Adiponectin and the Development of Type 2 Diabetes

The Atherosclerosis Risk in Communities Study

Bruce B. Duncan,[1,2] Maria Inês Schmidt,[1,2] James S. Pankow,[3] Heejung Bang,[4] David Couper,[4] Christie M. Ballantyne,[5] Ron C. Hoogeveen,[5] and Gerardo Heiss[2]

Adipocyte-derived secretory proteins have been increasingly linked to diabetes. To investigate whether adiponectin, a major adipocyte secretory protein, predicts diabetes, we conducted a case-cohort study representing the ~9-year experience of the 10,275 middle-aged, U.S. African-American and white participants of the Atherosclerosis Risk in Communities (ARIC) study. Adiponectin was measured on stored plasma of 581 incident diabetes case subjects and 572 noncase subjects. Overall hazard ratios (95% CIs) for developing diabetes, for those in the second, third, and fourth (versus the first) quartile of adiponectin were 0.57 (0.41–0.78), 0.39 (0.27–0.56), and 0.18 (0.11–0.27), respectively, after adjustment for age, sex, ethnicity, study center, parental history of diabetes, and hypertension and 0.72 (0.48–1.09), 0.67 (0.43–1.04), and 0.58 (0.34–0.99), respectively, after additional adjustment for BMI, waist-to-hip ratio, fasting glucose, insulin, and a score composed of six inflammation markers. The association was of similar magnitude in men and women and in whites and African Americans, but was absent in smokers and in those with a greater inflammation score (interaction $P < 0.01$ for each). In conclusion, in this community-based sample of U.S. adults, higher adiponectin levels were associated with a lower incidence of diabetes. *Diabetes* 53:2473–2478, 2004

Type 2 diabetes is a leading cause of morbidity and mortality. Prevention of diabetes and its associated burden, primarily cardiovascular morbidity and mortality, has become a major health issue worldwide (1).

Obesity has long been recognized as a major risk factor for diabetes, but only very recently have the underpinnings of this association begun to be unraveled. Extensive work now demonstrates that adipocytes have functions that go beyond the mere storage of energy as fat. Adipocyte-derived secretory proteins, many proinflammatory, may explain at least part of the relationship of obesity with insulin resistance (2), type 2 diabetes, and atherosclerotic disease (3–5). Yet, the association of inflammation with these diseases appears not to be a simple one. For example, we have previously demonstrated (6) that this association of a proinflammatory state with incident diabetes varies importantly by ethnicity and smoking status.

Adiponectin, one of the more recently described secretory proteins, has important metabolic and anti-inflammatory actions that suggest a protective role in diabetes development (7,8). The few epidemiologic studies (9–12) reported to date support this contention, as they relate a lower incidence of diabetes for those with higher adiponectin levels. However, this association has not yet been reported in African Americans, and previous studies lacked potentially important covariates as well as power to investigate potentially important variability in risk across categories of BMI and smoking.

Thus, the purpose of this study is to evaluate the association of adiponectin with incident type 2 diabetes in a sample well characterized with respect to several other risk factors for diabetes. To gain further insight, we also aimed to investigate possible differences in levels of adiponectin and in adiponectin associations with incident diabetes across categories of sex, ethnicity, BMI, smoking status, baseline glucose and insulin, and systemic inflammation.

RESEARCH DESIGN AND METHODS

In 1987–1989 the Atherosclerosis Risk in Communities (ARIC) study recruited a population-based cohort of 15,792 men and women, 45–64 years of age, from four U.S. communities (13). All subjects were invited to return to three clinic visits, at ~3-year intervals, at which incident diabetes was ascertained. Human-subject research review committees at the involved institutions approved the study, and all participants gave written consent.

We chose the same case-cohort design previously used to investigate the role of inflammation in the development of diabetes (6), which permits evaluation of the adiponectin/incident diabetes association within the context of a sample well characterized with respect to inflammation markers. Before sampling, we excluded 2,018 participants with prevalent diabetes, 95 members of minority ethnic groups with small numbers, 853 not returning to any follow-up visit, 26 with no valid diabetes determination at follow-up, 7 with restrictions on stored plasma use, 12 with missing baseline anthropometrics, and 2,506 participants in previous ARIC case-control and case-cohort studies involving cardiovascular disease for whom stored plasma was either previously exhausted or held in reserve. This resulted in a final sample of 10,275 individuals (75% of those in the full cohort without diabetes at baseline), of whom 1,155 (11.2%) were ascertained as developing diabetes during follow-up. From these 10,275 eligible members of this baseline cohort, we selected and measured analytes on ethnicity-stratified (50% white, 50% African American) random samples of both cases of incident diabetes and eligible members

From the [1]Graduate Studies Program in Epidemiology, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; the [2]Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, North Carolina; the [3]Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, Minnesota; the [4]Department of Biostatistics, School of Public Health, University of North Carolina, Chapel Hill, North Carolina; and the [5]Department of Medicine, Baylor College of Medicine, Houston, Texas.

Address correspondence and reprint requests to Dr. Bruce B. Duncan, Graduate Studies Program in Epidemiology, UFRGS, Av. Luiz Manoel Gonzaga, 630/8, Porto Alegre, RS 90470-280 Brazil. E-mail: bbduncan@orion.ufrgs.br.

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AMPK, AMP-activated protein kinase; ARIC, Atherosclerosis Risk in Communities; IL, interleukin.

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of the full cohort (1,198 individuals in total). A few of the incident cases of diabetes overlapped with the cohort random sample, and a few were selected only via the cohort sample. Of those sampled, we excluded 45 for incomplete fasting (<8 h) or for not having values for all covariates, leaving a total of 1,153 subjects, including 581 diabetes case and 572 noncase subjects, for analysis. The cohort random sample contained 4.4% of eligible white participants and 13.9% of eligible African Americans. Among those with incident diabetes, 40% of whites and 70% of African Americans were included in the study sample.

We measured glucose at baseline and at follow-up visits by a hexokinase method and fasting serum insulin by nonspecific radioimmunoassay. We measured waist girth at the umbilical level and hip circumference at the maximum hip girth to obtain the waist-to-hip ratio. We defined parental history of diabetes as a report of diabetes in either parent. The definitions and methods for other baseline measurements (height, weight, smoking status, systolic blood pressure, hypertension, physical activity, triglycerides, HDL cholesterol, insulin, white blood cell count, fibrinogen, GAD antibody, interleukin [IL]-6, sialic acid, C-reactive protein, and orosomucoid) have been previously reported (6,14).

Adiponectin and additional markers of inflammation were measured at a central lab on plasma specimens frozen at baseline. These samples, stored for ~15 years at −70°C, were thawed and maintained at 4°C until measured, which was no longer than 24 h later. We measured total adiponectin in duplicate by radioimmunoassay (Linco Research, St. Charles, MO); this assay uses 125I-labeled murine adiponectin as a tracer and a multispecies adiponectin rabbit antiserum for detection of adiponectin in human plasma calibrated against recombinant human adiponectin standards. The adiponectin reliability coefficient (measuring between-person variance to the total variance and obtained analyzing replicate pairs of samples obtained at baseline on a subset of 35 subjects) was 0.95.

As in our previous report (6), we created a score to indicate low-grade systemic inflammation ranging from 0 to 6, attributing one point for a value greater than the median of the cohort sample for each of the six inflammation markers: IL-6, C-reactive protein, orosomucoid, sialic acid, white blood cell count, and fibrinogen.

We defined diabetes on the basis of *1*) a reported physician diagnosis, *2*) use of antidiabetic medications, *3*) a fasting (≥8 h) glucose value ≥7.0 mmol/l, or *4*) a nonfasting glucose value of ≥11.1 mmol/l. The date of diabetes incidence was estimated by linear interpolation using glucose values at the ascertaining visit and the previous one, as previously described (6).

Statistical analysis was based on our case-cohort sampling design. We used weighted ANCOVA to compute adjusted means and proportions of sociodemographic variables and risk factors and used weighted Spearman correlations to describe unadjusted associations between study variables. In these analyses, weights were defined as the inverse of the ethnicity-specific sampling fractions, permitting statistical estimation and inference relevant to the entire cohort. The adjusted relative risk of developing diabetes at different levels of adiponectin was estimated in proportional hazards models fit using SUDAAN (Survey Data Analysis) to account for the case-cohort design, with stratified sampling both from the whole cohort and from the incident diabetes case subjects (15). We used the Wald test of interaction terms in these models to test heterogeneity in associations. Analyses were performed using SAS (16) and SUDAAN (17).

RESULTS

The median (interquartile range) follow-up was 3.0 years (1.7–5.9) for those who did and 8.9 (8.8–9.0) for those who did not develop diabetes. Of incident case subjects, 499 (86%) were ascertained by a fasting glucose ≥7.0 mmol/l, 5 (1%) by a nonfasting glucose ≥11.1 mmol/l, and 77 (13%) by reported physician diagnosis or medication use. Among case subjects, 153 (26%) were white men, 151 (26%) white women, 76 (13%) African-American men, and 201 (35%) African-American women. Among noncase subjects, the corresponding numbers were 123 (22%), 203 (35%), 76 (13%), and 170 (30%), respectively. Additional characteristics of case and noncase subjects are shown in Table 1.

Median (interquartile range) values for adiponectin were 8.84 μg/ml (6.24–11.70) in the cohort random sample. Medians and proportions of principal covariates for those who developed, or did not develop, incident diabetes have been previously reported (6). Smokers constituted 21% of case subjects and 22% of noncase subjects. Among case subjects, 60% had inflammation scores ≥4 and, among noncase subjects, 37%.

TABLE 1
Weighted medians (interquartile ranges) or proportions of baseline sociodemographic variables and risk factors in participants who developed diabetes and those who did not: ARIC study, 1987–1989

Variable	Developed diabetes	No diabetes
n	581	572
Age (years)	53 (49–58)	52 (48–57)
Male	42.6	36.4
White	66.3	80.7
Parental history diabetes	34.3	21.1
Fasting glucose (mmol/l)	6.04 (5.61–6.38)	5.34 (5.05–5.66)
Current smokers	20.7	22.2
Ex-smokers	34.9	31.3
BMI (kg/m^2)	30.0 (26.7–33.9)	26.1 (23.7–28.9)
Waist-to-hip ratio	0.96 (0.92–1.00)	0.91 (0.85–0.96)
Fasting insulin (pmol/l)	100.4 (71.7–157.8)	57.4 (35.9–86.1)
Hypertension	46.3	24.9

Data are medians (interquartile ranges) or percent.

Modest correlations were observed for adiponectin with elements of the metabolic syndrome (in absolute value from 0.23 to 0.43), fasting insulin (0.37), and inflammation markers (0.14 to 0.25), but were generally negative for all elements, except for HDL cholesterol (Table 2). The largest correlations with adiponectin were seen with HDL cholesterol, triglycerides, and waist circumference, among syndrome elements, and with orosomucoid and C-reactive protein, among the inflammation markers.

Mean values of adiponectin, adjusted for age, sex, ethnicity, educational level, smoking status, and BMI, varied considerably across subgroups (Table 3). The mean was 52% higher for women than men ($P < 0.001$) and 22% lower in African Americans than whites ($P < 0.001$). The overweight and obese subjects had values 13 and 22% lower, respectively, than those with BMI <25 kg/m^2 ($P < 0.001$). Those with a high inflammation score (four or more of six markers with above-median values) had a mean

TABLE 2
Correlation of adiponectin with elements of the metabolic syndrome and inflammation in the cohort random sample

Factor	Spearman correlation coefficient*
Inflammation markers	
Orosomucoid	−0.25
C-reactive protein	−0.23
IL-6	−0.18
White blood cell count	−0.17
Sialic acid	−0.14
Fibrinogen	−0.14
Metabolic syndrome elements	
HDL cholesterol	0.42
Fasting insulin	−0.37
Waist circumference	−0.33
Triglycerides	−0.33
Glucose	−0.29
BMI	−0.27
Systolic blood pressure	−0.24

*Weighted to reflect sampling strategy; $P < 0.001$ for all correlations.

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TABLE 3
Adjusted means of adiponectin (μg/ml) in different subgroups of the cohort random sample: ARIC study*

Group	Mean (95% CI)	*P*
Overall	9.72 (9.38–10.07)	
Sex		<0.001
Men	7.31 (6.83–7.80)	
Women	11.10 (10.63–11.58)	
Ethnicity		<0.001
White	10.17 (9.75–10.58)	
African American	7.95 (7.49–8.42)	
BMI (kg/m^2)		<0.001
<25	10.79 (10.15–11.44)	
25 to <30	9.35 (8.83–9.87)	
≥30	8.46 (7.76–9.15)	
Systemic inflammation		<0.001
Three or fewer markers above median	10.51 (9.98–11.04)	
Four or more markers above median	8.38 (7.86–8.90)	
Smoking status		0.24
Smokers	9.35 (8.63–10.07)	
Nonsmokers	9.83 (9.44–10.22)	

*Adjusted through a weighted ANCOVA for age, sex, ethnicity, educational level, smoking status, and BMI.

value 20% less than those with lower inflammation scores ($P < 0.001$). Values were similar in current smokers and nonsmokers, and no important differences were noted across educational levels (data not shown).

The ethnicity difference was consistently found in men and women, and, for each sex, in the normal-weight, overweight, and obese categories. For instance, for normal-weight, overweight, and obese white women, the adjusted means were 12.6, 11.1, and 9.7 μg/ml, respectively, whereas for similarly categorized African-American women, adjusted means were 10.0, 8.5, and 7.6 μg/ml, respectively.

Figure 1 shows the association of adiponectin with incident diabetes, adjusted sequentially for covariates. In the model adjusting initially for age, center, ethnicity, sex, hypertension, and a parental history of diabetes, a strong, graded, protective association was seen for adiponectin (hazard ratio [HR] comparing quartile extremes = 0.18; 95% CI 0.11–0.27) (solid line in the figure). With adjustment for the obesity indexes of BMI and waist-to-hip ratio and fasting glucose and insulin, this protective association, while remaining statistically significant, decreased considerably (0.50, 0.30–0.83). Final adjustment for the inflammation score produced little further change (0.58, 0.34–0.99). Of note, in this final model, adiponectin, the inflammation score, BMI, and WHR all remained independently associated with the development of diabetes ($P < 0.05$). The HR for a one-unit difference in the inflammation marker score was 1.11 (1.01–1.22). For instance, this is equivalent to an estimated risk of developing diabetes ~50% greater (1.52) for an individual with five of the six markers with above-median values versus one with only one marker with an above-median value.

Figure 2 demonstrates the magnitude of the association when analyzed separately by sex, ethnicity, BMI, smoking, and systemic inflammation categories. Here, due to reduced sample size of the individual strata, adiponectin levels were expressed in tertiles. The association was similar in men and women, in African Americans and whites, and across categories of BMI. The slightly larger protective association suggested in the figure for African Americans was not statistically significant ($P = 0.60$), was less prominent in less-adjusted analyses, and was not consistent across sex. The association was also of similar strength in those with and without impaired fasting glucose at baseline, as well as in those with insulin values above and below the 75th percentile (93 pmol/l) of the cohort random sample.

By contrast, important heterogeneity was seen in the association in different categories of smoking status. High adiponectin levels were associated, in adjusted analyses, with a lower incidence of diabetes in nonsmokers (HR [third versus first quartile] 0.42, 95% CI 0.25–0.73 for never smokers, and 0.78, 0.40–1.54 for ex-smokers), but not in current smokers (1.15, 0.45–2.98; interaction $P = 0.006$).

Additionally, even after exclusion of current smokers, the association was present only in those with less systemic inflammation (inflammation score ≤3: HR [third versus first tertile] 0.25, 95% CI 0.13–0.48; and inflammation score ≥4: 1.18, 0.68–2.0; interaction $P = 0.002$).

The strength of adiponectin's protective association was similar for case subjects diagnosed at the first and at the last follow-up visit, and the association improved slightly when GAD antibody–positive case subjects were excluded. When the definition of an incident case was restricted to those with fasting glucose >140 mg/dl, the association was somewhat stronger.

The adiponectin association in a fully adjusted model was slightly stronger (HR comparing quartile extremes = 0.56; 95% CI 0.33–0.96) when adjustment for inflammation was performed by adding quartiles of all six inflammation makers, rather than the inflammation score, to the model. In this model, higher white blood cell counts (HR [fourth versus first quartile] 1.66; 95% CI 1.05–2.63) and higher levels of sialic acid (HR [third versus first quartile] 1.73;

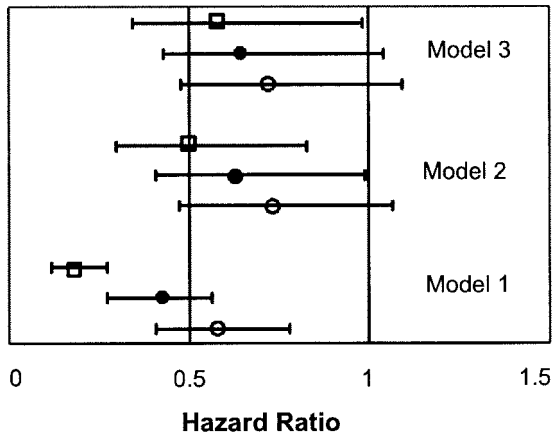

FIG. 1. Risk of developing diabetes across quartiles of adiponectin: ARIC study, 1987–1998. Model 1 was adjusted, in proportional hazards modeling, for age, center, sex, race, family history of diabetes, and hypertension. Model 2 was additionally adjusted for baseline fasting glucose and insulin, BMI, and waist-to-hip ratio. Model 3 was additionally adjusted for an inflammation score based on six inflammation markers. Lines show HRs and 95% CIs. For each model, the bottom line (○) indicated risk for participants with adiponectin values in the second quartile, the middle line (●) for those with values in the third quartile, and the top line (□) for those with values in the fourth quartile. The first quartile is the reference group.

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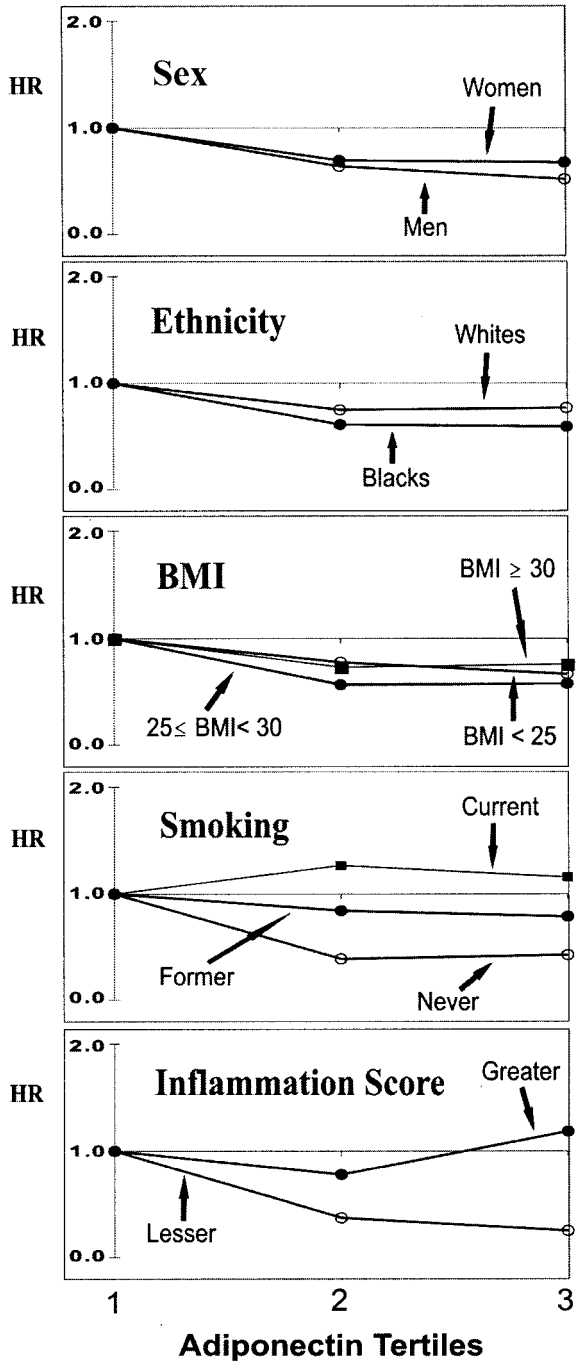

FIG. 2. Adjusted associations of adiponectin with incident diabetes in different subgroups of the sample: ARIC study, 1987–1998. Analyses were adjusted through proportional hazards modeling for ethnicity, family history of diabetes, baseline fasting glucose, BMI, waist-to-hip ratio, and an inflammation score based on the number of six inflammation markers having an above-median value. The first quartile is the exposure reference. Current smokers were excluded from analyses stratified by the inflammation score.

95% CI 1.08–2.78; HR [fourth versus first quartile] 1.54; 95% CI 0.97–2.46) were associated with a greater risk of developing diabetes. No association was seen for IL-6, C-reactive protein, fibrinogen, or orosomucoid.

An oral glucose tolerance test was performed on a fraction of the original cohort at the last ARIC visit. Exclusion of the 27 individuals otherwise classified as noncase subjects but having 2-h glucose values >11.1 mmol/l did not change the magnitude or the statistical significance of the association of adiponectin with incident diabetes.

DISCUSSION

These results demonstrate a graded inverse association between adiponectin levels and risk of diabetes in a cohort of middle-aged African-American and white men and women, representative of four U.S. communities. In fully adjusted analyses, those in the highest quartile of adiponectin had an ~40% lower risk of developing diabetes than those in the lowest quartile. This protective association was similar in men and women, African Americans and whites, and groups of participants characterized by different levels of BMI. However, it was absent in smokers and in those with a higher inflammation score.

A purported protective effect in the development of type 2 diabetes conferred by higher adiponectin levels has been previously shown in four observational studies involving diverse ethnic groups, i.e., Pima Indians (9), white Europeans (11), Japanese (10), and Asian Indians (12). Our results demonstrate this association also in African Americans. Additionally, the larger sample size, greater number of incident diabetes cases, and longer follow-up of our study, compared with previous investigations, permit greater confidence with respect to the temporality of the association, precision of the estimates, and adjustment for confounding factors.

The glucose-lowering effect of adiponectin has been shown to be due in part to its activation of the AMP-activated protein kinase (AMPK) cascade. AMPK, a likely target for metformin and other antidiabetic drugs as well as for exercise-related glucose transport, is an insulin-independent, phylogenetically ancient mechanism of stimulating glucose transport. Best thought of as a means of maintaining intracellular energy levels, AMPK stimulates both the catabolism of existing intracellular energy stores, such as triglycerides, and an insulin-independent influx of extracellular energy sources, such as glucose (18). Two adiponectin receptors have been cloned and shown (19) to mediate increased fatty acid oxidation in muscle and increased glucose uptake in the liver.

Adiponectin has also been shown (20,21) to have insulin-sensitizing and anti-inflammatory actions. Low-grade systemic inflammation, intimately related to obesity and insulin resistance, precedes and predicts the development of both diabetes and atherothrombotic diseases (5). Thus, adiponectin may act, in part, by counteracting these mechanisms, as supported by the findings of this report, at least at lower levels of systemic inflammation. However, the fact that both adiponectin and the inflammation score associations remained statistically significant in simultaneous modeling of diabetes risk suggests that both adiponectin and systemic inflammation provide independent contributions to the development of diabetes.

Another potential mechanism for adiponectin's protective effect is improved insulin secretion, in that it has been recently shown (22) to counteract cytokine- and fatty acid–induced β-cell dysfunction.

The heterogeneity observed in the adiponectin/incident diabetes association may have important implications. The protective association of adiponectin was absent in cur-

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rent smokers. We have no firm explanation for this finding. However, it adds to the list of epidemiologic findings suggesting an important effect of smoking on adipocyte biology. Smoking is associated with lower weight in adults and quitting smoking with dramatic weight gains (23). We have previously reported that low-grade, chronic, systemic inflammation is a risk factor for diabetes only in nonsmokers and that mild, chronic, systemic inflammation is associated with weight gain to a much greater extent in ex-smokers than in current smokers.

Nicotine, through the nicotinic receptor, has recently been shown to have anti-inflammatory effects (24–26) in macrophages and adipocytes (27). Adipocytes, at least in rodents, appear to have nicotinic receptors that when exposed to nicotine lead to markedly decreased intracellular tumor necrosis factor-α levels and increased adiponectin secretion (28). Thus smoking, although generating an overall proinflammatory state, could selectively alter the effects of inflammation on adipocyte metabolism and adipocytokine production. How this mechanism might contribute to our findings of similar adjusted levels of circulating adiponectin in smokers and nonsmokers and of a similar risk of diabetes in smokers with high and low adiponectin levels is not clear at this point.

The absence of a protective adiponectin association among individuals with greater systemic inflammation was not postulated a priori. Adiponectin and inflammation mediators have several antagonistic functions. Thus, it would not be surprising if inflammatory mediators inhibit not only adiponectin expression (29), perhaps in part leading to the lower levels of adiponectin we found in those with greater systemic inflammation, but also its effects. Lowering of adiponectin levels has been shown (30) to be an early occurrence in the progression from normal carbohydrate metabolism to diabetes in rhesus monkeys. Our results permit speculation that once a state of chronic systemic inflammation is more fully established, increased adiponectin levels are less effective in counteracting the metabolic derangements that accompany it and lead to diabetes.

Adjusted levels of adiponectin were lower in African Americans and in men and were also lower with increasing levels of BMI and a higher inflammation score. Our findings of lower levels in men and in obese individuals are consistent with previous reports (10–12,31). Differences between whites and African Americans have been less investigated. A previous report (32) of similar levels among obese African-American and white women in a much smaller sample may have resulted from selection bias, as this finding was not replicated here. An additional small study (33) showed lower adiponectin levels in African-American, compared with white, boys. The lower levels we found among African Americans, especially if present from childhood, could explain in part their higher diabetes risk.

Basic science research and previous clinical and small population-based studies (34,35) suggest an inverse relationship between adiponectin and markers of inflammation. The inverse association of adiponectin with diverse markers of inflammation that we found was modest in nature. Nonetheless, the difference in adjusted levels of adiponectin between those with low and high inflammation scores was similar in magnitude to that seen between those of normal weight and those who were obese.

Whether the protective association of adiponectin is causal cannot be affirmed at this point. The magnitude of the association (a reduction in risk of 40% in fully adjusted models) appears to be of potential clinical relevance. Moreover, it may underestimate the association's true magnitude. First, the model not adjusting for factors that could be interpreted as mediators of adiponectin's actions (BMI, fasting glucose and insulin, and the inflammation score) demonstrated a relative protection of much greater magnitude (~80%). Adiponectin, as mentioned above, may exert its protective effect through increasing insulin action and secretion (7,20), as well as opposing the effects of tumor necrosis factor-α (8), thus preventing increases in insulin and glucose levels. Studies in rodents (36), though not replicated in Pima Indians (37), suggest that adiponectin prevents weight gain. Thus, baseline differences in these factors may thus represent the previous action of adiponectin and may be mediating in part an adiponectin effect. Additionally, the determination of adiponectin at one point may represent its customary circulating level less well than measurement of BMI at one point estimates adiposity. This difference in measurement error could result in an erroneous underestimation of the importance of adiponectin in BMI-adjusted analyses.

Additional support for causality is found in the consistency of findings across different ethnic groups, the graded nature of the association found across quartiles of adiponectin, and the existence of plausible biologic mechanisms.

Potential limitations to our study merit comment. First, selection bias, due either to a participant not returning for follow-up or not having samples available for measurement, could conceivably have influenced our results. However, we have little a priori reason to believe that the association between adiponectin and incident diabetes should be stronger or weaker among those without available samples or lost to follow-up. Second, epidemiologic studies are in general restricted in their ability to assess independent effects of interrelated variables, such as obesity, inflammation, glucose, and insulin resistance. Finally, as we ascertained diabetes without use of an oral glucose tolerance test, some misclassification in diabetes ascertainment was likely to have occurred. As our length of follow-up was relatively long, and as other studies showing adiponectin protection have ascertained diabetes using the oral glucose tolerance test, we believe it unlikely that this misclassification would explain our findings.

In conclusion, low adiponectin levels predict the development of type 2 diabetes, at least in nonsmoking subjects and in those with less systemic inflammation. These findings provide further evidence of the importance of adipocytokines in the development of diabetes in adults. Although the underlying signaling processes of this growing array of molecules are not yet well understood, this association with adiponectin, a molecule with important anti-inflammatory actions, provides further support for the hypothesis that diabesity is an inflammatory as well as a metabolic disease process. A better understanding of these pleiotropic signaling molecules and their interaction is important for the development of effective strategies for diabetes treatment and prevention.

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