each adipokine. The  $\chi^2$  tests of trend were used to assess differences in insulin resistance prevalence across adipokine tertiles. We used metabolic syndrome as the primary prediabetes phenotype and used the same approach for association analyses stratified by presence or absence of metabolic syndrome. We tested the interaction of metabolic syndrome on the association of adipokine level with insulin resistance using first-order (metabolic syndrome by adipokine) interaction terms in linear or logistic regression models for the continuous and dichotomous parameterizations of insulin resistance. Logistic regression analysis related insulin resistance to metabolic syndrome (presence *vs.* absence) and/or tertiles of each adipokine modeled as ordinal (0, 1, 2) variables. We developed models by considering first the association of metabolic syndrome and adipokines individually and then in combination, with the fully specified model including metabolic syndrome and all three adipokines. Analyses were age-sex adjusted and then further adjusted for BMI.

**TABLE 1.** Characteristics of 2356 Framingham Offspring Study participants, overall and stratified by metabolic syndrome status

	Overall		No metabolic syndrome		Metabolic syndrome		P value <sup>a</sup>
n (%)	2356		1373 (58%)		983 (42%)		
Age (mean yr, sd)	60	9.5	59	9.6	62	9.0	<0.0001
Women, %	55.0		58.1		49.8		<0.0001
BMI (mean kg/m <sup>2</sup> , sd)	27.8	5.0	25.9	4.1	30.4	5.0	<0.0001
HOMA-IR (mean U, sd)	3.3	1.8	2.6	1.6	4.6	1.7	<0.0001
Insulin resistance (%)	25.0		8.8		47.5		<0.0001
Adiponectin (mean μg/ml, sd)	8.75	1.85	10.2	1.79	7.04	1.82	<0.0001
Resistin (mean ng/ml, sd)	12.8	1.49	12.3	1.48	13.6	1.50	<0.0001
TNFα (mean pg/ml, sd)	1.25	1.63	1.18	1.61	1.34	1.65	<0.0001

<sup>a</sup> P values are for comparisons between groups with and without metabolic syndrome.

We performed subsidiary analyses to assess assumptions made in the main analyses. Insulin resistance was defined, by convention, using the 75th percentile. We recently reported that using a HOMA-IR threshold around the 92nd percentile provides better specificity (>90%) than the 75th percentile to predict type 2 diabetes (25). Therefore, we repeated the analyses using the 90th percentile of HOMA-IR to define insulin resistance. Prediabetes may be defined in several ways. Prior data show metabolic syndrome to be a powerful predictor of diabetes beyond IFG alone (26). Therefore, we used metabolic syndrome to define the prediabetic state in our main analysis, but we also conducted the analysis using IFG. In another subsidiary analysis, we used waist circumference instead of BMI to adjust for adiposity. Next, because adipokine levels differ between male and female, interactions between sex and each individual adipokine on the level of insulin resistance were tested in the age-sex-BMI adjusted models using first-order sex-by-adipokine interaction terms. Finally, we conducted the analysis for the original individual adipokine models adjusting further for hypertensive and dyslipidemic medications. We considered  $P < 0.05$  to indicate statistical significance. We performed all analyses using SAS software (version 8.1; SAS Institute, Cary, NC).

## Results

Characteristics of the study subjects are displayed in Table 1. By definition, 25% of subjects were classified as having insulin resistance. Metabolic syndrome criteria were fulfilled by 42% of subjects; those with metabolic syndrome were older, had a higher mean BMI, were more likely to be men, had higher HOMA-IR values, and were more likely to be classified as insulin resistant. Among the individuals with metabolic syndrome, 47.5% were classified as insulin resistant. In those with insulin resistance, 79.6% fulfilled the metabolic syndrome criteria. In the overall cohort, 31% were using antihypertensive medication, whereas 17% were taking drugs for lipid disorders; in individuals with metabolic syndrome the proportions were 50 and 32%, respectively. Subjects with metabolic syndrome had lower mean levels of adiponectin and higher levels of resistin and TNFα than subjects without metabolic syndrome.

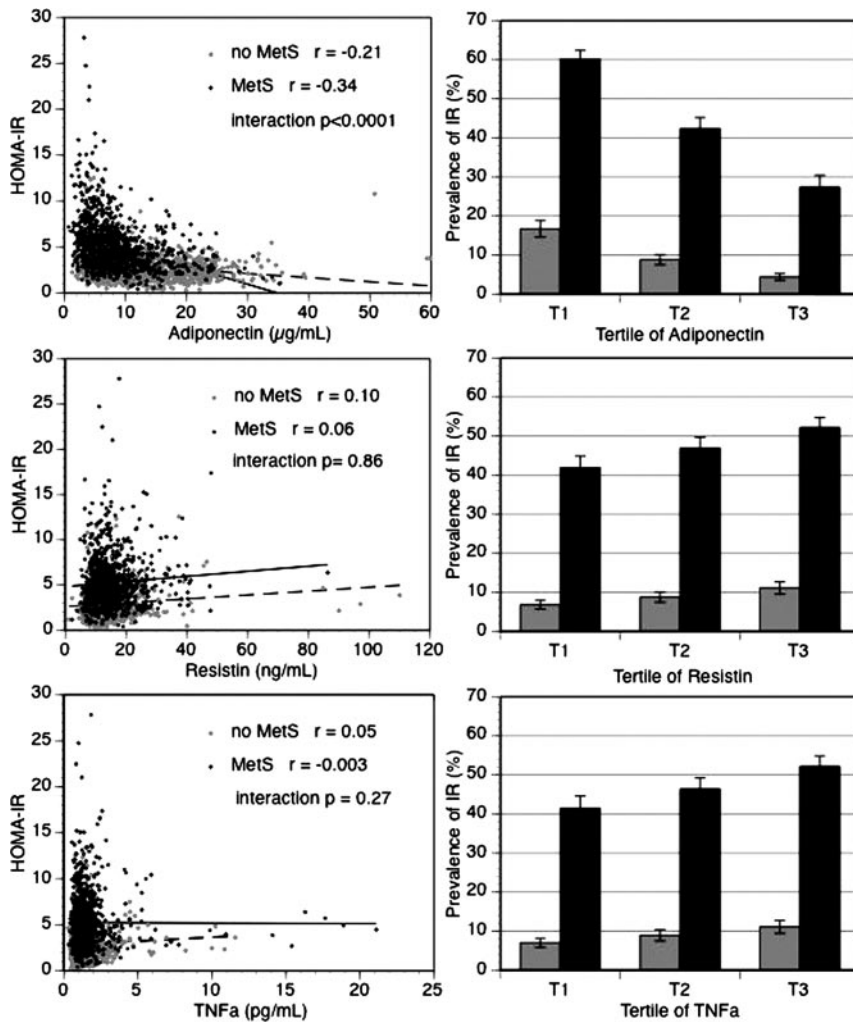
Each component of the metabolic syndrome, age, and BMI were all significantly associated with HOMA-IR in Spearman correlations (all  $P < 0.0001$ ). In age-sex adjusted analysis, with variables as continuous, adiponectin was inversely related to HOMA-IR ( $r = -0.40$ ,  $P < 0.0001$ ), whereas resistin ( $r = 0.13$ ,  $P < 0.0001$ ) and TNFα ( $r = 0.12$ ,  $P < 0.0001$ ) were positively related.

We tested associations between increasing tertile of each adi-

pokine and the prevalence of insulin resistance. The prevalence of insulin resistance decreased with increasing level of adiponectin, with a prevalence of 43.8% in the lowest tertile, 22.0% in the middle, and 10.1% in the highest tertile ( $P < 0.0001$  for trend). The prevalence of insulin resistance increased positively with increasing level of resistin (18.7, 26.3, and 29.8% in ascending tertiles,  $P < 0.0001$ ) and TNF-α (18.7, 25.7, and 31.5% in ascending tertiles,  $P < 0.0001$ ). The same analyses were carried out with age, sex, and BMI adjustment with similar results ( $P$  values for trends  $< 0.0001$  for adiponectin, 0.03 for resistin, and 0.0002 for TNFα). Adjusting for adiposity with waist circumference instead of BMI resulted in similar trends and  $P$  values ( $P$  values for trends  $< 0.0001$  for adiponectin, 0.03 for resistin, and 0.0001 for TNFα).

The distributions of HOMA-IR and each adipokine according to the presence or absence of metabolic syndrome are illustrated in Fig. 1 (*left hand column*). In the adiponectin scatter plot in particular, we noticed that few individuals without the metabolic syndrome appear in the high range of HOMA-IR; likewise, few individuals with the metabolic syndrome are in the high range of adiponectin. The negative correlation between adiponectin levels and HOMA-IR was stronger in the group with metabolic syndrome ( $r = -0.34$  with metabolic syndrome *vs.*  $r = -0.21$  without metabolic syndrome, interaction  $P < 0.0001$ ). In the resistin and TNFα scatter plots, stratifying the group with and without the metabolic syndrome reduced the correlations between adipokines and insulin resistance, but in both cases, there was no significant interaction by metabolic syndrome on the adipokine-insulin resistance association ( $P > 0.2$  for interaction).

The prevalence of insulin resistance by level of adipokines and stratified by presence or absence of the metabolic syndrome is shown in Fig. 1 (*right hand column*). In each panel, the presence of metabolic syndrome was associated with a higher prevalence of insulin resistance. The prevalence of insulin resistance decreased with increasing level of adiponectin in individual with or without metabolic syndrome ( $P < 0.0001$  for trends in both groups). In the resistin and TNFα panels, the prevalence of insulin resistance increased with increasing adipokine level in people with or without metabolic syndrome ( $P < 0.01$  for trends in all categories). For all three adipokines, these associations were similar comparing groups with *vs.* without metabolic syndrome (interaction  $P > 0.3$ ).



**FIG. 1.** Adverse adipokine levels are associated with insulin resistance in individuals with metabolic syndrome (MetS) and without (no MetS). The left-hand column shows scatter plots (crude data) of plasma adiponectin (top), resistin (middle), and TNF- $\alpha$  (bottom) in relation to insulin resistance measured by HOMA-IR according to metabolic syndrome status (gray circle, no MetS; black circle, MetS; dashed line, no MetS; solid line, MetS). The right-hand column shows the prevalence (and ses) of insulin resistance (IR) (age-sex adjusted) defined by HOMA-IR greater than 75th percentile in relation to adipokines grouped into tertiles of increasing plasma concentration, according to metabolic syndrome status (light gray, no metabolic syndrome; dark gray, metabolic syndrome). For trends within metabolic syndrome categories,  $P < 0.0001$  for decreasing prevalence of insulin resistance across adiponectin tertiles in both groups; for resistin (middle) and TNF- $\alpha$  (bottom), all  $P < 0.01$  for trends in insulin resistance prevalence across tertiles in both groups.

Results of multivariable logistic regression modeling testing the hypothesis that adipokines levels are associated with insulin resistance are shown in Table 2. In individual age-sex adjusted models, metabolic syndrome and levels of each adipokine were significantly associated with insulin resistance. Further adjustment for BMI substantially decreased the magnitude of the insulin resistance association for metabolic syndrome (25% decrease in the size of the  $\beta$ -coefficient), resistin (52% decrease), and TNF $\alpha$  (23% decrease). The insulin resistance-adiponectin association appeared less affected by BMI adjustment (14% decrease). In models including the metabolic syndrome plus one adipokine, each adipokine remained significantly associated with insulin resistance, even when further adjusted for BMI. In the age-sex adjusted full model including metabolic syndrome and all three adipokines, all showed significant association with

insulin resistance. When this last model was further adjusted for BMI, resistin lost significant association with insulin resistance, whereas the associations with metabolic syndrome, adiponectin, and TNF $\alpha$  all remained statistically significant.

### Subsidiary analyses

By definition, when using the 90th percentile, the prevalence of insulin resistance was lower overall and in adipokine categories, but the significance of associations of adipokines and insulin resistance were the same as in the main analysis (see online supplemental Table 1, published as supplemental data on The Endocrine Society's Journals Online Web site at <http://jcem.endojournals.org>).

We also conducted the analysis using IFG (40% of the study sample) vs. normal glucose tolerance (NGT) to define prediabetes (see online supplemental Table 2 for complete results). Individuals with IFG were older (62 vs. 59 yr) and more likely to be male (58 vs. 37%). IFG presented lower adiponectin levels and higher resistin and TNF $\alpha$  levels (all  $P < 0.01$ ). The trends in HOMA-IR and in prevalence of insulin resistance across tertiles of adipokines were similar in individuals with NGT or with IFG.

We also conducted all multivariable models analysis using waist circumference to adjust for adiposity. Substituting waist circumference for BMI in all the models did not alter the results (see online supplemental Table 3). We also tested the presence of a sex interaction with each adipokine on risk of insulin resistance in the BMI-adjusted individual models; no significant interaction was detected ( $P = 0.09$ – $0.5$  for sex by adipokine interaction terms). Looking at the

individual adipokine models, we further adjusted for hypertensive and dyslipidemic medications. The results remained very similar (adiponectin,  $P < 0.0001$ ; resistin,  $P = 0.05$ ; TNF $\alpha$ ,  $P = 0.0002$ ) once adjusted for age-sex-BMI plus medications for both hypertension and dyslipidemia.

### Discussion

We have demonstrated that adverse levels of the adipokines adiponectin, resistin, and TNF $\alpha$  are associated with insulin resistance in a community-based cohort. This association persisted, albeit attenuated, after adjustment for BMI. Elevated levels of resistin and TNF $\alpha$  had an additive effect with metabolic syndrome, with higher levels of insulin resistance in those with met-



**TABLE 2.** Logistic regression models predicting insulin

	Adjusted for sex and age				Adjusted for sex, age, and BMI			
	Metabolic syndrome (yes vs. no) <sup>a</sup>	Adiponectin (per tertile) <sup>b</sup>	Resistin (per tertile)	TNF $\alpha$ (per tertile)	Metabolic syndrome (yes vs. no)	Adiponectin (per tertile)	Resistin (per tertile)	TNF $\alpha$ (per tertile)
	Individual models (metabolic syndrome or adipokines) <sup>c</sup>							
Coefficient <sup>d</sup>	2.26				1.69			
OR (95% CI)	9.62 (7.62–12.15)				5.43 (4.23–6.98)			
P value	<0.0001				<0.0001			
Coefficient		-1.01				-0.87		
OR (95% CI)		0.36 (0.32–0.42)				0.42 (0.36–0.49)		
P value		<0.0001				<0.0001		
Coefficient			0.31				0.15	
OR (95% CI)			1.36 (1.21–1.53)				1.16 (1.02–1.33)	
P value			<0.0001				0.03	
Coefficient				0.35				0.27
OR (95% CI)				1.42 (1.25–1.61)				1.32 (1.14–1.52)
P value				<0.0001				0.0002
	Metabolic syndrome + one adipokine model							
Coefficient	2.00				1.49			
OR (95% CI)	7.41 (5.82–9.43)				4.42 (3.41–5.72)			
P value	<0.0001				<0.0001			
Coefficient	2.25		0.26		1.69		0.16	
OR (95% CI)	9.49 (7.51–12.00)		1.30 (1.14–1.48)		5.44 (4.23–6.99)		1.17 (1.02–1.35)	
P value	<0.0001		0.0002		<0.0001		0.03	
Coefficient	2.22			0.28	1.67			0.24
OR (95% CI)	9.24 (7.22–11.81)			1.32 (1.15–1.52)	5.32 (4.09–6.92)			1.27 (1.09–1.48)
P value	<0.0001			0.001				0.002
	Full model							
Coefficient	1.97	-0.73	0.23	0.19	1.48	-0.70	0.13	0.17
OR (95% CI)	7.19 (5.57–9.27)	0.48 (0.41–0.56)	1.25 (1.09–1.45)	1.21 (1.04–1.40)	4.39 (3.35–5.76)	0.50 (0.42–0.58)	1.14 (0.98–1.33)	1.19 (1.01–1.39)
P value	<0.0001	<0.0001	0.002	0.01	<0.0001	<0.0001	0.09	0.03

Age, sex, and BMI were modeled as continuously distributed covariates.

<sup>a</sup> Odds ratios (OR) for metabolic syndrome are for subjects with metabolic syndrome relative to those without.

<sup>b</sup> Adipokine levels are modeled as ordinal variables (0, 1, 2) with regression coefficients and associated odds ratios (OR) and 95% confidence intervals (CI) expressed as risk per tertile increase. Age-sex adjusted mean adipokine values for each tertile were: adiponectin = 4.8, 8.9, and 15.8  $\mu\text{g}/\text{ml}$ ; resistin = 8.5, 12.6, and 19.7  $\text{ng}/\text{ml}$ ; TNF $\alpha$  = 0.78, 1.2 and 2.1  $\text{pg}/\text{ml}$ .

<sup>c</sup> Individual models are using each variable (metabolic syndrome or each adipokine levels by tertile) by itself to predict prevalence of insulin resistance, adjusted for age and sex, or age, sex, and BMI according to the models.

<sup>d</sup> Coefficients are the  $\beta$ -coefficients for each variable when included in the multivariable logistic regression analysis.

abolic syndrome at every level of adipokine. Metabolic syndrome appeared to modify the association of adiponectin with insulin resistance. Multivariable logistic regression analysis showed that metabolic syndrome and each of adiponectin, resistin, and TNF $\alpha$  made independent contributions to the presence of insulin resistance. By use of a large, unselected population sample and simultaneous measurement of type 2 diabetes risk factors and several major adipokines, we extend the current literature to show that multiple adipose-tissue derived signaling molecules are correlates of insulin resistance. Furthermore, these associations are not explained by the presence of other risk factors for type 2 diabetes and can be seen in groups at either low or high future risk of diabetes.

Our findings are consistent with the prior literature on adiponectin. Cross-sectional studies have shown that adiponectin was positively associated with insulin sensitivity measured by hyperinsulinemic clamp (4, 27), whereas prospective studies demonstrated that hypoadiponectinemia was associated with an increase in insulin resistance (28) and higher risk of developing diabetes (5, 6). Our data also confirm the association between low adiponectin and the presence of metabolic syndrome (29). We add to current knowledge by showing that adiponectin was associated with insulin resistance either in the presence or absence of elevated diabetes risk as embodied in metabolic syndrome. Furthermore, in individuals with metabolic syndrome, adiponectin seemed to have a stronger negative correlation with HOMA than in individuals without the metabolic syndrome. As we observed in Fig. 1 (*right hand panel*), the presence of metabolic syndrome was associated with a very high risk of insulin resistance, but being in the highest tertile of adiponectin decreased this risk by more than half, compared with the lowest tertile of adiponectinemia (prevalence of insulin resistance of 60.1% decreased to 27.3%). The multivariable models demonstrated that adiponectin was associated with insulin resistance, even when age, sex, BMI, metabolic syndrome, and the other adipokines were accounted for. Each increase in tertile of adiponectin is associated with a lower prevalence of insulin resistance by half (odds ratio 0.50). Those observations suggest that low adiponectin levels are related to insulin resistance above the usual clinical markers of prediabetes.

We demonstrated that TNF $\alpha$  is associated with insulin resistance in the community. TNF $\alpha$  has been suspected to be involved in the pathophysiology of obesity-induced insulin resistance based on animal models (30) and increased TNF $\alpha$  expression in obese human adipose tissue (14). Subsequently, conflicting results were reported relating circulating TNF $\alpha$  to insulin resistance in humans (15, 16). The small number of subjects in those studies might have limited their power to detect an association. Also, previous immunoassays might not have been sensitive enough to measure adequately the usual low circulating TNF $\alpha$  levels in healthy individuals. We used a high-sensitive assay to measure TNF $\alpha$  in the very low range (limit of detection as low as 0.06 pg/ml), which might have helped to detect the association that others have not seen. Our results are in concordance with smaller studies reporting that TNF $\alpha$  was related to insulin resistance measured by HOMA-IR (18) or insulin clamp (17, 31), and to metabolic syndrome status (29). We added to the current

knowledge by demonstrating that TNF $\alpha$  remained significantly associated with insulin resistance above metabolic syndrome status and even when adjusted for adiponectin, resistin, and BMI.

The association between resistin and insulin resistance in humans has been controversial. Many studies did not find an association between resistin and measures of insulin resistance (10, 11), whereas others found a relationship that was attenuated when adjusting for adiposity (8, 32). A lot of those studies included small numbers of participants, which limited their power. Our finding is in accordance with small studies showing a significant association between resistin and HOMA-IR (33, 34) and one recent large study in the general Japanese population (9). We extend these data by showing that elevated levels of resistin are associated with not only insulin resistance in the community but also remain associated with insulin resistance, even after accounting for metabolic syndrome and levels of adiponectin and TNF $\alpha$ . Only with simultaneous adjustment for all risk factors and BMI does the association weaken to not significant, although it can be argued that these fully specified models are overadjusted for adiposity because they include terms both for BMI and waist circumference (as part of the metabolic syndrome phenotype).

In all the models, having metabolic syndrome was associated with an increased prevalence of insulin resistance. Nevertheless, the crude data showed that more than half of the individuals with metabolic syndrome were not classified as insulin resistance. We hypothesized that this discrepancy might be partly explained by variability in adipokines. The full model suggests that even when identifying individuals at risk by the clinical definition of metabolic syndrome, the variability of insulin resistance can be explained by both antiinflammatory (adiponectin) and proinflammatory (TNF $\alpha$ ) pathways. Some authors have suggested that a paracrine loop exists in the adipose tissue by demonstrating that adipocytes cocultured with macrophages up-regulate proinflammatory cytokines (TNF $\alpha$ ) and in counterpart, those proinflammatory proteins down-regulate adiponectin expression (35). Some have proposed that the interrelations between the metabolic and immune pathways could be explained by resistin (13), whereas others have suggested TNF as the missing link (36). In adipose tissue, macrophages appear to be the main source of both resistin (37) and TNF $\alpha$  (35). Macrophages are primary mediators of immune response and represent up to 40% of infiltrating cells in adipose tissue of obese human (38). Also, both resistin and TNF $\alpha$  have been shown to impair insulin signaling in adipocytes and skeletal muscle (13, 36). Our findings argue in favor of the hypothesis that resistin and TNF $\alpha$  are both part of the complex system of adiposity-induced proinflammatory pathways that contribute to insulin resistance.

Strengths of this study include a large sample size from a representative community. Clinical measurements were taken under standardized protocol and biomarkers were measured using assays with good precision. We had adequate sample size to observe effects of the adipokines in tertiles and subgroups with or without the metabolic syndrome. Limitations of the study include the fact that we used surrogate markers for insulin resistance (HOMA-IR) and prediabetes (metabolic syndrome or IFG). Also, we measured total adiponectin and not high molec-

ular weight fraction, which has been proposed to have a stronger correlation with insulin resistance, compared with total adiponectin (39). All these limitations would have reduced our chance to observe significant associations. Furthermore, the cross-sectional design of our analyses does not allow us to make conclusions regarding causality. Finally, the Framingham cohort is largely white and middle-aged to elderly, so findings may have limited generalizability to other ethnic and age groups.

## Conclusion

Adverse levels of several key adipokines representing different adipose tissue cellular components are associated with insulin resistance in free-living people at low or high risk of future type 2 diabetes. The joint combination of metabolic syndrome and low adiponectin levels is associated with especially high levels of insulin resistance. The information gained from adipokines levels explained part of the insulin resistance variability above metabolic syndrome, but a large part of the variability of this association remains unexplained. The observations made in the multivariable models point toward the complexity of the anti- and proinflammatory pathways related to excess adiposity in free-living humans and reinforce the importance of analyzing several adipokines in analyses of the physiopathology of insulin resistance in humans.

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