

Inflammatory Markers, Adiponectin, and Risk of Type 2 Diabetes in the Pima Indian

JONATHAN KRAKOFF, MD¹
TOHRU FUNAHASHI, MD, PHD²
COEN D.A. STEHOUWER, MD³
CASPER G. SCHALKWIJK, MD³
SACHIYO TANAKA, BS²
YUJI MATSUZAWA, MD, PHD²

SAYUKO KOBES, BS¹
P. ANTONIO TATARANNI, MD¹
ROBERT L. HANSON, MD, MPH¹
WILLIAM C. KNOWLER, MD, DRPH¹
ROBERT S. LINDSAY, MB, PHD¹

OBJECTIVE — To examine the association between adiponectin, a known predictor of diabetes in Pima Indians, and markers of inflammation and endothelial function in nondiabetic subjects and to assess whether these markers predict later diabetes in a case-control study within a longitudinal health study in Pima Indians.

RESEARCH DESIGN AND METHODS — Participants with normal glucose tolerance at baseline were selected. Case subjects (who later developed type 2 diabetes), and control subjects ($n = 71$ pairs) were matched for BMI, age, and sex. Adiponectin, C-reactive protein (CRP), interleukin (IL)-6, tumor necrosis factor- α , phospholipase A2 (sPLA2), soluble E-selectin (SE-selectin), soluble intracellular adhesion molecule-1, soluble vascular adhesion molecule-1, and von Willebrand factor (vWF) were measured in baseline samples.

RESULTS — Adiponectin was negatively correlated with CRP ($r = -0.25$, $P < 0.05$), IL-6 ($r = -0.20$, $P < 0.05$), sPLA2 ($r = -0.22$, $P < 0.05$), and SE-selectin ($r = -0.20$, $P < 0.05$). CRP and IL-6 did not predict diabetes. Only vWF predicted the development of diabetes (incidence rate ratio 0.67 for a 1-SD difference, 95% CI 0.41–1.00, $P = 0.05$), but this was not significant after adjustment for age, glucose, HbA_{1c}, waist circumference, and fasting insulin (hazard rate ratio 0.73, 95% CI 0.46–1.16, $P = 0.18$).

CONCLUSIONS — Adiponectin is negatively correlated with markers of inflammation in vivo. In case and control subjects matched for BMI, with the exception of vWF, none of the inflammatory markers predicted diabetes. Adiponectin may be the link between adiposity, inflammation, and type 2 diabetes.

Diabetes Care 26:1745–1751, 2003

Chronic inflammation has been postulated to play a role in the pathogenesis of type 2 diabetes (1). Cross-sectional studies have shown that obesity

and insulin resistance are associated with higher levels of markers of inflammation and endothelial function (2–6). Recent prospective studies have shown a rela-

From the ¹National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona; the ²Department of Internal Medicine and Molecular Science, Osaka University, Osaka, Japan; and the ³Department of Internal Medicine, University Hospital Vrije Universiteit, Amsterdam, the Netherlands.

Address correspondence and reprint requests to Jonathan Krakoff, MD, Diabetes and Arthritis Epidemiology Section, Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, 1550 East Indian School Rd., Phoenix, AZ 85014. E-mail: jkrakoff@mail.nih.gov.

Received for publication 18 July 2002 and accepted in revised form 5 March 2003.

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; IRR, incidence rate ratio; OGTT, oral glucose tolerance test; SE-selectin, soluble E-selectin; sICAM, soluble intracellular adhesion molecule; sPLA2, phospholipase A2; sVCAM, soluble vascular adhesion molecule; TNF- α , tumor necrosis factor- α ; vWF, von Willebrand factor; WBC, white blood cell count.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

© 2003 by the American Diabetes Association.

tionship between various inflammatory markers, specifically sialic acid, orosomucoid, C-reactive protein (CRP), and interleukin (IL)-6, and the risk of developing type 2 diabetes (7–10). In the Pima Indians of Arizona, elevations in serum immunoglobulins and white blood cell count (WBC) have also been found to predict diabetes (11,12). Adiponectin is a 244 amino acid adipose-specific protein (13) that has been shown to downregulate inflammatory responses in vitro (14,15), but it also improves glucose tolerance and insulin resistance in mouse models of diabetes (16). Adiponectin is related to insulin resistance and adiposity in humans (17,18). Recently, we have shown in the subjects of this report that adiponectin is protective against later development of diabetes (19).

We hypothesized that adiponectin might underpin relationships of markers of inflammation, endothelial dysfunction, and obesity and later risk of type 2 diabetes. Therefore, in the same nested case control study mentioned above (19), we examined the relationship of adiponectin to a variety of markers of inflammation and endothelial dysfunction and assessed the relationship of these markers to later incidence of diabetes.

RESEARCH DESIGN AND METHODS

Subjects

Members of the Gila River Indian Community are invited to participate in a longitudinal study of diabetes and its complications. Every 2 years, residents aged ≥ 5 years are asked to participate regardless of health status. Each participant undergoes a physical examination including measurements of height, weight, and waist circumference and a 75-g oral glucose tolerance test (OGTT). Participants were eligible for inclusion in this nested case-control study if they had a normal OGTT (defined as fasting plasma glucose < 110 mg/dl and 2-h plasma glucose < 140 mg/dl) (20) between June of 1990

Table 1—Baseline characteristics for case and control subjects

	Case subjects	Control subjects	Pr > t
n (women/men)	71 (47/24)	71 (47/24)	
Age (years)	32.6 ± 9.3	32.0 ± 8.8	0.38
BMI (kg/m ²)	36.33 ± 6.9	36.29 ± 6.9	0.14
Waist (cm)	112.7 ± 16.9	113.0 ± 17.3	0.81
FPG (mg/dl)	5.31 ± 0.44	5.29 ± 0.36	0.69
2hPG (mg/dl)	6.07 ± 1.01	5.92 ± 1.00	0.36
HbA _{1c} (%)	5.5 ± 0.4	5.3 ± 0.4	0.005
Fasting insulin (pmol/l)	256 (179–338)	265 (179–338)	0.99
2-h insulin (pmol/l)	794 (509–1,167)	937 (517–1,285)	0.82
Follow-up time (years)	4.6 ± 2.2	6.8 ± 2.2	<0.0001
Sample storage time (years)	8.7 ± 1.7	8.7 ± 1.8	0.94

Data are mean ± SD and median (range 25–75%). Follow-up time = last exam or exam at diabetes diagnosis – data of baseline exam; sample storage time = date samples were thawed and aliquoted for measurements (1 June 2001) – date of baseline exam. Pr > |t| is the result of the paired *t* test. 2hPG, 2-h plasma glucose.

and June of 1997 and if their heritage was at least one-half Pima or closely related Tohono O’odham (Papago).

The goal of the present analysis, as a case-control study nested within a longitudinal study, was to efficiently estimate the incidence rate ratio (IRR) for diabetes associated with specific inflammatory markers, i.e., the same IRRs that would be obtained in a longitudinal cohort study. To estimate these parameters without bias, case and control subjects must be selected in a specific fashion (21–23). In essence, case subjects must be incident case subjects (i.e., free of disease at baseline), and for each case subject at least one matched control must be selected from among those who are at risk for the disease at the time the case subject developed the disease (i.e., among those who could contribute person-time in an incidence analysis). In the present study, case subjects who developed incident diabetes according to 1985 World Health Organization criteria (24) following the baseline exam were selected. For each case subject, a control subject (who had not developed type 2 diabetes at the time of the follow-up of the case) was selected from the baseline population matched for age (within 10 years), BMI (within 1 kg/m²), and sex. Where more than one control subject fulfilled these criteria, the subject most closely matched for BMI was chosen.

Because in an incidence analysis someone without disease at one time point is still at risk for disease later, they are included as free of disease at one point and then as having disease at another point. Similarly, if they remain without

disease they are counted as not having disease at all time points for which they have been followed. Therefore, in keeping with this in the analogous nested case-control study, the same control subject can be matched to more than one case subject (in the present study eight control subjects were used twice and one three times). Control subjects can subsequently become case subjects themselves (as occurred twice, one subject was selected as a control twice and then became a case, the other was a control once and a case). Thus, the 71 pairs are derived from 129 participants. If case and control subjects are selected in this manner, the analytic approach calculates a true IRR (23).

Laboratory methods

Plasma glucose was measured by the potassium ferricyanide method (Technicon) or after October 1991 by the hexokinase method (Ciba-Corning). Insulin was measured by immunoassay using a Concept four analyzer (ICN Pharmaceuticals, Costa Mesa, CA). Samples from the 2-h blood draw of the OGTT, stored as EDTA plasma at –70° centigrade, were batch analyzed in duplicate as follows. CRP was measured with a highly sensitive sandwich enzyme immunoassay, using rabbit anti-human CRP immunoglobulin as a catching and detecting antibody (Dako, Copenhagen, Denmark) with intra- and interassay CVs of 4.7 and 2.4%, respectively. IL-6 and tumor necrosis factor-α (TNF-α) were measured by sandwich enzyme immunoassay (Quantikine High Sensitivity; R&D Systems, Oxon, U.K.). Intra- and interassay CVs for IL-6 are

<11.1% and 16.5% and for TNF-α <14.3% and <22.6%, respectively, as reported by the manufacturer. Phospholipase A2 (sPLA2) was measured by enzyme-linked immunosorbent assay (ELISA) with inter- and intra-assay CVs of 7.9 and 6.3%. Soluble E-selectin (SE-selectin), soluble intracellular adhesion molecule (sICAM)-1, and soluble vascular adhesion molecule (sVCAM)-1 were measured by sandwich enzyme immunoassay (R&D Systems). Intra- and interassay CVs were as follows: SE-selectin 1.3 and 5.5%, sICAM-1 3.5 and 5.1%, and sVCAM-1 <8.0 and <8.0%, respectively. Plasma von Willebrand factor (vWF) antigen was measured by ELISA using rabbit anti-vWF antigen IgG as a catching antibody and a peroxidase-conjugated rabbit anti-vWF antigen as a detecting antibody (Dako, Copenhagen, Denmark). Levels of vWF are expressed as a percentage of vWF antigen in normal pooled plasma, which is defined as 100%. The intra- and interassay CVs are 2.3 and 3.8%, respectively. Adiponectin was measured using an ELISA with an adiponectin-specific antibody, as described previously (24). Intra- and interassay CVs were 3.3 and 7.4%, respectively.

Statistical analysis

Statistical analysis was performed using software from the SAS Institute (Cary, NC). All inflammatory and endothelial markers and fasting insulin were log transformed to achieve a normal distribution. A paired *t* test was used to compare baseline metabolic measurements and levels of markers of inflammation and endothelial dysfunction between case and control subjects. Spearman correlations were calculated to examine the relationship between inflammatory and endothelial markers and between baseline metabolic measurements and these markers for the 129 individuals in the study (so each individual was only included once in this analysis). The IRRs for the development of diabetes were calculated by conditional logistic regression, maintaining the pairwise matching of case and control subjects. To facilitate comparisons between measurements, IRRs were calculated for a 1-SD difference for all variables except age.

Table 2—Relationships between inflammatory and endothelial markers

	Adiponectin	CRP	IL-6	TNF- α	sPLA2	sE-selectin	sICAM-1	sVCAM-1	vWF
Adiponectin	1.0	-0.25 [†]	-0.20 *	0.002	-0.22 *	-0.20 *	-0.13	0.02	0.03
CRP		1.0	0.56 [†]	0.11	0.43 [†]	0.14	0.29 [†]	0.16	-0.03
IL-6			1.0	0.14	0.36 [†]	0.13	0.22 *	0.11	-0.0005
TNF- α				1.0	0.25 [†]	0.16	0.12	0.14	0.05
sPLA2					1.0	0.21 *	0.34 [†]	0.18 *	0.08
sE-selectin						1.0	0.36 [†]	0.26 [†]	0.14
sICAM-1							1.0	0.30 [†]	0.06
sVCAM-1								1.0	0.52 [†]
vWF									1.0

Spearman correlations: * $P < 0.05$, $\dagger P < 0.01$, $\ddagger P < 0.001$. Spearman r values in bold when $P < 0.05$. Each individual was included only once, because of missing values there were 127 individuals with measurements of CRP, TNF- α , sPLA2, sE-selectin, sICAM-1, sVCAM-1, and vWF; 126 with measurements of IL-6; and 128 with measurements of adiponectin.

RESULTS

Study subjects

Table 1 shows the baseline characteristics of the case and control subjects. Case and control subjects had similar age, BMI, fasting and 2-h glucose, and fasting and 2-h insulin concentrations. Baseline HbA_{1c} was slightly higher in case compared with control subjects (5.5 vs. 5.3%, $P = 0.005$). The mean follow-up time was longer for the control subjects, but there was no systematic difference in sample storage time between case and control subjects.

Correlations between variables

Relationships between the markers in all subjects are shown in Table 2. Adiponectin was significantly and negatively correlated with CRP, IL-6, sPLA2, and SE-selectin ($r = -0.25, -0.20, -0.22,$ and -0.20 , respectively, $P < 0.05$). CRP was highly correlated with IL-6 ($r = 0.56, P < 0.001$), sPLA2 ($r = 0.43, P < 0.001$) and was also positively related to sICAM-1 ($r = 0.29, P < 0.01$). IL-6 also positively correlated with sICAM-1 ($r = 0.22, P < 0.05$). SE-selectin was correlated with sICAM-1 and sVCAM-1 ($r = 0.36, P < 0.001$ and $r = 0.26, P < 0.01$, respectively). VWF was strongly associated with sVCAM-1 ($r = 0.52, P < 0.001$). Relationships of these markers to baseline BMI, waist circumference, and fasting and 2-h glucose and insulin concentrations are shown in Table 3. BMI and waist circumference were negatively correlated with adiponectin and positively correlated with CRP, IL-6, sPLA2, and SE-selectin. Fasting insulin was negatively correlated with adiponectin and TNF- α and positively correlated with SE-selectin. Partial correlations with adjustments for

the effect of sex or group (whether a subject was a case or a control) did not alter the results (data not shown). The effect of the interaction between group and each variable was also tested in a general linearized model and was not significant. Only adiponectin was significantly different between case and control subjects at baseline, i.e., lower in case subjects (Table 4). Correlations between baseline fasting and 2-h plasma glucose and HbA_{1c} were also calculated. There was no statistically significant correlation between these variables at the baseline exam.

Predictors of diabetes

In a conditional logistic regression analysis of baseline variables (Figs. 1 and 2), CRP and IL-6 were not predictive of the development of diabetes in the univariate model (IRR 1.03, 95% CI 0.68–1.55, $P = 0.90$ and RR 0.91 95% CI 0.60–1.38, $P = 0.65$, respectively), or in the multivariate

model adjusted for age, fasting, and 2-h glucose, HbA_{1c}, waist circumference, and fasting insulin (IRR 0.96, 95% CI 0.60–1.55, $P = 0.88$ and IRR = 0.75 95% CI 0.45–1.30, $P = 0.28$, respectively). In comparison, our previously reported findings in these subjects (18) showed that adiponectin predicted type 2 diabetes in both the univariate and multivariate models (IRR 0.63, 95% CI 0.43–0.92, $P = 0.02$ for univariate model and IRR 0.63, 95% CI 0.41–0.98, $P = 0.04$ for multivariate model). In the present study, the IRR is slightly different in the multivariate model because of the inclusion of HbA_{1c}. VWF was marginally protective for the development of diabetes in the univariate model (IRR 0.67, 95% CI 0.45–1.00, $P = 0.05$), and this effect was attenuated after adjustment for the same covariates (IRR 0.73, 95% CI 0.46–1.16, $P = 0.19$). Although SE-selectin had an IRR > 1.0 , it was not significantly predic-

Table 3—Relationships between inflammatory and endothelial markers and baseline measurements

	BMI	Waist	FPG	2hPG	HbA _{1c}	Fasting insulin	2-h insulin
Adiponectin	-0.22 *	-0.18 *	-0.16	-0.20 *	0.01	-0.25 [†]	-0.29 [†]
CRP	0.53 [†]	0.50 [†]	0.05	0.12	0.12	0.04	0.08
IL-6	0.57 [†]	0.56 [†]	0.09	0.07	0.05	0.09	0.14
TNF- α	-0.08	-0.14	0.03	-0.11	-0.28 [†]	-0.19 *	-0.06
sPLA2	0.23 [†]	0.20 *	0.10	0.02	-0.05	0.04	0.11
sE-selectin	0.22 *	0.24 [†]	0.29 [†]	-0.04	-0.07	0.21 *	0.05
sICAM-1	0.17	0.19 *	0.16	0.13	-0.14	0.14	0.17
sVCAM-1	0.21 *	0.22 *	0.05	0.03	-0.05	0.09	0.24 [†]
vWF	0.05	0.03	0.08	0.03	-0.12	-0.03	0.08

Spearman correlations: * $P < 0.05$, $\dagger P < 0.01$, $\ddagger P < 0.001$. Spearman r values in bold when $P < 0.05$. 2hPG, 2-h plasma glucose. Each individual was included only once, because of missing values there were 127 individuals with measurements of CRP, TNF- α , sPLA2, sE-selectin, sICAM-1, sVCAM-1, and vWF; 126 with measurements of IL-6; and 128 with measurements of adiponectin.

Table 4—Comparison of inflammatory and endothelial markers between case and control subjects

	Case subjects	Control subjects	Pr> t
Adiponectin (μg/ml)	4.3 (3.6–5.8)	5.3 (4.3–6.3)	0.01
CRP (mg/l)	4.5 (2.5–8.3)	4.9 (2.1–8.6)	0.98
IL-6 (pg/ml)	5.2 (3.3–6.2)	4.9 (4.0–6.2)	0.86
TNF-α (pg/ml)	3.2 (2.4–5.2)	3.0 (2.4–4.1)	0.80
sPLA2 (ng/ml)	3.0 (2.2–4.1)	3.3 (2.2–4.5)	0.72
sE-selectin (ng/ml)	75 (66–94)	76 (59–94)	0.68
sICAM-1 (ng/ml)	324 (263–364)	338 (298–385)	0.40
sVCAM-1 (ng/ml)	499 (413–550)	490 (444–562)	0.26
vWF (%)	92 (65–128)	106 (80–132)	0.17

Data are median ± interquartile range. Calculations are from 70 case-control subject pairs for adiponectin and from 68 case-control subject pairs for all other markers except IL-6, which was calculated for 66 case-control subject pairs.

tive for the development of diabetes in the univariate and multivariate models (IRR 1.12, 95% CI 0.82–1.55, *P* = 0.53 and IRR 1.34 95% CI 0.91–1.99, *P* = 0.14, respectively). The protective effect of adiponectin was not altered by including CRP, SE-selectin, vWF, or any other markers to the models either alone or in combination (data not shown).

CONCLUSIONS— The finding that low adiponectin level is associated with increased risk of diabetes in Pimas has been previously published (18) and recently corroborated in another population (25). In the present analysis, we extend this observation by assessment of both the correlation of adiponectin to markers of inflammation and endothelial

function and the relationship between these markers and incidence of diabetes. Despite strong correlations between many of these markers and the metabolic measurements at baseline, only adiponectin and vWF were significantly related to the later development of type 2 diabetes, and the effect of vWF was attenuated after controlling for multiple variables. Other inflammatory markers, most notably CRP and IL-6, were not related to later diabetes. Furthermore, the relationship between adiponectin and diabetes incidence persisted with control for these markers.

The design of the present analysis is a standard epidemiological design for a case-control study nested within a longitudinal study. Although certain aspects of this design may be counterintuitive (in

that control subjects may be selected at one time point and then become case subjects at a subsequent time point and also that control subjects may be selected more than once), the assumptions in this design are the same as for a longitudinal cohort study of disease incidence (21–23). Because case and control subjects were selected in this manner, the risk ratio we calculated is a true unbiased IRR that takes into account the effect of time (23). Furthermore, if we ignore the matching and analyzed each individual in a conventional proportional hazards model, the results are very similar to those presented here (data not shown).

Our results provide further in vivo evidence that adiponectin concentrations correlate with markers of inflammation and endothelial dysfunction (26). These correlations are modest but present in four of the markers measured including three (CRP, IL-6, and sPLA2) generally associated with subclinical inflammation. In vitro, adiponectin modulates the immune system in several ways. Adiponectin is structurally similar to TNF-α (27) and inhibits TNF-α production by macrophages (14). Adiponectin also inhibits the expression of sICAM-1, sVCAM-1, and SE-selectin in cultured endothelial cells (15) acting via necrosis factor-κB signaling pathways (28), a pathway crucial to inflammatory response (29). Thus, the inverse correlations of adiponectin with inflammatory markers observed in the present study support the idea that adiponectin is associated with anti-inflammatory activity.

The other markers in this study were intercorrelated, with strong relationships of CRP to IL-6 and between the endothelial markers. Although the CVs for IL-6 and TNF-α were larger than for other markers, in general, relationships between markers and metabolic measures correspond to those previously reported in other series. In particular, adiponectin was negatively correlated with BMI and fasting and 2-h insulin, consistent with previous observations in the Pima Indians (17). CRP and IL-6 were positively associated with both BMI and waist circumference, and coefficients were similar to those in other studies (30,31). The exception to this is TNF-α, which was negatively correlated with fasting insulin. Although TNF-α has been thought to play an important role in mediating the insulin resistance of obesity (32), the in vivo re-

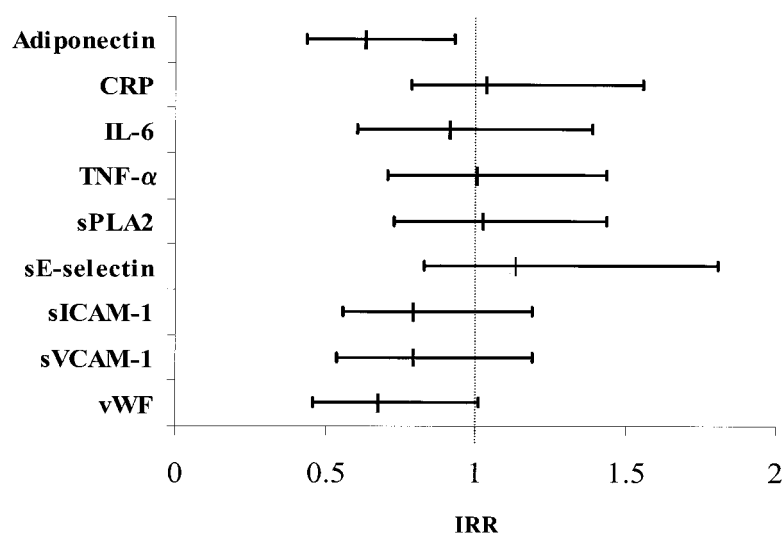


Figure 1—Prediction of type 2 diabetes by inflammatory markers, univariate model. All markers were log transformed. IRR was calculated using conditional logistic regression with values standardized to a mean of 0 and an SD of 1.

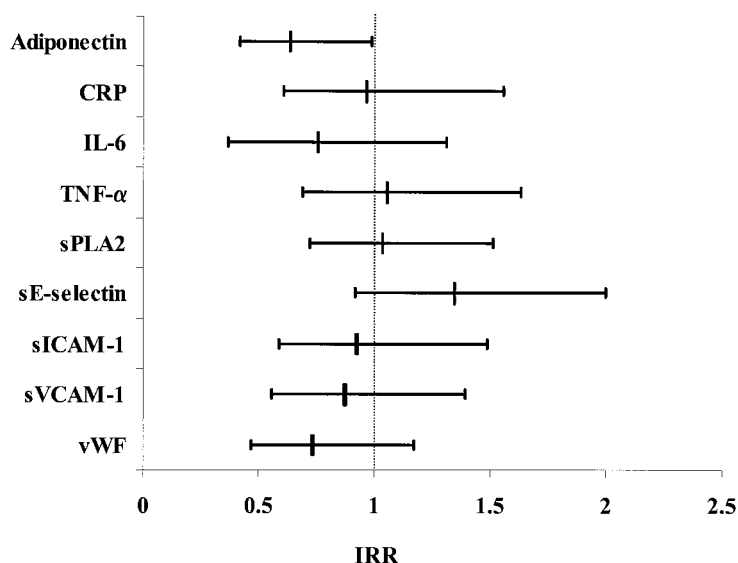


Figure 2—Prediction of type 2 diabetes by inflammatory markers, multivariate model. All markers were log transformed. IRR was calculated using conditional logistic regression with values standardized to a mean of 0 and an SD of 1. The model was adjusted for age, waist circumference, fasting and 2-h plasma glucose, HbA_{1c}, and fasting insulin.

relationship of TNF- α to insulin sensitivity has been less clear. TNF- α is inversely correlated with insulin sensitivity (33,34) in some studies (33,34) but not in others (35,36).

A number of explanations for the relationship of markers of inflammation with both obesity and insulin resistance have been proposed. TNF- α has been proposed as a mediator of obesity-related insulin resistance (32,37). However, knockout of TNF- α receptors in mouse models (38) and investigations using anti-TNF- α antibodies in humans (39) have not supported this. More recently, activation of the I κ K kinase β (I κ K β) pathway by inflammation has been found to influence insulin resistance in mouse models (40) and via other pathways perhaps mediated via TNF- α (32). In our study, despite the correlation between markers and adiposity and insulin resistance, only low adiponectin predicted diabetes after adjustment for other baseline variables. In other longitudinal studies, markers of inflammation did predict later type 2 diabetes. WBC, sialic acid, and orosomucoid predicted the development of type 2 diabetes in subjects in the Atherosclerosis Risk in Communities Study (ARIC) (7), as did CRP in the Cardiovascular Health Study (9), the Women's Health Study (8), and the West of Scotland Coronary Prevention Study (10). Although these studies also controlled statistically for the

effects of obesity, there were substantial baseline differences in the degree of adiposity between subjects who developed diabetes and those who did not.

In contrast, in our study, we closely matched case and control subjects by BMI and they did not differ by waist circumference. Having matched for adiposity, there were no predictive effects for CRP or IL-6 and diabetes. Taken together, our findings suggest an alternate hypothesis: that the relationship of these other markers of subclinical inflammation to the development of type 2 diabetes is mediated by adiponectin. Adiponectin, while having anti-inflammatory activity, may mediate diabetes risk via mechanisms other than inflammation. If so, given the negative correlations between adiponectin and CRP and SE-selectin, the predictive value of inflammatory markers in other studies may have been because they are associated with obesity and were also acting as surrogate markers of hypo adiponectinemia. Thus, they may be only indirectly associated with the development of diabetes.

It is possible that an effect of CRP and SE-selectin on the development of diabetes might have been apparent in a larger study. The data from the Women's Health Study (8) and the Cardiovascular Health Study (9) were drawn from larger cohorts, although the number of subjects who developed type 2 diabetes was only substan-

tially higher in the Women's Health Study (188 cases of diabetes). This suggests that the relationship of these markers to the development of diabetes is relatively weak and that adiponectin plays a far larger and potentially biologically important role than these other markers.

Compared with other populations, Pima Indians are at very high risk for diabetes and obesity. If inflammatory markers are higher in general, this might reduce the power of the study to detect an effect of such a marker. For example, the levels of CRP at the baseline exams are higher than in other studies (8,9,41). This is expected given the higher BMIs in Pimas and the relationship of CRP to adiposity. This may limit the generalizability of our results to other populations. Nevertheless, our data suggest at least within our population during this follow-up period, inflammatory markers such as CRP and IL-6 are less important than adiponectin. As with any observational study, the present analysis can determine to what extent inflammatory markers are predictive of diabetes in this population, but the effects of any interventions targeting inflammation would need to be assessed by clinical trials.

HbA_{1c} was slightly higher in case than control subjects at baseline, even though concomitant OGTTs showed both to be normal glucose tolerant, and there was no difference in fasting or 2-h glucose. Despite this difference, the IRRs did not change substantially in the models that included HbA_{1c}.

The higher levels of vWF in control subjects and its protective effect for the development of diabetes are at odds with previous reports. A number of studies have found that vWF is higher in subjects with type 2 diabetes (42,43). In the ARIC study, higher levels of vWF were predictive for the development of diabetes, particularly in women, but the effect decreased substantially after adjusting for BMI and waist-to-hip ratio (39). A smaller study in the Pima population found that vWF is positively correlated with insulin resistance, but not correlated with percentage body fat (6). Studies in other populations have found no difference in vWF levels between insulin-resistant relatives of subjects with type 2 diabetes and control subjects (44). Therefore, the correlation between vWF and adiposity and insulin resistance, two major risk factors for type 2 diabetes, is not clear. VWF was

not strongly correlated with any of the metabolic measurements in the present study (Table 3) but was protective for the development of diabetes. While vWF is a marker of endothelial dysfunction, it is elevated in conditions of vascular insult and exercise to a similar extent (45). Low levels of physical activity itself are a risk factor for the development of diabetes (46). Therefore, subjects who are less physically active may have lower vWF levels.

In summary, we have extended our previous findings on adiponectin, demonstrating that adiponectin is negatively correlated with markers on inflammation in vivo. Moreover, once adiposity is taken into account, these other markers have little or no predictive value for the development of diabetes in Pima Indians. Adiponectin may be an important link between adiposity and inflammation and type 2 diabetes.

Acknowledgments— T.F., Y.M., and S.T. have collaborated with the Otsuka Pharmaceutical Company to establish a kit for measuring plasma adiponectin.

We thank the members of the Gila River Indian Community for their continued support and participation and the staff of the Diabetes and Arthritis Epidemiology Section for help in this study.

References

- Pickup JC, Crook MA: Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia* 41:1241–1248, 1998
- Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB: Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 282:2131–2135, 1999
- Hak AE, Stehouwer CD, Bots ML, Polderman KH, Schalkwijk CG, Westendorp IC, Hofman A, Witteman JC: Associations of C-reactive protein with measures of obesity, insulin resistance, and subclinical atherosclerosis in healthy, middle-aged women. *Arterioscler Thromb Vasc Biol* 19:1986–1991, 1999
- Roytblat L, Rachinsky M, Fisher A, Greemberg L, Shapira Y, Douvdevani A, Gelman S: Raised interleukin-6 levels in obese patients. *Obes Res* 8:673–675, 2000
- Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE: Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res* 9:414–417, 2001
- Weyer C, Yudkin JS, Stehouwer CD, Schalkwijk CG, Pratley RE, Tataranni PA: Humoral markers of inflammation and endothelial dysfunction in relation to adiposity and in vivo insulin action in Pima Indians. *Atherosclerosis* 161:233–242, 2002
- Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G: Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet* 353:1649–1652, 1999
- Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286:327–334, 2001
- Barzilay JI, Abraham L, Heckbert SR, Cushman M, Kuller LH, Resnick HE, Tracy RP: The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. *Diabetes* 50:2384–2389, 2001
- Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GD, O'Reilly DS, Packard CJ, Sattar N: C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. *Diabetes* 51:1596–1600, 2002
- Lindsay RS, Krakoff J, Hanson RL, Bennett PH, Knowler WC: Gamma globulin levels predict type 2 diabetes in the Pima Indian population. *Diabetes* 50:1598–1603, 2001
- Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA: High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 51:455–461, 2002
- Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M: Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *J Biochem (Tokyo)* 120:803–812, 1996
- Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y: Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 96:1723–1732, 2000
- Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y: Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 100:2473–2476, 1999
- Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadawaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946, 2001
- Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA: Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 86:1930–1935, 2001
- Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83, 1999
- Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J: Adiponectin protects against development of type 2 diabetes in the Pima Indian population. *Lancet* 360:57–58, 2002
- Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
- Wacholder S, Silverman DT, McLaughlin JK, Mandel JS: Selection of controls in case-control studies. III. Design options. *Am J Epidemiol* 135:1042–1050, 1992
- Lubin JH, Gail MH: Biased selection of controls for case-control analyses of cohort studies. *Biometrics* 40:63–75, 1984
- Miettinen O: Estimability and estimation in case-referent studies. *Am J Epi* 103:226–235, 1975
- World Health Organization: *Second Report of the WHO Expert Committee on Diabetes Mellitus*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
- Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boenig H, Pfeiffer AF: Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 361:226–228, 2003
- Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, Okamoto Y, Ohashi K, Nagaretani H, Kishida K, Nishizawa H, Maeda N, Kobayashi H, Hiraoka H, Matsuzawa Y: Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* 107:671–674, 2003
- Shapiro L, Scherer PE: The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor ne-

- crosis factor. *Curr Biol* 8:335–338, 1998
28. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y: Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF- κ B signaling through a cAMP-dependent pathway. *Circulation* 102:1296–1301, 2000
 29. Barnes PJ, Karin M: Nuclear factor- κ B: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336:1066–1071, 1997
 30. Festa A, D'Agostino R, Howard G, Mykkanen L, Tracy RP, Haffner SM: Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: the Insulin Resistance Atherosclerosis Study. *Kidney Int* 58:1703–1710, 2000
 31. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW: C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 19:972–978, 1999
 32. Hotamisligil GS: The role of TNF α and TNF receptors in obesity and insulin resistance. *J Intern Med* 245:621–625, 1999
 33. Mishima Y, Kuyama A, Tada A, Takahashi K, Ishioka T, Kibata M: Relationship between serum tumor necrosis factor- α and insulin resistance in obese men with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 52:119–123, 2001
 34. Lofgren P, van Harmelen V, Reynisdottir S, Naslund E, Ryden M, Rossner S, Arner P: Secretion of tumor necrosis factor- α shows a strong relationship to insulin-stimulated glucose transport in human adipose tissue. *Diabetes* 49:688–692, 2000
 35. Ronnema T, Pulkki K, Kaprio J: Serum soluble tumor necrosis factor- α receptor 2 is elevated in obesity but is not related to insulin sensitivity: a study in identical twins discordant for obesity. *J Clin Endocrinol Metab* 85:2728–2732, 2000
 36. Blüher M, Kratzsch J, Paschke R: Plasma levels of tumor necrosis factor- α , angiotensin II, growth hormone, and IGF-I are not elevated in insulin-resistant obese individuals with impaired glucose tolerance. *Diabetes Care* 24:328–334, 2001
 37. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor- α in human obesity and insulin resistance. *J Clin Invest* 95:2409–2415, 1995
 38. Schreyer SA, Chua SC, Jr., LeBoeuf RC: Obesity and diabetes in TNF- α receptor-deficient mice. *J Clin Invest* 102:402–411, 1998
 39. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R: Effects of an engineered human anti-TNF- α antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* 45:881–885, 1996
 40. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE: Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of I κ B β . *Science* 293:1673–1677, 2001
 41. Festa A, D'Agostino R, Howard G, Mykkanen L, Tracy RP, Haffner SM: Chronic subclinical inflammation as part of the insulin resistance syndrome. *Circulation* 102:42–47, 2000
 42. Duncan BB, Schmidt MI, Offenbacher S, Wu KK, Savage PJ, Heiss G: Factor VIII and other hemostatic variables are related to incident diabetes in adults. *Diabetes Care* 22:767–772, 1999
 43. Seligman BG, Biolo A, Polanczyk CA, Gross JL, Clausell N: Increased plasma levels of endothelin 1 and von Willebrand factor in patients with type 2 diabetes and dyslipidemia. *Diabetes Care* 23:1395–1400, 2000
 44. Mansfield MW, Heywood DM, Grant PJ: Circulating levels of factor VII, fibrinogen, and von Willebrand factor and features of insulin resistance in first-degree relatives of patients with NIDDM. *Circulation* 94:2171–2176, 1996
 45. van Mourik JA, Boertjes R, Huisveld IA, Fijnvandraat K, Pajkrt D, van Genderen PJ, Fijnheer R: von Willebrand factor propeptide in vascular disorders: a tool to distinguish between acute and chronic endothelial cell perturbation. *Blood* 94:179–185, 1999
 46. Burchfiel CM, Sharp DS, Curb JD, Rodriguez BL, Hwang LJ, Marcus EB, Yano K: Physical activity and incidence of diabetes: the Honolulu Heart Program. *Am J Epidemiol* 141:360–368, 1995