

CLINICAL STUDY

Resistin is associated with biomarkers of inflammation while total and high-molecular weight adiponectin are associated with biomarkers of inflammation, insulin resistance, and endothelial function

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Abstract

Objective: Adiponectin and resistin have been linked to inflammation, endothelial dysfunction, and/or insulin secretion or resistance. It remains to be elucidated which of these adipokines is associated primarily with biomarkers of all or only some of these categories, i.e. biomarkers of inflammation, endothelial dysfunction, and/or insulin secretion or insulinemia.

Design and methods: We studied 1065 healthy women, Nurses' Health Study participants, who provided blood samples in 1989–1990. A cross-sectional analysis was conducted to assess the relationships between total and high-molecular weight (HMW) adiponectin and resistin with inflammatory markers and biomarkers of endothelial dysfunction, insulin secretion, and insulinemia.

Results: Resistin was positively associated with the inflammatory markers soluble tumour necrosis factor- α receptor II and interleukin-6 but not with any biomarkers of endothelial function, glycemia, insulinemia, or markers of insulin secretion after multivariate adjustment for age and body mass index (BMI). In both crude and multivariate analyses, total adiponectin was inversely associated with insulin, proinsulin, C-peptide, HbA1c, sE-selectin, and C-reactive protein (CRP) levels. HMW adiponectin was inversely associated with circulating insulin, proinsulin, C-peptide, HbA1c, sE-selectin, and CRP concentrations, even after adjustment for age, BMI, lifestyle factors, exercise, the use of medications as well as the other biomarkers of interest. Total and HMW adiponectin demonstrated negative associations with soluble intercellular adhesion molecule-1, which became nonsignificant after adjustment for confounders, whereas positive associations between soluble vascular cell adhesion molecule-1 and total adiponectin became significant only after multivariate adjustment.

Conclusions: Total and HMW adiponectin are inversely associated with markers of insulin secretion/insulinemia, endothelial function, and inflammation. Resistin is positively associated only with markers of inflammation.

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Introduction

The discovery of leptin, the prototypical adipose tissue-secreted adipokine, in the mid-90s altered our understanding of adipose tissue from that of an inert storage organ to that of an active endocrine organ (1). Adipose tissue secretes several adipokines, most of them, such as tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6), negatively affecting insulin resistance and others, such as adiponectin, having a beneficial effect. Adiponectin is a metabolically active adipokine, which is inversely associated with obesity, insulin resistance, and atherosclerosis (2–4). Prospective studies have shown that higher plasma adiponectin

concentrations are associated with decreased risk of type 2 diabetes mellitus (5), cardiovascular disease (CVD) in men with type 2 diabetes mellitus (6), and myocardial infarction in men with or without diabetes (7). Adiponectin is present in plasma in three forms: a trimer, a hexamer, and a high-molecular weight (HMW) form, which has been proposed to be the most active adiponectin form (8). Total adiponectin levels have previously been shown to be associated with better glycemic control and reduced inflammation in type 2 diabetic women from the Nurses' Health Study (9), but associations of adiponectin with biomarkers of inflammation, such as TNF- α and IL-6, endothelial dysfunction, such as soluble intercellular adhesion

molecule-1 and soluble vascular cell adhesion molecule-1 (sICAM-1 and sVCAM-1), and insulin resistance and secretion in healthy women have yet to be examined. Moreover, relationships between HMW adiponectin and biomarkers of inflammation, endothelial dysfunction, and insulin resistance have not been previously explored in either healthy or diabetic women.

Resistin was originally discovered as an adipokine, which was found to be elevated in the diet-induced obese mouse model and was proposed to induce insulin resistance or impaired hepatic sensitivity to insulin (10). However, data in humans remain controversial. In contrast to mice, resistin in humans is expressed in lower levels in adipocytes but at relatively higher levels in circulating blood monocytes (11). Several studies have failed to detect increased serum resistin levels in obese or insulin-resistant subjects (12). Although its role in insulin resistance has been challenged, it has been subsequently proposed that resistin may promote endothelial cell activation and upregulate several adhesion molecules and cytokines (13, 14). Since both inflammation and insulin resistance play critical roles in the development of atherosclerosis (15), associations with biomarkers of inflammation and/or endothelial function would imply that resistin could possibly be directly or indirectly related to CVD. Associations between resistin and biomarkers of inflammation, endothelial dysfunction, and insulin resistance and secretion have not been previously explored in healthy women.

In view of these data, we utilized a large sample of healthy nondiabetic women studied in the context of the Nurses' Health Study to assess whether circulating total and HMW adiponectin and resistin levels are associated with biomarkers of insulin resistance or insulin secretion as well as biomarkers of inflammation and endothelial function, independently of known risk factors. Thus, we analyzed the cross-sectional associations of total and HMW adiponectin and resistin with biomarkers of inflammation, endothelial dysfunction, and insulin resistance.

Methods

Study population

The Nurses' Health Study was initiated in 1976 with the enrollment of 121 700 USA nurses, aged 30–55 years at baseline. This cohort study involves biennially mailed questionnaires related to lifestyle factors and health outcomes. Between 1989 and 1990, 32 826 study participants provided blood samples by overnight courier. Study participants of the current analysis were a subset of the prospective Nurses' Health Study, all of whom were healthy at baseline. We identified and confirmed all women who developed type 2 diabetes over a 12-year period of follow-up, as well as an equal number of women who were diabetes-free at baseline

and never developed diabetes during this 12-year period of follow-up. All subjects were free of diabetes and CVD at blood draw. In order to keep sample size constant throughout models in multivariate adjustment, we excluded women who had missing data for biomarkers of interest, including plasma total and HMW adiponectin, resistin, soluble TNF- α receptor II (sTNF- α RII), C-reactive protein (CRP), IL-6, sICAM-1, sVCAM-1, sE-selectin, and ferritin. We further excluded women with missing data on confounders (age, body mass index (BMI), smoking status, alcohol intake, physical activity, fasting status, postmenopausal status, postmenopausal hormone use, aspirin use, history of hypertension, or hypercholesterolemia). After such exclusions, 576 healthy women who developed type 2 diabetes later in life and 489 healthy women who never developed type 2 diabetes were available for analysis. First, we analyzed data from all 1065 subjects with adjustment for an indicator variable indicating development of diabetes later in life. An additional, independent analysis was conducted in the subset of 489 of these women who never developed type 2 diabetes mellitus using established criteria (16). The study was approved by the Institutional Review Board (IRB) of Brigham and Women's Hospital and the biochemical measures by the IRB of Beth Israel Deaconess Medical Center, both in Boston, MA, USA.

Blood collection and processing

Blood samples were collected between 1989 and 1990 as previously described (16). Briefly, a phlebotomy kit was sent to all the women who agreed to provide blood samples. The samples were returned by overnight mail in a frozen water bottle, processed on arrival, and stored in liquid nitrogen (-130°C or less) until time of analysis. The samples were analyzed in a random order, and laboratory personnel were blinded to the study hypothesis. All assays for each analyte run for this study for the first time (i.e., total adiponectin, HMW adiponectin, and resistin) were run in one run. All assays have been used and described in detail elsewhere (6, 7, 9, 12, 17–19). Briefly, total adiponectin concentration was measured using RIA (Linco Research, Inc., St Charles, MO, USA), which has a sensitivity of 2 $\mu\text{g}/\text{ml}$ and intra-assay coefficient of variation (CV) of 1.8–6.2%. Serum HMW adiponectin levels were determined by ELISA (Millipore, St Charles, MO, USA) with a sensitivity of 0.5 ng/ml . Resistin was assayed by ELISA (Linco Research, Inc). The minimum detectable range of this assay is 0.16 ng/ml for a sample size of 10 μl (diluted 1:10); intra-assay CV%, 3.2–7.0%. Intra-assay precision was determined using four samples of known concentration tested ten times on one plate. Assays for sTNF- α RII, CRP, IL-6, sICAM-1, sVCAM-1, sE-selectin, insulin, HbA_{1c}, C-peptide, proinsulin, and ferritin had been run previously and are described in detail elsewhere (16, 20–22).

Assessment of lifestyle exposures

Body weight, BMI, lifestyle, and medical history variables were determined as previously described (23). Waist-to-hip ratio was determined using self-reported waist and hip circumference information in 1986 (24). Physical activity was computed as metabolic equivalent tasks (METs) per week from data on duration and intensity of exercise collected during the period 1986–2000 (16). The validity of self-reported Nurses' Health Study data has been previously reported (23, 24).

Statistical analysis

Descriptive characteristics were compared across quartiles of resistin levels using one-way ANOVA for continuous variables and χ^2 tests for categorical variables. To evaluate the relationships between biomarkers, Spearman's correlations (r) were performed. Multivariate linear regression analyses were then performed to adjust for age (continuous), diabetes status (yes, no), BMI (continuous), smoking (never, past, and current), alcohol intake (0.0, 0.1–4.9, 5.0–9.9, 10.0–14.9, and ≥ 15.0 g/day), physical activity (<3, 3–8, 9–17, 18+ METs/week), fasting status (<8 or ≥ 8 h since

last meal), postmenopausal status, postmenopausal hormone use, aspirin use, history of high blood pressure, history of high blood cholesterol, and additionally for sTNF- α RII, IL-6, CRP, sE-selectin, sICAM-1, sVCAM-1, and ferritin. Since results were similar for log-transformed and non-transformed biomarkers and parameter estimates from non-transformed markers were more meaningful for interpretation, we only presented nontransformed results in the current analysis. Additional analysis was also conducted among the subgroup of those who remained free of type 2 diabetes. All statistical analyses were performed using SAS statistical software version 9.0 (SAS Institute, Inc., Cary, NC, USA). A study with 489 subjects would have an 80% power to detect associations with $r \geq 0.11$ at the conventional $\alpha = 0.05$ level, whereas a study with 1065 subjects would have similar power to detect associations with $r \geq 0.08$.

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Table 1 Characteristics by quartiles of resistin in 1065 women without diabetes and cardiovascular disease at baseline.

Variable	Quartiles of resistin concentration				P value
	Q1	Q2	Q3	Q4	
Resistin (ng/ml)	<i>n</i> =266	<i>n</i> =266	<i>n</i> =267	<i>n</i> =266	
Range	2.25–11.73	11.73–14.93	14.93–20.97	20.97–99.36	
Biomarkers					
Adiponectin (μ g/ml)	16.3 \pm 8.3	14.8 \pm 7.4	14.0 \pm 7.4	13.8 \pm 7.4	0.0004
HMW adiponectin (μ g/ml)	6.97 \pm 5.54	5.75 \pm 4.23	5.38 \pm 4.43	5.33 \pm 4.31	<0.0001
CRP (mg/dl)	0.32 \pm 0.44	0.35 \pm 0.36	0.42 \pm 0.42	0.52 \pm 0.59	<0.0001
Interleukin-6 (ng/ml)	2.32 \pm 2.16	2.56 \pm 2.49	2.83 \pm 2.12	3.39 \pm 3.34	<0.0001
sTNF- α RII (pg/ml)	2424.0 \pm 767.4	2577.6 \pm 799.9	2674.7 \pm 853.9	2944.0 \pm 974.6	<0.0001
sE-selectin (ng/ml)	53.4 \pm 28.3	57.4 \pm 30.7	57.6 \pm 24.6	63.0 \pm 30.0	0.002
sICAM-1 (ng/ml)	262.0 \pm 74.3	268.2 \pm 100.3	279.2 \pm 97.5	278.1 \pm 69.6	0.06
sVCAM-1 (ng/ml)	550.2 \pm 149.9	565.4 \pm 259.3	575.9 \pm 154.4	578.0 \pm 169.3	0.30
Ferritin (ng/ml)	91.1 \pm 87.2	96.4 \pm 89.8	81.7 \pm 79.4	91.7 \pm 88.3	0.26
Insulin (μ U/ml)	11.4 \pm 6.8	13.0 \pm 9.6	12.1 \pm 8.5	12.4 \pm 7.4	0.36
Proinsulin (fmol/ml)	16.7 \pm 15.5	19.8 \pm 17.9	16.0 \pm 16.3	26.5 \pm 22.2	0.005
C-peptide (pmol/ml)	0.69 \pm 0.55	0.84 \pm 0.75	0.73 \pm 0.41	0.92 \pm 0.58	0.04
HbA1c (g/dl)	0.59 \pm 0.22	0.59 \pm 0.20	0.57 \pm 0.18	0.60 \pm 0.19	0.55
Descriptive variables					
Age (year)	57.9 \pm 6.6	56.9 \pm 6.7	56.5 \pm 6.9	56.6 \pm 7.0	0.07
BMI	26.7 \pm 5.4	28.4 \pm 5.8	29.1 \pm 6.1	30.2 \pm 6.7	<0.0001
Physical activity (METs/week)	14.7 \pm 16.7	15.5 \pm 38.6	13.4 \pm 16.5	13.6 \pm 18.8	0.74
Alcohol (g/day)	5.9 \pm 11.6	4.1 \pm 7.8	3.6 \pm 6.7	3.8 \pm 7.8	0.007
Current smoker, <i>n</i> (%)	28 (10.5)	23 (8.7)	38 (14.2)	52 (19.6)	0.0009
Fasting (≥ 8 h), <i>n</i> (%)	184 (69.2)	197 (74.1)	171 (64.0)	170 (63.9)	0.04
Postmenopausal, <i>n</i> (%)	213 (80.1)	195 (73.3)	187 (70.0)	191 (71.8)	0.05
Current postmenopausal hormone use, <i>n</i> (%)	81 (30.5)	68 (25.6)	68 (25.5)	64 (24.1)	0.36
Current aspirin use, <i>n</i> (%)	143 (53.8)	114 (42.9)	111 (41.6)	114 (42.9)	0.02
History of high blood pressure	100 (37.6)	119 (44.7)	111 (41.6)	120 (45.1)	0.26
History of high cholesterol	123 (46.2)	115 (43.2)	108 (40.5)	119 (44.7)	0.58

Values shown are mean \pm s.d. for continuous variables and frequency (percentage) for categorical variables.

Results

In this cross-sectional study of 1065 women who were free of diabetes and CVD at blood draw, resistin levels were significantly and positively associated with BMI ($P < 0.0001$) and smoking ($P = 0.0009$), inversely associated with alcohol consumption ($P = 0.007$), and inversely but marginally associated with age ($P = 0.07$; Table 1). Women with lower resistin levels were more likely to be in fasting status ($P = 0.04$), postmenopausal ($P = 0.05$), and taking aspirin ($P = 0.02$), and these

factors were adjusted for in the analysis, as shown in Table 2. Resistin levels were not associated with physical activity, postmenopausal hormone use, history of high blood pressure or high cholesterol. Resistin levels were significantly and inversely associated with total ($P = 0.0004$) and HMW adiponectin ($P < 0.0001$), and positively associated with CRP ($P < 0.0001$), IL-6 ($P < 0.0001$), sTNF- α RII ($P < 0.0001$), sE-selectin ($P = 0.002$), proinsulin ($P = 0.005$), and C-peptide ($P = 0.04$). Resistin was not associated with sICAM-1, sVCAM-1, ferritin, insulin, and HbA1c in this crude analysis.

Table 2 Parameter estimates and P values for 10 unit increase in adipokine levels in relation to biomarkers in 1065 women.

Biomarkers	<i>n</i>	Model 1 ^a		Model 2 ^b		Model 3 ^c		Model 4 ^d	
		Estimate	<i>P</i> value	Estimate	<i>P</i> value	Estimate	<i>P</i> value	Estimate	<i>P</i> value
β estimates and P values for 10 μ g/ml increase in adiponectin levels									
HMW adiponectin (μ g/ml)	1065	5.28	<0.0001	5.11	<0.0001	5.06	<0.0001	5.01	<0.0001
Resistin (ng/ml)	1065	-1.40	0.004	-0.77	0.13	-0.59	0.27	-0.26	0.62
sTNF- α RII (pg/ml)	1065	-141.4	<0.0001	-98.4	0.006	-56.6	0.13	-27.5	0.45
Interleukin-6 (ng/ml)	1065	-0.39	0.0002	-0.27	0.01	-0.25	0.03	-0.04	0.72
CRP (mg/dl)	1065	-0.14	<0.0001	-0.09	<0.0001	-0.09	<0.0001	-0.06	0.0008
sE-selectin (ng/ml)	1065	-10.9	<0.0001	-8.0	<0.0001	-7.2	<0.0001	-5.6	<0.0001
sICAM-1 (ng/ml)	1065	-16.5	<0.0001	-14.0	0.0001	-10.2	0.005	-3.08	0.29
sVCAM-1 (ng/ml)	1065	-0.48	0.95	4.94	0.53	13.3	0.10	35.3	<0.0001
Ferritin (ng/ml)	1065	-11.6	0.0007	-13.0	0.0002	-12.2	0.0007	-5.84	0.11
Insulin (μ U/ml)	606	-3.48	<0.0001	-2.69	<0.0001	-2.52	<0.0001	-1.76	<0.0001
Proinsulin (fmol/ml)	293	-9.68	<0.0001	-8.0	<0.0001	-7.24	<0.0001	-4.53	0.0006
C-peptide (pmol/ml)	417	-0.28	<0.0001	-0.21	<0.0001	-0.20	<0.0001	-0.13	0.0004
HbA1c (g/dl)	631	-0.08	<0.0001	-0.07	<0.0001	-0.07	<0.0001	-0.05	<0.0001
β estimates and P values for 10 μ g/ml increase in HMW adiponectin levels									
Total adiponectin (μ g/ml)	1065	14.2	<0.0001	14.0	<0.0001	13.8	<0.0001	13.6	<0.0001
Resistin (ng/ml)	1065	-2.22	0.005	-1.10	0.19	-0.75	0.39	-0.22	0.80
sTNF- α RII (pg/ml)	1065	-210.1	0.0002	-123.0	0.04	-46.9	0.44	-31.3	0.60
Interleukin-6 (ng/ml)	1065	-0.66	0.0001	-0.44	0.01	-0.39	0.04	-0.13	0.46
CRP (mg/dl)	1065	-0.22	<0.0001	-0.13	<0.0001	-0.13	<0.0001	-0.09	0.005
sE-selectin (ng/ml)	1065	-17.1	<0.0001	-11.8	<0.0001	-10.4	<0.0001	-9.5	<0.0001
sICAM-1 (ng/ml)	1065	-20.4	0.0003	-14.6	0.02	-7.0	0.25	0.34	0.94
sVCAM-1 (ng/ml)	1065	10.7	0.38	23.6	0.07	38.4	0.004	68.6	<0.0001
Ferritin (ng/ml)	1065	-16.3	0.004	-17.2	0.003	-15.8	0.008	-66.3	0.27
Insulin (μ U/ml)	606	-5.42	<0.0001	-3.91	<0.0001	-3.69	<0.0001	-2.56	0.001
Proinsulin (fmol/ml)	293	-17.6	<0.0001	-13.4	<0.0001	-11.7	<0.0001	-6.85	0.01
C-peptide (pmol/ml)	417	-0.43	<0.0001	-0.28	<0.0001	-0.25	0.0002	-0.17	0.01
HbA1c (g/dl)	631	-0.10	<0.0001	-0.09	<0.0001	-0.08	<0.0001	-0.05	0.003
β estimates and P values for 10 ng/ml increase in resistin levels									
Total adiponectin (μ g/ml)	1065	-0.57	0.004	-0.28	0.13	-0.20	0.27	-0.09	0.62
HMW adiponectin (μ g/ml)	1065	-0.34	0.005	-0.15	0.19	-0.09	0.39	-0.03	0.80
sTNF- α RII (pg/ml)	1065	138.9	<0.0001	123.8	<0.0001	117.7	<0.0001	103.3	<0.0001
Interleukin-6 (ng/ml)	1065	0.38	<0.0001	0.34	<0.0001	0.33	<0.0001	0.25	<0.0001
CRP (mg/dl)	1065	0.05	<0.0001	0.03	0.007	0.03	0.009	0.01	0.38
sE-selectin (ng/ml)	1065	1.89	0.01	0.90	0.20	0.84	0.22	0.45	0.44
sICAM-1 (ng/ml)	1065	2.28	0.30	1.09	0.62	-0.0003	0.99	-3.27	0.05
sVCAM-1 (ng/ml)	1065	3.74	0.44	2.07	0.67	2.37	0.62	-1.94	0.62
Ferritin (ng/ml)	1065	1.41	0.52	0.77	0.72	1.02	0.63	0.07	0.97
Insulin (μ U/ml)	606	0.23	0.44	-0.19	0.53	-0.25	0.40	-0.42	0.14
Proinsulin (fmol/ml)	293	2.75	0.010	1.49	0.16	1.11	0.29	0.95	0.31
C-peptide (pmol/ml)	417	0.05	0.10	-0.002	0.92	-0.01	0.76	-0.01	0.56
HbA1c (g/dl)	631	0.01	0.10	0.004	0.51	0.003	0.62	0.005	0.46

^aUnadjusted.

^bAdjusted for age and BMI.

^cAdditionally adjusted for smoking (never, current, past), alcohol (0.0, 0.1–4.9, 5.0–14.9, 15.0+g), physical activity (<3, 3–8, 9–17, 18+ METs/week), fasting (Y/N), postmenopausal status (Y/N), postmenopausal hormone usage (Y/N), aspirin usage (Y/N), history of hypertension, and history of hypercholesterolemia (Y/N).

^dAdditionally adjusted for sTNF- α RII, IL-6, CRP, sE-selectin, sICAM-1, sVCAM-1, and ferritin.

Table 3 Spearman correlation between biomarkers in 1065 women.

	Resistin	Adiponectin	HMW	CRP	Interleukin-6	sTNF- α RII	sE-selectin	sICAM-1	sVCAM-1	Ferritin	Insulin	Proinsulin	C-peptide	HbA1c
Resistin	1													
Adiponectin		1												
HMW			1											
CRP				1										
Interleukin-6					1									
sTNF- α RII						1								
sE-selectin							1							
sICAM-1								1						
sVCAM-1									1					
Ferritin										1				
Insulin											1			
Proinsulin												1		
C-peptide													1	
HbA1c														1

* $P < 0.05$, † $P < 0.0001$.

Spearman's correlation analyses generated similar results (Table 3). Resistin was significantly positively correlated with CRP ($r = 0.22$), IL-6 ($r = 0.26$), sTNF- α RII ($r = 0.28$), and sE-selectin ($r = 0.16$) at $P < 0.0001$, and with sICAM-1 ($r = 0.11$), sVCAM-1 ($r = 0.08$), proinsulin ($r = 0.12$), and C-peptide ($r = 0.14$) at $P < 0.05$. Resistin was also significantly inversely correlated with total ($r = -0.12$) and HMW ($r = -0.14$) adiponectin ($P < 0.0001$ for both). Resistin was not correlated with ferritin, insulin, or HbA1c. Total and HMW adiponectin were both significantly inversely correlated with biomarkers of inflammation, endothelial function, and insulinemia or insulin resistance/secretion, with the sole exception of sVCAM-1.

Multivariate analyses showed that resistin was significantly positively associated with sTNF- α RII and IL-6 ($P < 0.0001$), and was not associated with CRP, sE-selectin, sICAM-1, sVCAM-1, or ferritin, controlling for confounding effects from all anthropometric and inflammatory parameters (Table 2, Model 4). For each 10 ng/ml of increase in resistin level, sTNF- α RII increases by 103.3 pg/ml ($P < 0.0001$) and IL-6 increases by 0.25 ng/ml ($P < 0.0001$). Among all anthropometric parameters, age and BMI were the most significant confounders on the relationships between resistin and inflammatory markers; the parameter estimates and P values of sTNF- α RII, IL-6, and CRP did not change substantially when all anthropometric parameters were introduced in Model 3, in addition to age and BMI in Model 2. When all inflammatory markers were controlled for in Model 4, the associations between resistin, sTNF- α RII, and IL-6 decreased in magnitude but remained highly significant ($P < 0.0001$), and the association between resistin and CRP became insignificant. Secondary analyses on insulin, proinsulin, C-peptide, and HbA1c showed that resistin was not associated with any of these four biomarkers. Controlling for confounding from all anthropometric factors and inflammatory markers did not change these results.

There were statistically significant inverse associations between total adiponectin levels and sE-selectin, insulin, proinsulin, and C-peptide in both crude and multivariate analysis (Table 2). Total adiponectin was not significantly associated with sVCAM-1 in crude analysis, but a significant positive association between adiponectin and sVCAM-1 was evident after adjustment for lifestyle variables and mutual adjustment for the markers of inflammation and endothelial function ($P < 0.0001$).

Associations between HMW adiponectin and the other biomarkers were very similar to those of total adiponectin. HMW adiponectin was significantly associated with sE-selectin, insulin, and sVCAM-1 after adjustment for possible confounders in multivariate analysis (Table 2). As seen with total adiponectin, a positive relationship between HMW adiponectin and sVCAM-1 became significant after multivariate

adjustment. Both total and HMW adiponectin were inversely associated with HbA1c (Table 2).

Additional analysis conducted among the women who did not develop diabetes later in life produced similar results, and thus, only results from the entire cohort are presented herein. No notable effect modification was detected for obesity, fasting, or HbA1c levels.

Discussion

Resistin is derived almost exclusively from adipose tissue in rodents (25), but in contrast to rodents, resistin is expressed primarily in inflammatory cells, especially macrophages (26), in humans. Although resistin mRNA is detectable in human adipocytes (27), expression levels are much higher in human inflammatory cells (26, 28). Similar to a previous study in healthy men and women with a family history of coronary artery disease (29), we detected significant associations between resistin and sTNF- α RII, IL-6, and CRP, inflammatory markers derived mainly from monocytes and vascular endothelium rather than adipocytes. Resistin levels have previously been reported to be associated with plasma CRP levels in healthy men and women, as well as patients with coronary artery disease or diabetes, or patients at high risk for diabetes (19, 30–33). In diabetics, the magnitude of the previously reported associations is similar to the associations in healthy women reported herein. In this cohort of women, however, the relationship between resistin and CRP was attenuated after adjustment for the other biomarkers of inflammation and endothelial function studied. This suggests that the previously observed association between resistin and CRP may be mediated in part by the positive relationships between resistin, sTNF- α RII, and IL-6, both of which are upstream of CRP, the levels of which depend on sTNF- α RII and IL-6 activity. Laboratory investigations have demonstrated that inflammatory responses may stimulate resistin secretion (28, 34, 35). Conversely, resistin may also promote production of proinflammatory mediators such as IL-6 and sTNF- α RII, in part by activation of the nuclear receptor factor κ -B signaling pathway (36). Data in this study are consistent with those prior laboratory investigations but cannot prove causality and/or direction of association given the cross-sectional nature of the study.

In healthy women, adiponectin levels are significantly and inversely associated with plasma CRP, a marker of systemic inflammation induced by proinflammatory cytokines in the liver (37). Adiponectin has been shown to be inversely associated with CRP concentrations in recent cross-sectional studies among patients with type 2 diabetes (38), patients who are diabetic or at risk for diabetes (19), patients with vascular disease or dyslipidemia (39), and among apparently healthy men (40) but this is the first study in women. In addition,

significant inverse associations between adiponectin and plasma sE-selectin concentrations are shown herein and remain significant after multivariate analysis. This finding confirms the inverse relationship seen previously in a cross-sectional investigation of 62 patients with coronary heart disease (41). We also reported that adiponectin is significantly positively associated with sVCAM-1 after multivariate adjustment, similar to a recent cross-sectional study among 264 patients with vascular disease or dyslipidemia (39). This association has been attributed to a possible involvement of adiponectin in the shedding of ectodomains of sVCAM-1 from the endothelial surface. Adiponectin may downregulate effects of sVCAM-1 (39), but the full effects of adiponectin on sVCAM-1 need to be confirmed and the underlying mechanisms need to be fully elucidated. Similar to total adiponectin, we also observed significant inverse relationships of HMW adiponectin with CRP and sE-selectin and a positive association of HMW adiponectin with sVCAM-1, which were independent of other risk factors and biomarkers of inflammatory disease. These data support a recent report that higher HMW adiponectin concentrations are associated with improved endothelial function (42). HMW adiponectin was also independently inversely associated with insulin resistance, as seen previously (8). Thus, the relationships between total and HMW adiponectin with inflammation and insulin resistance may be mediated by direct effects on CRP, sE-selectin, and biomarkers of insulin resistance. Women with higher plasma total and HMW concentrations also had significantly lower HbA1c levels, despite the fact that all women were healthy and within the normal range of HbA1c. This is consistent with adiponectin's role as an insulin sensitizer, as previously proposed.

Strengths of this study include its relatively large size and the use of a well-known cohort of women with excellent validation of diabetes diagnosis (or lack thereof), and measurements of an extensive list of risk factors (including detailed information on diet and lifestyle) as well as biomarkers associated with diabetes and CVD that we could adjust for in the models. Although we have detailed information on lifestyle exposures, which allowed us to control for known CVD and diabetes risk factors in analysis, the potential for residual confounding by unknown factors cannot be eliminated. Analytes were measured using state of the art techniques but random misclassification remains a possibility. This could have attenuated effect estimates but cannot account for the significant results reported herein. We recognize several limitations of this study. We obtained only a single blood measure of adipokines, but this has shown to be a reliable indicator of more than one measurement over time and to be reflective of long-term hormone concentrations. Within-individual variability has been reported to be small for resistin and adiponectin, with correlations of 0.95 and 0.73

respectively over 3 years (43). Biomarkers of insulinemia and insulin secretion were used in lieu of clinical measurements of insulin resistance, but the indicators used herein have been proven to be reliable predictors of insulin resistance in large epidemiology studies such as this one. Observational studies such as the one herein cannot prove causality since they report only associations. Nevertheless, the associations observed in this study were independent of the effects of confounders, including physical activity and other modifiable lifestyle factors. In addition, results of this study may not be generalizable to the entire population since the analysis was limited to healthy women only. Healthy women were the focus of the study since no studies have assessed these adipokines as predictors of risk in healthy women, who either develop or never develop diabetes later in life.

In conclusion, our observations in healthy women confirm previously reported associations of adiponectin with biomarkers of inflammation, endothelial function, and insulin resistance and secretion in other populations and further extend that the association with sE-selectin is independent of lifestyle and medical history factors as well as the other biomarkers of inflammation, endothelial function, and insulin resistance/secretion. We report for the first time that in healthy women, HMW adiponectin is also independently associated with CRP, sE-selectin, and sVCAM-1 concentrations and biomarkers of insulin resistance. We also report that there is a close association between resistin and two biomarkers of inflammation, IL-6 and sTNF- α RII, but circulating resistin concentrations are not associated with biomarkers of endothelial function, insulinemia, insulin secretion, or glycemia. These findings are of both physiological and potential prognostic significance. Future prospective studies are needed to establish and/or confirm prospectively the value of these markers as predictors of risk for developing disease states associated with insulin resistance such as diabetes, CVD, and certain malignancies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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