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The association of tumor necrosis factor α receptor 2 and tumor necrosis factor α with insulin resistance and the influence of adipose tissue biomarkers in humans

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Abstract

Tumor necrosis factor α (TNFα) is a proinflammatory adipokine hypothesized to link obesity with insulin resistance. Functional studies suggest that TNFα acts through pathways involving adipokines and fatty acids to induce insulin resistance. We tested the hypothesis that the association of measures of TNFα activity with insulin resistance is independent of obesity and adipose tissue biomarkers. We analyzed data from 2131 participants (without diabetes) of the Framingham Offspring Study examination 7. The outcome of interest was insulin resistance, measured using the homeostasis model assessment (HOMA-IR). Tumor necrosis factor α activity was measured by plasma tumor necrosis factor α receptor 2 (TNFr2) or TNFα; possible confounders included adipose tissue biomarkers (plasma adiponectin, resistin, and triglycerides). We used multivariable age- and sex-adjusted linear regression analyses to adjust for waist circumference and for biomarkers individually and simultaneously, and in biomarker-stratified (above and below median) models. We found that TNFr2 was positively associated with HOMA-IR ($r = 0.21$, $P < .0001$). In age- and sex-adjusted model, for each increase of 1 standard deviation of TNFr2 (SD = 746 pg/mL), the log (HOMA-IR) value was increased by 0.11 units ($P < .0001$). Adjustment for waist circumference reduced the TNFr2 β-coefficient (by about 45%), but the association between TNFr2 and HOMA-IR remained significant ($P < .0001$). Tumor necrosis factor α receptor 2 was still associated to HOMA-IR after adding adiponectin, resistin, and triglycerides (individually and simultaneously). We found similar associations with plasma levels of TNFα. We conclude that, in a representative community sample, measures of TNFα activity are associated with insulin resistance, even after accounting for central adiposity and other adipose tissue biomarkers.

1. Introduction

Adipose tissue is recognized to be an energy storage tissue as well as an endocrine tissue that secretes proteins known as *adipokines*. It has been proposed that excess fat accumulation alters the pattern of adipokines secretion causing a low-grade proinflammatory state that induces

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insulin resistance and endothelial dysfunction, leading to diabetes and cardiovascular disease [1]. Tumor necrosis factor α (TNFα) is produced primarily by adipose-infiltrating macrophages [2] and was one of the first proteins to be identified as part of the adipose tissue inflammatory pathways [3]. In circulation, TNFα is bound to a number of circulating proteins, including the soluble form of TNFα receptor 2 (TNFr2) that might act as a buffer system (or slow-release reservoir) for TNFα [4]. Increased circulating levels of both TNFα [3,5] and TNFr2 [6,7] have been associated with markers of insulin resistance.

Based on in vitro data and animal studies, several pathways have been proposed to explain how TNFα could induce insulin resistance [2,5]. Tumor necrosis factor α decreases adipocyte production of adiponectin [8], an adipokine with anti-inflammatory and insulin-sensitizing properties [9]. Tumor necrosis factor α also induces the production of proinflammatory proteins including resistin, an adipokine secreted mainly by macrophages in human adipose tissue [10,11]. Another potential mechanism linking TNFα to insulin resistance is related to TNFα action on lipid regulation and adipocyte lipolysis. In mouse models fed a high-fat, high-calorie diet, TNFα knockout protects against the development of hyperinsulinemia [12], whereas rats given a TNFα infusion (causing insulin resistance) showed an increase in both circulating nonesterified fatty acids (NEFA) and triglycerides [13]. In fact, these diverse adipokine interrelations are likely to be all involved in the pathophysiology of insulin resistance.

Data linking TNFα or TNFr2, other adipose tissue–derived pathways, and insulin resistance have come primarily from in vitro, animal model, and small physiologic studies in humans; but there is less evidence that these relations operate at the population level. Using data from the seventh examination of the community-based Framingham Offspring Study, we have shown previously that TNFα is associated with insulin resistance, even after adjustment for adiposity [14]. To address the question of adipokine interrelations, we conducted analyses to test the hypothesis that the relation between measures of TNFα activity (plasma TNFr2 or TNFα) and insulin resistance is influenced by biomarkers (adiponectin, resistin, triglycerides) reflecting different adipose tissue pathways.

2. Methods

2.1. Study participants

Started in 1971, the Framingham Offspring Study is a community-based, prospective, observational study following the children of the original Framingham Heart Study cohort and their spouses to investigate cardiovascular disease and its risk factors [15]. At baseline, 5124 participants, mainly white and of European ancestry, were recruited. Participants were invited to return for follow-up measurements on a periodic basis. We used data from the seventh examination cycle, conducted between 1998 and 2001 (n = 3539 attendees). Evaluation included fasting blood samples, a standardized medical examination, and a medical history. For this analysis, we excluded participants with prevalent diabetes (n = 389). We present here analysis of the participants (total n = 2061) with both TNFr2 and TNFα levels measured, after excluding individuals with missing values for adipokines levels (n = 483) or metabolic risk factors (n = 311). The study protocol was approved by the Institutional Review Boards of the Boston University Medical Center and of the Massachusetts General Hospital; all participants provided written informed consent.

2.2. Exposure and outcome definitions

The primary outcome of interest was insulin resistance, measured using the homeostasis model assessment (HOMA-IR, calculated by [fasting glucose × fasting insulin]/22.5) [16], which was considered as a continuous variable. Activity of the TNFα pathway was represented by measurements of circulating plasma TNFr2 or TNFα. Potential confounders included adipose tissue biomarkers (adiponectin, resistin, and triglycerides), which we chose because literature suggested that they reflect the potential pathways though which TNFα could induce insulin resistance. We used levels of triglycerides as a proxy for NEFA flux. In humans, elevated plasma triglycerides reflect increased NEFA flux: in physiologic studies, rising plasma NEFA increased circulating triglycerides [17]; circulating NEFA and triglycerides are modestly correlated in population studies [18]; and hypertriglyceridemia is a well-known characteristic of insulin-resistant states [19].

Laboratory methods for glucose, insulin, and lipid assays have been published previously [20]. Fasting plasma glucose 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, St Louis, MO). Triglycerides levels were determined by commercial assay (Spectrum CCX, Abbott). Total adiponectin, resistin, TNFr2, and TNFα were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). We used a high-sensitivity TNFα assay to capture variation in TNFα in the very low levels that we expected to find in a community-based sample. In our laboratory, intraassay coefficients of variation were less than 3% for glucose, 6.1% for insulin, 1.2% for triglycerides, 5.8% for adiponectin, 9.0% for resistin, 2.2% for TNFr2, and 6.6% for TNFα. Based on the assay kit we used (R&D Systems), interassay values and sensitivities were 5.8% to 6.9% and 0.246 ng/mL for adiponectin, 7.8% to 9.2% and 0.026 ng/mL for resistin, 3.5% to 5.1% and 0.6 pg/mL for TNFr2, and 6.3% to 8.3% and 0.391 pg/mL for TNFα.

Other covariates included standardized physical examination measures. We measured height, weight, and waist circumference (at the umbilicus) with the subject standing. We calculated body mass index (BMI) as weight in kilograms divided by the square of height in meters.

2.3. Statistical analysis

We used means and standard deviations (SDs) to describe continuous characteristics and percentages for categorical characteristics. Variables not normally distributed were log transformed. We used Pearson correlations to assess associations between TNFr2 or TNFα and various clinical and biochemical variables. For linear regression, we modeled HOMA-IR as a continuous variable and the adipokines as per change of 1 SD to be able to compare their relative effects on a unit change in HOMA-IR. We performed all analyses using circulating TNFr2 or TNFα as markers of TNFα activity, but we present results from TNFr2 in the main article because our results with Pearson correlations (showing stronger associations with metabolic traits) were concordant with previous literature [6,7]. We present all the results using TNFα in online supplementary tables. We first tested the age- and sex-adjusted association of TNFr2 with HOMA-IR. Knowing that both TNFr2 and insulin resistance are related to central adiposity, we added waist circumference to the model to assess the impact of adjusting for adiposity on the TNFr2–HOMA-IR association. We then tested the effect of adding adipose tissue biomarkers (adiponectin, resistin, and triglycerides) individually and together to the models adjusted for age, sex, and waist circumference. We assessed whether adding adipose tissue biomarker variables to the models altered the size, direction, or statistical significance of the TNFr2 β-coefficient association with HOMA-IR.

We then stratified the analysis to examine whether the adipose tissue–derived biomarkers modified the effect of TNFr2 on insulin resistance by testing the association in individuals with low or high levels of adipose biomarkers. We divided the TNFr2 distribution by tertiles and examined the level of HOMA-IR in each tertile by 2 strata of adipose tissue biomarker defined as *below* or *above* the median level. We examined the trends within each stratum and tested statistical significance of TNFr2-biomarker interactions using first-order interaction terms in multivariable linear regression models. We adjusted all the stratified analysis for age, sex, and waist circumference. We considered P values < .05 to indicate statistical significance. We performed all analyses using SAS software (version 8.1; SAS Institute, Cary, NC).

3. Results

3.1. Participant characteristics

The characteristics of the Framingham Offspring Study participants are presented in Table 1. At examination 7, participants were on average 61 years old, mean BMI was in the overweight range, and about half were women. Pearson correlations of (log-transformed) TNFr2 and TNFα with several clinical and biochemical factors are shown in Table 2. Circulating levels of TNFr2 and TNFα were modestly correlated with each other ($r = 0.36$, $P < .0001$). In general, the correlations were stronger with TNFr2 than with TNFα levels. The characteristics that showed the strongest associations with TNFr2 were age ($r = 0.39$, $P < .0001$) and resistin levels ($r = 0.31$, $P < .0001$).

Table 1
Characteristics of 2131 participants free of diabetes at Framingham Offspring Study examination 7

	Mean (SD) or %
n	2061
Women (%)	51
Age (y)	61 (10)
BMI (kg/m^2)	27.8 (5.1)
Waist circumference (cm)	97.3 (13.3)
HOMA-IR	3.86 (2.46)
TNFα (pg/mL)	1.45 (1.26)
TNFr2 (pg/mL)	2120 (746)
Adiponectin (μg/mL)	10.48 (6.56)
Resistin (ng/mL)	14.09 (7.14)
Triglycerides (mg/dL)	132.9 (82.0)

3.2. Multivariate models: TNFα measures association with insulin resistance

In a series of linear regression models, we estimated the relation of adiposity and biomarkers with the association of TNFr2 with HOMA-IR. As shown in Table 3, age- and sex-adjusted levels of TNFr2 were associated with HOMA-IR. Adjusting for waist circumference, the β-coefficient for TNFr2 decreased substantially (going from 0.11 to 0.06, a 45% decrease); but TNFr2 remained significantly associated with HOMA-IR. We then tested the influence of adding adiponectin, resistin, or triglycerides (one at a time) on the TNFr2–HOMA-IR association. The age-, sex-, and-waist-adjusted β-coefficient for TNFr2 was barely influenced by adding biomarkers individually. When modeling the biomarkers simultaneously, the age-, sex-, and waist-adjusted TNFr2 β-coefficient was still significantly associated with

Table 2
Pearson correlation coefficients between TNFα or TNFr2 and clinical or biochemical factors in 2131 participants free of diabetes at Framingham Offspring Study examination 7

Clinical and biochemical factors	Correlation with TNF-r2	P	Correlation with TNFα	P
n	2061		2131	
Age	0.39	<.0001	0.19	<.0001
BMI	0.19	<.0001	0.07	.001
Waist circumference	0.22	<.0001	0.09	<.0001
Triglycerides	0.12	<.0001	0.09	<.0001
Adiponectin	−0.05	.01	−0.06	.009
Resistin	0.31	<.0001	0.20	<.0001
HOMA-IR	0.21	<.0001	0.09	<.0001
TNFα	0.36	<.0001	–	–

Table 3
Association of TNFr2 with HOMA-IR in the Framingham Offspring Study, adjusted for age and sex, and further adjusted for waist circumference and adipose tissue biomarkers

	TNFr2	Adiponectin	Resistin	Triglycerides
Age and sex adjusted				
β (SE)[a]	0.11 (0.01)			
P value	<.0001			
Age, sex, and waist circumference adjusted				
β (SE)	0.06 (0.01)			
P value	<.0001			
Age, sex, and waist circumference adjusted; adding adipose tissue biomarkers individually				
β (SE)	0.06 (0.01)	−0.12 (0.01)		
P value	<.0001	<.0001		
β (SE)	0.05 (0.01)		0.02 (0.01)	
P value	<.0001		.12	
β (SE)	0.05 (0.01)			0.11 (0.01)
P value	<.0001			<.0001
Age, sex, and waist circumference adjusted; adding all 3 adipose tissue biomarkers simultaneously[b]				
β (SE)	0.05 (0.01)	−0.10 (0.01)	0.01 (0.01)	0.09 (0.01)
P value	<.0001	<.0001	.28	<.0001

[a] β-Coefficient in linear regression models predicting HOMA-IR (log transformed) reflects an SD change in each biomarker. By example, in the age- and sex-adjusted model, for each increase of 1 SD of TNFr2 (SD = 746 pg/mL, nontransformed), the log (HOMA-IR) value would be increased by 0.11 units.

[b] Estimated variance of the full model: $r^2 = 0.36$.

HOMA-IR. Adiponectin and triglycerides levels were also significantly associated with HOMA-IR in the fully adjusted models, but not resistin levels.

We conducted the same analyses using TNFα instead of TNFr2 (Online Supplement Table 1). In all the models, TNFα was significantly associated with HOMA-IR; and the β-coefficient was influenced in a similar fashion with the successive adjustments. The association remained significant ($P = .04$) in the full model that included waist circumference and all 3 adipose tissue biomarkers.

3.3. Effect of biomarker stratification on the TNFr2–HOMA-IR association

Low adiponectin, high resistin, and high triglycerides levels have been associated with a higher-risk metabolic profile. We tested if the TNFr2–HOMA-IR association was modified in individuals with adverse levels of these adipose tissue biomarkers. Fig. 1 shows HOMA-IR values by TNFr2 tertiles, stratified by below or above median of the adipose tissue biomarkers (all analyses adjusted for age, sex, and

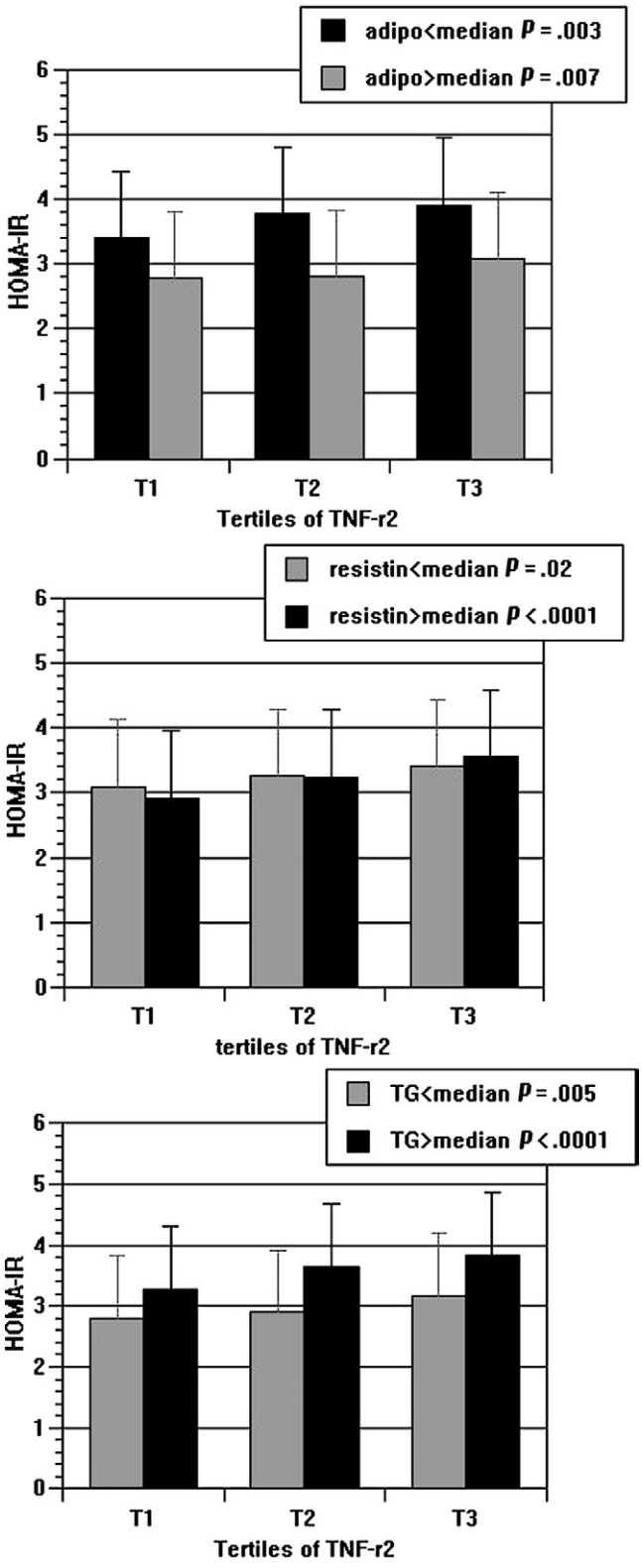

Fig. 1. Top panel: Insulin resistance measured by HOMA-IR in each TNFr2 tertile, further stratified by adiponectin level (below or above median). All data are adjusted for age, sex, and waist circumference. P values for trend of association with HOMA-IR across TNFr2 tertiles are shown in the figure; $P = .51$ for interaction (TNFr2 by adiponectin predicting HOMA-IR). Middle panel: Insulin resistance measured by HOMA-IR in each TNFr2 tertile, further stratified by resistin level (below or above median). P values for trend of association with HOMA-IR across TNFr2 tertiles are shown in the figure; $P = .08$ for interaction (TNFr2 by resistin predicting HOMA-IR). Bottom panel: Insulin resistance measured by HOMA-IR in each TNFr2 tertile, further stratified by triglyceride level (below or above median). P values for trend of association with HOMA-IR across TNFr2 tertiles are shown in the figure; $P = .60$ for interaction (TNFr2 by TG predicting HOMA-IR). TG indicates triglyceride.

waist circumference). The associations between TNFr2 and HOMA-IR remained significant in all the strata of biomarkers. No biomarker-TNFr2 interactions were statistically significant (all *P*s > .05), but the individuals with resistin levels above the median tended to show a stronger association between TNFr2 and HOMA-IR (interaction *P* value = .08).

4. Discussion

The association of insulin resistance with TNFα has been demonstrated in vitro and in animal studies, but has been less consistently shown in human studies [21-24]. We have shown previously that TNFα levels were associated with insulin resistance, adjusting for adiposity and metabolic syndrome status [14]. Here we have shown that HOMA-IR and other metabolic traits, including adipose tissue biomarkers, were correlated with circulating levels of TNFα, but showed stronger correlations with circulating TNFr2 levels in general, as a marker of TNFα activity. Tumor necrosis factor α receptor 2 is believed to stabilize circulating TNFα and to act as a "slow-release reservoir" of bioactive TNFα [4]. In accordance with our data, TNFr2 has been shown to have similar or stronger correlations with insulin resistance compared with circulating TNFα levels [7]. We extend understanding of the association between TNFα pathways and insulin resistance by taking into account circulating levels of adiponectin, resistin, and triglycerides, representing mediating pathways by which TNFα could act based on functional studies [2,5]. Our report lends support to the concept that TNFα is involved in the proinflammatory process linking adiposity to development of diabetes not only in animal models but also in community-dwelling adults.

4.1. Extending understanding of functional studies of adipose tissue

Numerous functional studies of adipose tissue have outlined the complex interactions of adipokines produced by adipocytes and macrophages. Adipocytes are the major triglyceride storage site and have the exclusive ability to secrete adiponectin [25]. Resistin and TNFα can be secreted by both adipocytes and macrophages; but in humans, these adipokines seem to be mainly derived from adipose tissue–infiltrating macrophages [2,26].

It has been shown that adiponectin decreases TNFα production in macrophages [27] and that TNFα decreases adiponectin expression [8,28] and secretion [29,30] in adipocytes, leading to a "vicious" proinflammatory cycle. In our data, adiponectin was weakly associated with TNFα and TNFr2 ($r = -0.06$ and $r = -0.05$, respectively); had minimal impact when added to the age-, sex-, and waist-adjusted models; and was still significantly associated with insulin resistance in fully adjusted models. This suggests that adiponectin and TNFα provide complementary information and that both influence pathways leading to insulin resistance.

Resistin was the biomarker with the strongest association with both TNFr2 and TNFα levels. We have shown in the past that resistin levels are associated with insulin resistance, even when accounting for adiposity [14]; but this association was not significant in the present analysis when including TNFr2 levels and central adiposity in the models. This could be due to the fact that in human adipose tissue both resistin and TNFα are produced by infiltrating macrophages and so could be participating in closely linked proinflammatory pathways. In vitro, resistin stimulates production of TNFα by human adipocytes [31] and macrophages [32]. Tumor necrosis factor α in turn can stimulate resistin in blood mononuclear cells [10,11]. A possible proinflammatory "feed forward" loop is supported by our data pointing to a stronger association between TNFr2 and HOMA-IR in individuals with high resistin.

In vitro, NEFA have been shown to decrease adiponectin secretion and to increase TNFα secretion in adipocytes [33]. In macrophages, saturated fatty acids increase TNFα expression [2]. In addition, cocultures of adipocytes and macrophages induced an increased production of NEFA, which could be decreased by blockage of TNFα action [2]. In animal models, TNFα infusion increased circulating plasma NEFA after 1 day of infusion and increased both plasma NEFA and triglycerides after 4 days of TNFα infusion [13]. Using triglycerides as a proxy for NEFA flux, we found that TNFr2 was associated positively with triglycerides and that both biomarkers were independently associated with HOMA-IR in multivariable models. Overall, we found evidence supporting insulin resistance–TNFα–adipokine associations in adults in the community, with directions of effects that were expected on the basis of known molecular and physiologic relationships.

4.2. Markers of TNFα activity and other adipokines in population-based studies

Population-based studies exploring the interrelations of diverse adipokines and inflammatory markers and their effect on insulin resistance are scarce, and few have considered a comprehensive set of biomarkers. Circulating TNFα levels have been inconsistently associated with insulin resistance in small cohorts, where some have found significant associations [21,24], whereas others have not [22,23]. We previously reported that elevated TNFα levels were associated with increased insulin resistance [14]; here, we took advantage of our large sample size to further characterize the influence of key adipose tissue biomarkers on this association. We found modest significant correlations markers of TNFα activity with adiponectin, resistin, and triglycerides of similar magnitude to those reported in another general population–based study of Chinese men [34]. Few population studies have simultaneously assessed more than one biomarker in relation to insulin resistance. In a

Swedish male cohort, TNFα and adiponectin levels were both associated with glucose infusion rate assessed by euglycemic hyperinsulinemic clamps [21]. Population studies have shown a significant positive correlation between resistin and TNFα [35,36], but did not model both in relation to insulin resistance.

4.3. Strength and limitations

Strengths of this study include a large sample size from a community-based cohort, clinical measurements taken under standardized protocols, and a comprehensive set of biomarkers measured using assays with good precision. We present results using TNFr2, which is known to represent a good reflection of TNFα activity [4,6,7]. We confirmed our finding by repeating the analysis using TNFα, measured with a high-sensitivity assay that detected low concentrations of the biomarker in the ranges typical of community-based sample. A limitation of our study is that formal power to detect statistical interactions might not have been sufficient in some of the stratified analyses. Another limitation includes the use of surrogate markers for insulin resistance (HOMA-IR) and for NEFA (triglycerides). We recognize that triglycerides can be influenced by many clinical situations, are not a direct measure of circulating fatty acids in individuals, and cannot reliably replace direct measurement of circulating fatty acids. As circulating fatty acids are not available in the Framingham Offspring Study, we used triglycerides as a surrogate of fatty acid flux. Like HOMA-IR that is not a precise measure of insulin resistance in individuals but does discriminate insulin resistance from insulin-sensitive groups at the population level, we used triglycerides to define groups with relatively low vs relatively high free fatty acid flux at the population level. If anything, this might have reduced our ability to observe associations; and our results may be underestimations of the true associations. We measured only total adiponectin and not the high–molecular-weight fraction, which has been proposed to have a stronger correlation with insulin resistance and metabolic syndrome compared with total adiponectin [37,38]. Some might raise the fact that we used a single measurement of each biomarker, but 1-point adipokine measurement has been shown to be reliable compared with more than one assessment over many seasons [34]. Further limitations include the cross-sectional design of our analyses that does not allow us to make conclusions regarding causal direction. Finally, the Framingham cohort is largely white and middle-aged or older; so findings may have limited generalizability to other ethnic and age groups.

In conclusion, we have shown in a large community-based sample that markers of TNFα activity are associated with insulin resistance, even after taking into account central adiposity status and other adipose tissue biomarkers. Our observations in this representative population add to the functional studies suggesting that anti-inflammatory, proinflammatory, and lipolytic pathways are implicated in the association of TNFα with insulin resistance. Prospective follow-up and physiologic studies are needed to help us untangle the causal relations and to understand the sequence of adipokines interactions leading to insulin resistance and diabetes.

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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.2009.08.017.

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