High White Blood Cell Count Is Associated With a Worsening of Insulin Sensitivity and Predicts the Development of Type 2 Diabetes

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Chronic low-grade inflammation may be involved in the pathogenesis of insulin resistance and type 2 diabetes. We examined whether a high white blood cell count (WBC), a marker of inflammation, predicts a worsening of insulin action, insulin secretory function, and the development of type 2 diabetes in Pima Indians. We measured WBC in 352 nondiabetic Pima Indians (215 men and 137 women, aged 27 ± 6 years [means ± SD], body fat 32 ± 8%, WBC 8,107 ± 2,022 cells/mm³) who were characterized for body composition (by hydrodensitometry or dual-energy X-ray absorptiometry), glucose tolerance (by 75-g oral glucose tolerance test), insulin action (M; by hyperinsulinemic clamp), and acute insulin secretory response (AIR; by 25-g intravenous glucose challenge). Among 272 subjects who were normal glucose tolerant (NGT) at baseline, 54 developed diabetes over an average follow-up of 5.5 ± 4.4 years. Among those who remained nondiabetic, 81 subjects had follow-up measurements of M and AIR. Cross-sectionally, WBC was related to percent body fat ($r = 0.32, P < 0.0001$) and M ($r = -0.24, P < 0.0001$), but not to AIR ($r = 0.06, P = 0.4$). In a multivariate analysis, when adjusted for age and sex, both percent body fat ($P < 0.0001$) and M ($P = 0.03$) were independently associated with WBC. A high WBC value predicted diabetes (relative hazard 90th vs. 10th percentiles [95%CI] of 2.7 [1.3–5.4], $P = 0.007$) when adjusted for age and sex. The predictive effect of WBC persisted after additional adjustment for established predictors of diabetes, i.e., percent body fat, M, and AIR (relative hazard 2.6 [1.1–6.2], $P = 0.03$). After adjustment for follow-up duration, a high WBC at baseline was associated with a subsequent worsening of M ($P = 0.003$), but not a worsening of AIR. A high WBC predicts a worsening of insulin action and the development of type 2 diabetes in Pima Indians. These findings are consistent with the hypothesis that a chronic activation of the immune system may play a role in the pathogenesis of type 2 diabetes. *Diabetes* 51:455–461, 2002

Activation of the immune system and inflammation may be detected by an increase in a number of markers, including white blood cell count (WBC) and cytokine and plasminogen activator inhibitor-1 (PAI-1) concentrations. Because of the cross-sectional relationship between markers of inflammation and insulin resistance and/or type 2 diabetes (1–4), it has been suggested that a chronic low-grade activation of the immune system may play a role in the pathogenesis of type 2 diabetes. To date, there is some evidence from prospective studies in Pima Indians and other populations to support the hypothesis that altered markers of inflammation, such as a high WBC, plasma fibrinogen, PAI-1, gamma globulin, and lower albumin concentrations, are associated with the later development of type 2 diabetes (5–8). However, the mechanisms by which these markers of inflammation contribute to the development of type 2 diabetes are largely unknown.

In the present study, we examined the pathophysiological role of an activated immune system, as assessed by elevated WBC, in the development of type 2 diabetes in Pima Indians, a population with marked insulin resistance and one of the highest reported prevalence rates of type 2 diabetes in the world (9). Our aim was to examine *1*) whether a high WBC was associated with later development of type 2 diabetes; *2*) if so, whether these associations were independent of adiposity, insulin action, and insulin secretion; and *3*) whether a high WBC was associated with a subsequent decrease in insulin sensitivity and/or insulin secretion.

RESEARCH DESIGN AND METHODS

Subjects in this study were participants in a longitudinal study of the pathogenesis of type 2 diabetes that was initiated in 1982 (10). All participants were Pima (or closely related Papago) Indians from the Gila River Indian Community near Phoenix, Arizona. Subjects were invited at approximately annual intervals for repeat 75-g oral glucose tolerance tests (OGTTs) and an assessment of body composition, insulin sensitivity, and insulin secretion. All subjects were between 18 and 50 years of age, nondiabetic (normal glucose tolerant [NGT] or impaired glucose tolerant [IGT]) at baseline according to OGTT values (World Health Organization 1985 criteria) (11), and nonsmokers at the time of the study. Acute and chronic inflammation was excluded on the basis of medical history, physical examination, and routine laboratory tests, including measurement of oral temperature, WBC, and urinalysis. Subjects who had evidence of serious medical conditions (including autoimmune, cerebrovascular, and ischemic heart disease) or were taking any medications were excluded. The protocol was approved by the Tribal Council of the Gila River Indian Community and by the Institutional Review Board of the National

TABLE 1
Physical and metabolic characteristics of the study populations in the cross-sectional, prospective, and longitudinal analyses

	Cross-sectional analysis		Prospective analysis		Longitudinal analysis	
	Mean ± SD	Range	Nonprogressors	Progressors	Baseline	Follow-up
n	352		218	54	81	81
Age (years)	27 ± 6	18–47	27 ± 6	27 ± 6	26 ± 6	30 ± 7
Body weight (kg)	94.3 ± 21.8	49.5–181.1	91.0 ± 22.2	97.8 ± 21.1*	92.9 ± 24.3	98.9 ± 26.0†
Body fat (%)	32 ± 8	13–49	30 ± 8	35 ± 7†	30 ± 8	32 ± 8†
Waist (cm)	42.4 ± 6.7	26.0–66.5	41.3 ± 7.0	43.6 ± 6.0	43.6 ± 7.5†	43.6 ± 7.5
Waist-to-thigh ratio	1.65 ± 0.17	1.21–2.25	1.63 ± 0.16	1.64 ± 0.17	1.62 ± 0.13	1.70 ± 0.15†
Fasting plasma glucose (mmol/l)	4.8 ± 0.6	3.4–6.6	4.7 ± 0.6	5.2 ± 0.6†	4.8 ± 0.5	4.9 ± 0.4*
Fasting plasma insulin (pmol/l)	240 ± 108	54–738	222 ± 102	246 ± 102	198 ± 90	228 ± 90†
2-h plasma glucose (mmol/l)	6.6 ± 1.6	2.6–10.9	5.8 ± 1.2	6.7 ± 0.8†	5.7 ± 1.1	6.8 ± 1.7†
M-low	2.7 ± 1.1	1.3–8.9	3.00 ± 1.31	2.37 ± 0.70‡	3.1 ± 1.4	2.6 ± 0.9†
M-high (mg/kg EMBS/min)	8.8 ± 2.1	3.2–14.2	9.28 ± 2.08	8.59 ± 1.82*	9.2 ± 2.1	8.7 ± 2.2*
AIR (pmol/l)	1,482 ± 996	6–7,518	1590 ± 954	1212 ± 792*	1,554 ± 942	1,632 ± 1,080
Basal EGO (mg/kg EMBS/min)	1.91 ± 0.23	1.32–2.60	1.91 ± 0.25	1.91 ± 0.26	1.91 ± 0.24	1.96 ± 0.25
WBC (cell/mm^3)	8,107 ± 2,021	3,700–1,1900	7,877 ± 1,993	8,555 ± 1,780†	7,643 ± 1,997	8,215 ± 2,089*
Neutrophil count (cell/mm^3)	4,977 ± 1,760	1,820–1,1587	4,900 ± 1,727	5,110 ± 1,655	4,637 ± 1,710	5,003 ± 1,6204
Lymphocyte count (cell/mm^3)	2,441 ± 78	168–5,593	2,373 ± 696	2,576 ± 813	2,357 ± 723	2,481 ± 789
Monocyte count (cell/mm^3)	440 ± 250	0–1,118	478 ± 235	352 ± 290§	433 ± 216	476 ± 185
Eosinophil count (cell/mm^3)	196 ± 157	0–720	189 ± 155	225 ± 204	217 ± 158	204 ± 144
Basophil count (cell/mm^3)	40 ± 52	0–246	47 ± 6	47 ± 13	50 ± 5	68 ± 8*

Data are means ± SD. Significant differences between progressors and nonprogressors in the prospective analysis and between baseline and follow-up variables in the longitudinal analysis are indicated as follows: *$P < 0.05$, †$P < 0.001$, ‡$P < 0.0001$, and §$P < 0.01$. EMBS = fat-free mass + 17.7 kg. EGO, endogenous glucose output.

Institute of Diabetes and Digestive and Kidney Diseases, and all subjects provided written informed consent before participation.

A total of 352 nondiabetic (272 NGT and 80 IGT) Pima Indian subjects, 137 women and 215 men, who had been characterized for total WBC, body composition, glucose tolerance, fasting plasma insulin concentrations, insulin action (M), and acute insulin secretory response (AIR), were included in the cross-sectional analyses (Table 1). A subgroup was characterized for differential WBC (209 subjects, 87 women and 122 men). Prospective analyses were performed in 272 Pima Indian subjects who were NGT at baseline and had a follow-up OGTT. Among them, 54 subjects had developed diabetes (progressors), whereas 218 had remained nondiabetic (nonprogressors) after an average follow-up of 5.5 ± 4.4 years (range 1 month to 17 years). Longitudinal analyses were performed in 81 nonprogressors, who were NGT at baseline and nondiabetic at follow-up and had a follow-up assessment of total WBC, body composition, fasting plasma insulin concentrations, M, and AIR.
Methods. All subjects were admitted for 8–10 days to the National Institutes of Health Clinical Research Unit in Phoenix, Arizona, were fed a weight-maintaining diet (50, 30, and 20% of daily calories provided as carbohydrate, fat, and protein, respectively), and abstained from strenuous exercise.

Total and differential WBC were evaluated on admission. WBC was measured in the local laboratory by an automated cell counter. Reliability coefficients, based on blind replicate control data, ranged from 0.96 to 1.00. Body composition was estimated by underwater weighing, with simultaneous determination of residual lung volume by helium dilution (12) or by total-body dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) as previously described (13).

At least 3 days after admission and after a 12-h overnight fast, subjects underwent a 2-h 75-g OGTT to exclude diabetes (10). Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA) and plasma insulin concentrations by an automated immunoassay (Access; Beckman Instruments).

Insulin action was assessed at physiological and supraphysiological insulin concentrations during a two-step hyperinsulinemic-euglycemic glucose clamp as previously described (14). In brief, after an overnight fast, a primed continuous intravenous insulin infusion was administered for 100 min at a constant rate of 290 pmol · m^{-2} body surface area · min^{-1} (40 mU · m^{-2} · min^{-1}; low dose), followed by a second 100-min infusion at a rate of 2,900 pmol · m^{-2} · min^{-1} (400 mU · m^{-2} · min^{-1}; high dose). These infusions achieved steady-state plasma insulin concentrations of 888 ± 252 pmol/l and 1,6320 ± 6,540 pmol/l (means ± SD), respectively. The rate of total insulin-stimulated glucose disposal (M) was calculated for the last 40 min of the low-dose (M-low) and high-dose (M-high) insulin infusions. M-low was also corrected for the rate of endogenous glucose output (measured by a primed [30 μCi], continuous [0.3 μCi/min] 3-[^{3}H]glucose infusion) (14).

M-values were adjusted for the steady-state plasma glucose and insulin concentrations as previously described (14) and were normalized to the estimated metabolic body size (EMBS = fat-free mass + 17.7 kg).

AIR was measured in response to a 25-g intravenous glucose tolerance test and calculated as the average incremental plasma insulin concentration from the 3rd to the 5th min after the glucose bolus (15). Because even mildly elevated glucose concentrations can secondarily affect insulin secretion, only data from NGT subjects were included in the analyses performed with AIR.
Statistical analyses. Statistical analyses were performed using the software of the SAS Institute (Cary, NC). Results are given as means ± SD (unless indicated otherwise). The values for WBC, fasting insulin, M-low, and AIR were logarithmically transformed before analysis to approximate normal distributions.

In the cross-sectional analyses, we examined the relationship between WBC and anthropometric and metabolic variables using Spearman correlations. Multiple linear regression models were used to examine the relationships after adjusting for covariates. Sex differences and differences between NGT and IGT subjects were assessed by unpaired t test.

In the prospective analyses, we assessed the metabolic predictors of diabetes. Risk factors for progression from NGT to diabetes were estimated by proportional-hazard analyses. The effects of total and differential WBC, M, and AIR were expressed as relative hazards and were evaluated at the 10th and 90th percentiles of predictor variables. We calculated the relative hazard estimates, i.e., the risk of developing diabetes of a hypothetical subject at the 90th percentile compared with the hazard of a hypothetical subject at the 10th percentile (or at the 10th and 90th percentiles in case of a negatively related variable). For each relative hazard, the 95% CI was given.

In the longitudinal analyses, the effect of baseline total WBC on change (follow-up adjusted for baseline) in percent body fat, fasting plasma insulin concentration, M-low, and AIR were evaluated using multiple linear regression models. Models were adjusted for sex, follow-up age, and the time of follow-up. Change in fasting insulin concentrations and M-low were additionally adjusted for change in percent body fat, whereas the change in AIR was adjusted for changes in percent body fat and M-low (Table 4).

RESULTS

The anthropometric and metabolic characteristics of the study populations for the cross-sectional, prospective, and longitudinal analyses are summarized in Table 1.
Cross-sectional analysis. In a cross-sectional analysis of 352 nondiabetic Pima Indians, the mean WBC tended to be higher in IGT subjects compared with NGT subjects

TABLE 2
Univariate relationships among markers of inflammation with selected anthropometric and metabolic characteristics (correlations by Spearman)

	WBC	Neutrophil count	Lymphocyte count	Monocyte count	Eosinophil count	Basophil count
n	352	209	209	209	209	209
Age	−0.19*	−0.05	−0.09	−0.05	−0.02	0.06
Body weight	0.27*	0.24†	0.08	0.01	0.07	0.05
Body fat	0.31*	0.21†	0.18‡	−0.18‡	0.09	0.06
Waist	0.29*	0.27†	0.08	−0.03	0.13	0.08
WTR	0.13‡	0.20†	−0.02	0.05	0.03	0.23‡
Fasting glucose	0.05	0.07	0.04	−0.39*	0.02	0.11
Fasting insulin	0.28*	0.24*	0.19†	−0.09	0.16‡	0.11
2-h glucose	0.12‡	0.08	0.01	−0.13	0.04	0.18
M-low	−0.21*	−0.11	−0.16‡	0.15‡	−0.13	0.01
M-high	−0.13‡	−0.01	−0.06	0.23†	−0.11	0.06
AIR (only NGT)	0.06	0.12	0.07	0.04	0.13	0.02
Basal EGO	0.07	0.05	0.04	0.18‡	0.12	0.06

Significant correlations are indicated as follows: $*P < 0.001$, $†P < 0.01$, and $‡P < 0.05$. EGO, endogenous glucose output; WTR, waist-to-thigh ratio.

$(8,000 \pm 1,973$ and $8,470 \pm 2,153$ cells/mm^3, respectively; $P = 0.07$) and in women compared with men, after adjustment for percent body fat ($8,054 \pm 2,171$ and $7,578 \pm 1,894$ cells/mm^3; $P = 0.06$).

Total WBC was positively correlated with measures of adiposity and fasting plasma insulin concentration and negatively correlated with the rate of insulin-stimulated glucose disposal (M), as assessed during the low-dose (M-low) and the high-dose (M-high) insulin infusions (Table 2, Fig. 1). In contrast, WBC was not correlated with AIR. In multiple linear regression models, measures of central adiposity (waist circumference and waist-to-thigh ratio) were not related to WBC independent of percent body fat ($P = 0.2$ for both). After adjustment for age, sex, and percent body fat, WBC was positively associated with fasting plasma insulin concentrations ($P = 0.01$) and negatively associated with M-low ($P = 0.03$) (percent body fat was significant in both models, $P < 0.0001$). The correlations between WBC and percent body fat, insulin, and M-low were not different between subjects with NGT and IGT (all $P > 0.1$ for interaction term of glucose tolerance status × percent body fat, insulin, or M, respectively).

The correlations of differential WBC with anthropometric and metabolic variables are shown in Table 2. After adjustment for age and sex, only neutrophil count was associated with percent body fat ($P = 0.001$). An association between neutrophil count and M-low was not independent of percent body fat ($P = 0.4$).

Prospective analysis. In proportional hazards analysis performed in 272 subjects, with adjustment for age and sex, a high WBC predicted the progression from NGT to diabetes (relative hazard 90th vs. 10th percentiles [95%CI] of 2.7 [1.3–5.5], $P = 0.007$). The predictive effect of high WBC persisted after additional adjustment for percent body fat, AIR, and M-low (Table 3). As previously reported, both low M-low (relative hazard 10th vs. 90th percentiles of 3.3 [1.2–9.5], $P = 0.02$) and low AIR (relative hazard 5.5 [1.9–10.8], $P = 0.0005$) were significant independent predictors of diabetes (in models including age, sex, and percent body fat, not including WBC). The Kaplan-Meier curve of the cumulative incidence of diabetes indicates that the subjects in the upper tertile of WBC (adjusted for

age, sex, and percent body fat) had a higher cumulative incidence at all time points of follow-up when compared with subjects in the lower tertile (Fig. 2). In 154 subjects (42 progressors), no part of differential WBC was predictive of diabetes either before or after adjustment for age, sex, and body fat (relative hazard 1.9 [0.9–4.2], $P = 0.3$, and 1.3 [0.6–2.7], $P = 0.5$, for neutrophils and lymphocytes, respectively; for monocytes, eosinophils, and basophils, all $P > 0.2$).

Longitudinal analysis. In a longitudinal analysis of 81 subjects who were NGT at baseline, a high WBC at baseline was associated with the decline in M-low after adjustment for time of follow-up ($P = 0.002$) (Table 4) and additional adjustment for follow-up age, sex, and change in percent body fat ($P = 0.003$) (Table 4, Fig. 3A). The effect of high WBC was independent of age. Figure 4 shows a graphical representation of the effect of high and low WBC (relative to baseline M-low) on the change in M-low by tertiles of baseline M-low. In each tertile of baseline M-low, subjects with a relatively high WBC (above median) had greater decline in M-low than those with a relatively low WBC (below median). A high WBC at baseline was not associated with the change in AIR (Fig. 3B), change in fasting plasma insulin concentrations ($P = 0.2$), or change in percent body fat ($P = 0.67$).

DISCUSSION

In the present study, we conducted a series of cross-sectional, prospective, and longitudinal analyses aimed at further delineating the potential role of inflammation in the development of type 2 diabetes. We found that among Pima Indians with normal glucose tolerance, a high WBC predicted the development of diabetes. Moreover, we demonstrated that a high WBC was associated with a decline in insulin sensitivity. Collectively, these data suggest a role of inflammation in the development of insulin resistance and subsequent type 2 diabetes.

The cross-sectional relationship between WBC and adiposity confirms previous findings in Pima Indians (1) and other populations (1,5,16–18). In accordance with two previous series (19,20), we found that the inverse relationship between WBC and insulin sensitivity was independent
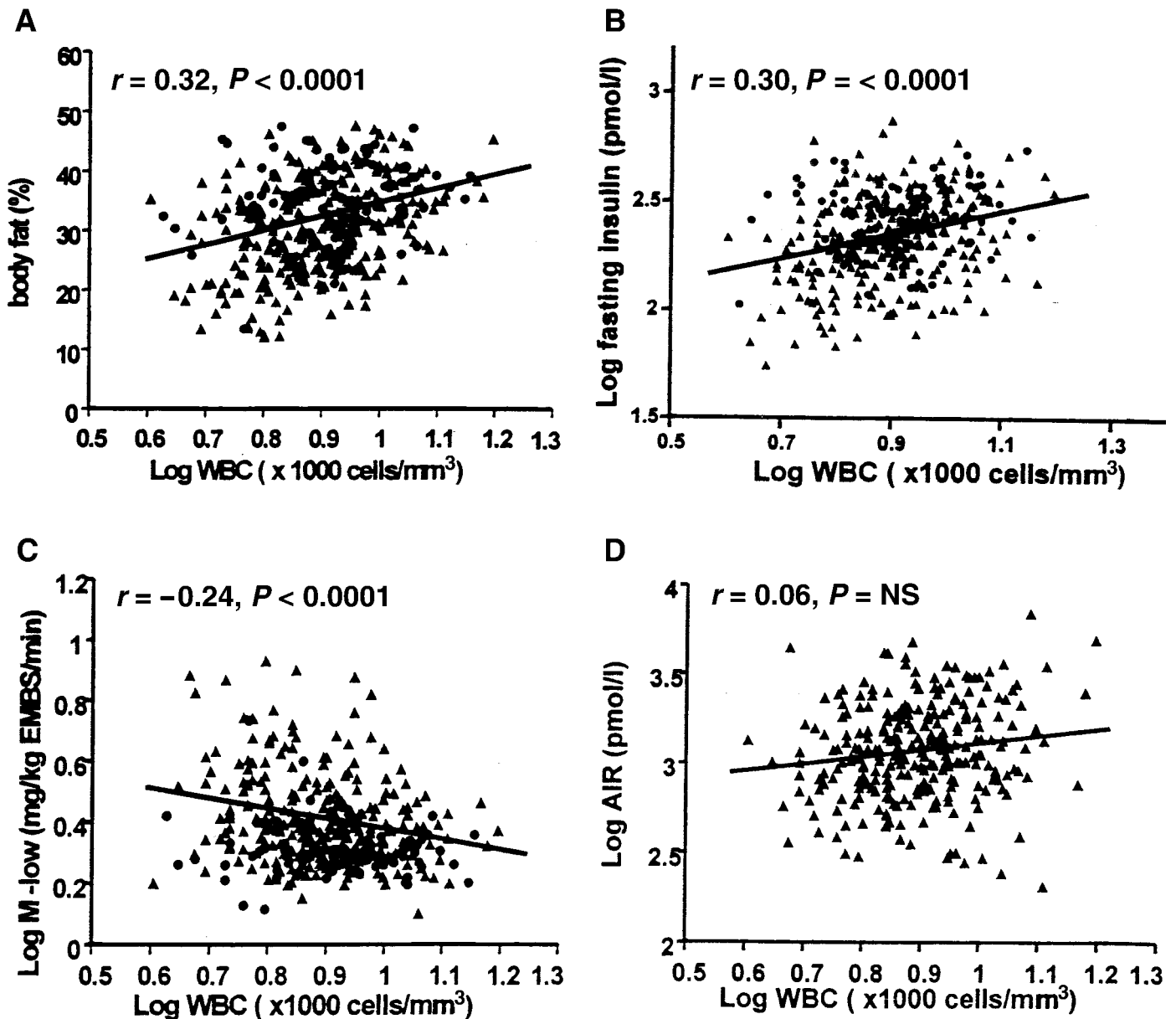
FIG. 1. Relationship between WBC and percent body fat (*A*), fasting plasma insulin (*B*), *M*-low (*C*), and AIR (*D*) in 352 nondiabetic Pima Indians (272 NGT [▲] and 80 IGT [●]). EMBS = fat-free mass + 17.7 kg. Because even mildly elevated glucose concentrations can secondarily affect insulin secretion, only data from NGT subjects were included in *D*.

of the degree of obesity. By contrast, a previous smaller study in Pima Indians (1) found that the relationship between WBC and fasting insulin, but not insulin action, was independent of adiposity. This partial disparity between the two studies may be attributable to the substantially smaller number of subjects in the previous study.

Prospective analyses revealed that a high total WBC predicts type 2 diabetes, which is in agreement with findings obtained in other populations (5,6). Our finding that the predictive effect of WBC was independent of the degree of adiposity agrees with findings by Schmidt et al. (5) in a mixed Caucasian and African-American popula-

TABLE 3
Independent predictive effects of WBC *M*-low, and AIR on the progression from NGT to diabetes in Pima Indians

Progression from NGT to diabetes	Value at 10th or 90th percentile*	Relative hazard†	95% CI	*P*
M-low (mg/kg EMBS/min)	1.8;4.4	2.5	0.85–7.0	0.1
AIR (pmol/l)	94;475	4.9	2.1–11.4	0.001
WBC (cell/mm³)	5,800;10,700	2.6	1.1–6.2	0.03

The model also includes age, sex, and percent body fat. *For *M* and AIR, the value at the 90th percentile is the value associated with the lower risk of developing diabetes, whereas for WBC, the value at the 10th percentile is associated with lower risk; †hazard rate for a subject at the percentile with higher risk is divided by the rate for a hypothetical subject at the percentile with the lower risk.
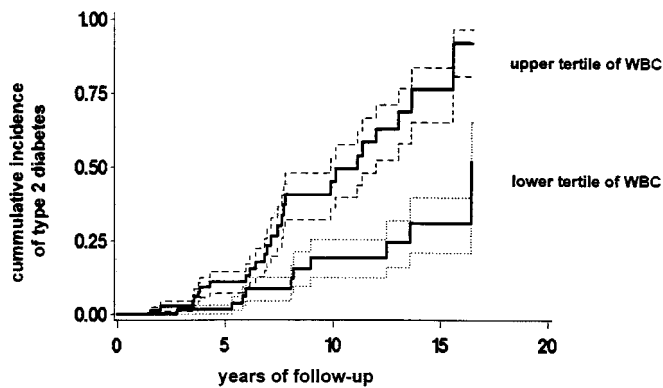
FIG. 2. Kaplan-Meier curves for cumulative incidence of type 2 diabetes (cumulative incidence ± 1 SE) in 272 NGT subjects with WBC in the upper and lower tertile, after adjustment for age, sex, and percent body fat. The number of events was 22 in the upper tertile, 21 in the middle tertile, and 11 in the lower tertile. For clarity, only the curves for the upper and the lower tertile are shown. The curve for the middle tertile (not shown) lays between the other two.

tion. Recently, a preliminary report by Festa et al. (8) described an effect of PAI-1, another marker of inflammation, on the development of type 2 diabetes. The effect was independent of obesity, insulin sensitivity (minimal model), and family history of diabetes. Neither of these studies, however, had measures of both insulin action and insulin secretion. Our finding confirms results obtained by Festa et al. (8) and shows that the predictive effect of a high WBC was independent of insulin resistance and insulin secretion, two previously identified predictors of diabetes (Table 3). Moreover, our results may give a new dimension to the predictive effect of WBC on the development of type 2 diabetes in an American Indian population. Fernandez-Real and Ricart (21) proposed an evolutionary link between inflammatory mediators and the risk of developing type 2 diabetes. In particular, an insulin-resistant genotype, associated with an activated immune system, may have been historically advantageous when the life span was short and injury and infectious disease prevalent, but it may be disadvantageous today. Therefore, it could be hypothesized that repeated epidemics in American Indians, after first contact with Europeans in the 15th century
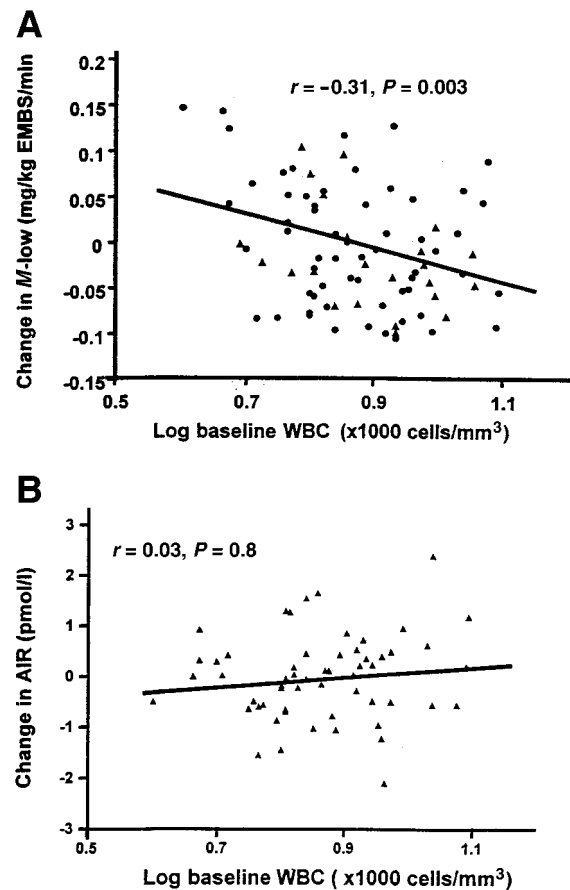

FIG. 3. A: Relationship between WBC at baseline and the subsequent change (follow-up adjusted for baseline) in M (adjusted for sex, follow-up age, change in body fat, and time of follow-up). B: Relationship between WBC at baseline and the change in AIR (adjusted for sex, follow-up age, M, change in body fat, and time of follow-up). ▲, NGT subjects at follow-up; ●, IGT subjects at follow-up.

and subsequent exposure to a range of new infectious diseases, may have led to the selection of individuals who were both resistant to infectious disease and prone to diabetes (7).

Having established that a high WBC is positively associated with adiposity and negatively associated with mea-

TABLE 4
Multivariate relationships between WBC at baseline and M at follow-up

| Dependent variable	Estimate	Determinant	SE	P
M at follow-up adjusted for time of follow-up $(mg \cdot kg \cdot EMBS^{-1} \cdot min^{-1})$				
	0.34	Intercept	0.0504	0.0001
	0.50	M at baseline (mg/kg EMBS/min)	0.05	0.0001
	−0.00004	Time of follow-up (years)	0.000009	0.0001
	−0.015	WBC at baseline (cells/mm^3)	0.05	0.002
M at follow-up adjusted for follow-up age, sex, and change in body fat $(mg \cdot kg \cdot EMBS^{-1} \cdot min^{-1})$				
	0.52	Intercept	0.0689	0.0001
	−0.0001	Age at follow-up (years)	0.001	0.87
	0.044	Sex	0.020	0.03
	−0.01	Body fat at follow-up (%)	0.001	0.0001
	0.004	Body fat at baseline (%)	0.002	0.03
	0.39	M at baseline (mg/kg EMBS/min)	0.05	0.0001
	−0.0003	Time of follow-up (years)	0.000008	0.0001
	−0.012	WBC at baseline (cells/mm^3)	0.004	0.003
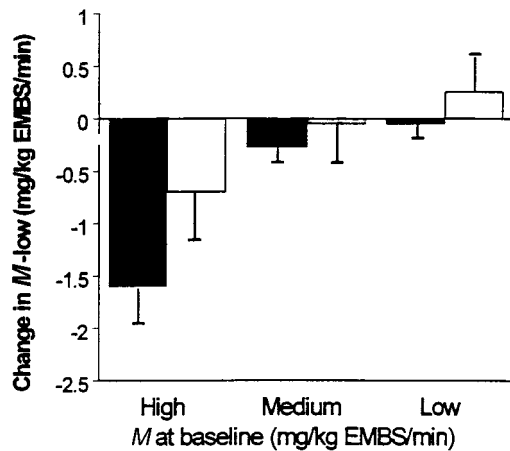
FIG. 4. The effect of high and low WBC (relative to baseline M) on the change in M (follow-up adjusted for baseline) by tertiles of baseline M. In each tertile, subjects are divided into a relatively high WBC (above median) and a relatively low WBC (below median) group. ■, high WBC; □, low WBC.

sures of insulin sensitivity and predicts the development of type 2 diabetes, we then examined mechanisms by which this occurs. Results from our longitudinal analyses show that a high WBC at baseline was associated with a decline in insulin action. In fact, subjects with a relatively high WBC for their insulin action at baseline had greater decrease in insulin action than those with a relatively low WBC. In contrast, WBC at baseline was not related to a decrease in AIR. Our data suggest that activation of the immune system causes the decline in insulin sensitivity and, therefore, contributes to the development of type 2 diabetes.

Why are WBC and insulin sensitivity associated? One possible explanation is that both a higher WBC and insulin resistance reflect an underlying activation of the immune system. It was shown, for instance, that interleukin-6 (IL-6), a potent white blood cell differentiation factor (22) that is produced mostly in adipose tissue (23) is associated with insulin resistance (24). Therefore, it could be hypothesized that IL-6 may be a factor that not only increases WBC but also causes insulin resistance. This notion is also supported by an observation that a single nucleotide polymorphism in the IL-6 gene was shown to be associated with an increased WBC and lower insulin sensitivity (25). Interestingly, it has been shown that WBC and other markers of inflammation aggregate in families, which suggests that genetic factors may be involved in the activation of the immune system (26). However, because relatives share not only genetic determinants but also environmental factors, such as exposure to infection, it is not possible to determine whether familiar associations are genetic or environmental. Because cytokines, such as IL-6, are produced by activated white blood cells, it is also possible that an activation of the immune system, caused by inflammation, could increase WBC and therefore cytokine production (2,27), which may decrease insulin sensitivity (2). Hormones are another possible link between WBC and insulin sensitivity. A variety of hormones have receptors on the surface of white blood cells and have been shown to play a role in their production and maturation (28–30). Some of them, such as insulin, cortisol, and

sex hormones, are also associated with insulin resistance (29). The role of cortisol and sex hormones as a possible link between WBC and insulin resistance cannot be resolved in this study. Insulin is an another possible link; however, we observed no difference in fasting plasma insulin concentrations between progressors and nonprogressors at baseline, nor a relationship between baseline WBC and follow-up insulin concentrations.

In conclusion, in Pima Indians a high WBC is associated with a worsening in insulin action and predicts the development of type 2 diabetes. These findings are consistent with the hypothesis that chronic activation of the immune system may play a role in the pathogenesis of type 2 diabetes.

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