Fasting Plasma Free Fatty Acids and Risk of Type 2 Diabetes

The Atherosclerosis Risk in Communities study

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OBJECTIVE — To evaluate whether plasma levels of free fatty acids (FFAs) are independently associated with incidence of type 2 diabetes.

RESEARCH DESIGN AND METHODS — A case-cohort design was used to randomly select 580 incident cases of diabetes and 566 noncases from 10,275 African-American and white men and women in the Atherosclerosis Risk in Communities study, aged 45–64 years and without prevalent diabetes at the baseline exam. Incident diabetes was ascertained at three exams over 9 years of follow-up. FFA levels were measured in plasma samples collected at the baseline exam.

RESULTS — At baseline, FFA level was inversely associated with height and positively associated with female sex, BMI, waist circumference, waist-to-hip ratio, heart rate, plasma triglycerides, and an inflammation score quantifying levels of six systemic inflammation markers. Relative risks for incident diabetes (fourth vs. first quartile of FFAs) were increased in a basic model adjusted for age, sex, race, and center (hazard ratio 1.68, 95% CI 1.20–2.34) and in a model further adjusted for baseline fasting glucose, insulin, BMI, waist circumference, triglycerides, and the inflammation score (1.63, 1.04–2.57). Relative risks associated with a greater FFA level were lowest among those of normal weight and highest among the obese, but a formal test of interaction between FFAs and BMI was not statistically significant.

CONCLUSIONS — Individuals with higher fasting levels of plasma FFAs were at modestly higher risk of type 2 diabetes in this cohort of middle-aged adults.

Diabetes Care 27:77–82, 2004

Several decades of basic science and animal research provide considerable support for a causal role of free fatty acids (FFAs) in the etiology of type 2 diabetes, possibly through detrimental effects on insulin and glucose metabolism (1). Higher FFAs may cause peripheral insulin resistance by interfering with the access of insulin to skeletal muscle or interfering with insulin signaling resulting in reduced glucose transport into muscle (2,3). Chronically elevated FFAs may also impair insulin secretory function through toxic effects on pancreatic β-cells as predicted by the "lipotoxicity hypothesis" (4). Finally, increased flux of FFAs into the liver, particularly from lipolysis of visceral adipose depots, may lead to excessive endogenous glucose production (5).

FFAs may mediate the effects of established and novel factors on risk of diabetes. For example, elevated FFAs in obesity (6,7), possibly associated with increased FFA flux from adipose to nonadipose tissues, may provide a mechanistic link between increased fat mass and the development of insulin resistance, glucose intolerance, and β-cell dysfunction that promote the onset of diabetes. Individuals with autonomic nervous system dysfunction may be at increased risk of diabetes because of higher circulating catecholamine levels, which in turn have major effects on rates of lipolysis in adipose tissue and FFA release (8). Finally, elevations in FFAs may accompany chronic, low-grade systemic inflammation associated with diabetes and atherothrombotic cardiovascular disease, possibly in part due to inhibition of adipocyte lipoprotein lipase activity and induction of hepatic triglyceride secretion by the proinflammatory cytokine interleukin-6 (9) and in part due to adipocytokine regulation of FFA uptake in muscle (10). These pathways may help explain associations between inflammation markers and incident diabetes that we (11–13) and others (14,15) have recently reported.

Although there is an extensive body of experimental research exploring the putative role of FFAs in insulin resistance, glucose intolerance, and type 2 diabetes, there are surprisingly few prospective epidemiologic studies (16–18). We investigated whether fasting plasma FFA concentration was an independent predictor of incident type 2 diabetes in the Atherosclerosis Risk in Communities (ARIC) study, a large population-based cohort of middle-aged African Americans and whites enrolled in four U.S. communities.

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Received for publication 11 July 2003 and accepted in revised form 15 September 2003.

Abbreviations: ARIC, Atherosclerosis Risk in Communities; FFA, free fatty acid; HOMA-IR, homeostasis model assessment of insulin resistance.

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

RESEARCH DESIGN AND METHODS

— In 1987–1989 the ARIC study recruited a population-based cohort of 15,792 men and women 45–64 years of age from four U.S. communities (19). Human subjects research review committees at the involved institutions approved the study, and all participants gave written consent. Follow-up examinations, ~3 years apart, were conducted in 1990–1992, 1993–1995, and 1996–1998.

To establish a sampling frame for this study, we excluded 2,018 participants who were with prevalent diabetes or missing diabetes status at baseline, 95 who were members of minority groups with small numbers, 879 who did not complete a follow-up examination or provided insufficient information to classify diabetes during at least one follow-up exam, 7 who restricted use of their stored biological specimens, 12 who had missing values for anthropometrics at baseline, and 2,506 who had plasma stores previously exhausted or held in reserve for case-control studies of cardiovascular disease. Among the remaining 10,275 subjects (i.e., the study base), a total of 1,155 incident cases of diabetes were identified. Participants in this sampling frame were stratified by race (African American or white). Due to limited resources, all diabetes case subjects were not selected for further study in our case-cohort design. Instead, a random sample of case subjects ($n = 581$) and a random sample of the cohort ($n = 693$) were selected within each race group. Sampling fractions were 72 and 39% of African-American and white cases and 15 and 5% of African-American and white members, respectively, of the eligible cohort. A total of 76 subjects selected in the sample of cases were independently selected in the sample of the cohort. A total of 23 subjects selected for the cohort random sample were incident cases of diabetes although not independently sampled as cases. A total of 50 participants were excluded from analysis because of missing values for FFA level, inflammation score, fasting glucose, insulin, or triglycerides, and 2 were excluded because of extreme values for insulin or triglycerides. After all exclusions, the final study population included 580 cases and 566 noncases.

Clinical and laboratory measurements

All participants in the present analysis reported fasting at least 8 h before the baseline examination. Venipuncture was between 7 A.M. and 12 P.M. in 97% of the participants. We analyzed free (nonesterified) fatty acid levels at a central laboratory on plasma specimens collected at the baseline exam and frozen at −70°C. FFA levels were measured in duplicate on a Hitachi 911 automated analyzer using an enzymatic colorimetric method (Wako Chemicals, Richmond, VA), with the mean of the duplicate measurements being taken as the value for that sample. The reliability coefficient for FFA level, measuring between-person variance to the total variance, was 0.92 based on replicate pairs of samples obtained from 36 participants at their baseline exam.

The definitions and methods used for other baseline measurements (education level, alcohol intake, cigarette smoking status and pack-years, sport activity index, hypertension, parental history of diabetes, height, BMI, waist and hip circumferences, HDL cholesterol, triglycerides, glucose, insulin, interleukin-6, C-reactive protein, orosomucoid, sialic acid, white cell count, and fibrinogen) have been previously reported (12,13). Heart rate was estimated from duration of R-R intervals from a 2-min rhythm strip with participants in a supine position. Information on multiple markers of systemic inflammation was integrated through an index of low-grade systemic inflammation (inflammation score). The score ranged from 0 to 6 and attributed one point for a value greater than the median of the cohort random sample for each of interleukin-6, C-reactive protein, orosomucoid, sialic acid, white cell count, and fibrinogen (12). The homeostasis model of insulin resistance (HOMA-IR) was computed as fasting glucose (mmol/l) times fasting insulin (μU/ml) divided by 22.5.

Diagnosis of diabetes

We defined diabetes on the basis of *1*) reported physician diagnosis, *2*) use of antidiabetes medications, *3*) fasting (≥8 h) serum glucose ≥7.0 mmol/l, or *4*) nonfasting glucose of ≥11.1 mmol/l. Because the actual date of diabetes onset was unknown, the date of diagnosis was interpolated between visits. For cases ascertained solely on the basis of an elevated glucose value (i.e., cases who were unaware of their diabetes status on the date of their visit), the incident date was estimated as the date at which fasting glucose first crossed the threshold for diabetes (e.g., ≥7.0 mmol/l for fasting values; ≥11.1 mmol/l for nonfasting values), assuming that glucose increased linearly between visits. For cases ascertained on the basis of physician diagnosis or use of antidiabetes medications, the fasting glucose on the date of the visit may have been affected by their knowledge of their diabetic status (and in some cases was <7.0 mmol/l). For these subjects, the date of diagnosis was interpolated based on their glucose value at the last visit during which diabetes was not diagnosed and the mean slope of glucose change among all incident cases who were unaware of their diabetes status on the date of their visit (i.e., cases ascertained solely on the basis of an elevated glucose value). Of the 580 cases of diabetes, 51 (9%) were first ascertained by self-reported physician diagnosis only, 3 (<1%) by use of antidiabetes medications only, 453 (78%) by glucose values only, and 73 (13%) by two or more of these criteria.

Statistical analysis

Statistical analysis was based on our case-cohort sampling design. To evaluate associations between FFAs and baseline levels of other risk factors, we used ANCOVA to compute age-, sex-, race-, and center-adjusted means or proportions of study variables across quartiles of FFAs, with appropriate weighting for the stratified sampling design. A test for linear trend across quartiles was conducted using the Surveyreg procedure in SAS (20). We computed hazard ratios (HRs) and 95% confidence intervals (CIs) for the time to the development of diabetes by using weighted, proportional hazards regression as implemented in SUDAAN (21). We evaluated the strength of association between FFA level and incident diabetes in a basic model adjusted for age, sex, race, and center and in models that adjusted additionally for other diabetes risk factors.

We also used the basic model described above to evaluate possible heterogeneity of the association between FFAs and type 2 diabetes according to levels of other risk factors, including sex, race (African American or white), parental history of diabetes (yes or no), normal or impaired baseline fasting glucose (<6.1 or ≥6.1 mmol/l), normal or elevated fasting triglycerides (<1.68 or ≥1.68 mmol/l), and BMI (<25 or ≥25, <30 or ≥30, and <35 or ≥35 kg/m^2). Interaction on the multiplicative scale was evaluated by comparing hazard ratios for FFAs (i.e., relative risk for those above the

Table 1—*Weighted and adjusted* means or proportions of baseline characteristics and risk factors in the cohort random sample according to fasting plasma FFA level*

	FFA quartiles (range in g/l)				
Characteristic or risk factor	1 (0.04–0.15)	2 (0.16–0.20)	3 (0.21–0.29)	4 (0.30–0.67)	*P*†
Age (years)‡	52.5	52.2	53.4	53.0	0.22
Female sex (%)‡	45.7	60.8	72.0	73.6	<0.01
African American (%)‡	24.1	20.8	20.8	22.0	0.39
Education (%)					
Less than high school graduate	21.7	23.2	21.2	26.1	
High school graduate	40.1	37.0	42.1	37.8	
Some college	38.3	39.9	36.7	36.1	0.60
Alcohol intake (g/week)§‖	48.9	76.3	76.5	64.2	0.25
Cigarette smoking (%)					
Never smoker	45.6	43.3	45.4	52.7	
Former smoker	31.2	33.8	31.3	24.8	
Current smoker	23.2	22.9	23.3	22.5	0.90
Cigarette pack-years§#	16.4	13.7	16.5	14.1	0.68
Sport activity index	2.48	2.32	2.31	2.36	0.27
Hypertension (%)	35.8	33.0	27.7	38.0	0.90
Heart rate (bpm)	65.0	66.4	67.6	68.7	<0.01
Parental history of diabetes (%)	24.4	23.9	25.8	20.3	0.49
Height (cm)	168.3	167.7	167.4	166.8	0.04
BMI (kg/m^2)	27.7	27.4	27.7	29.3	<0.01
Waist circumference (cm)	95.4	94.7	95.3	98.8	0.03
Waist-to-hip ratio	0.905	0.902	0.908	0.922	0.03
HDL cholesterol (mmol/l)	1.37	1.45	1.46	1.42	0.22
Triglycerides (mmol/l)§	1.04	1.06	1.10	1.28	<0.01
Fasting glucose (mmol/l)	5.50	5.42	5.44	5.47	0.77
Fasting insulin (pmol/l)§	64.6	62.5	59.0	61.8	0.52
HOMA-IR	2.89	2.74	2.57	2.95	0.96
Inflammation score	3.0	2.8	3.3	3.5	<0.01

*Adjusted for age, sex, race, and center; †test of linear trend across quartiles; ‡unadjusted means are shown; §geometric mean values are shown; ‖among current drinkers; #among ever smokers.

median of FFAs compared with those below the median) across levels of the other risk factor. Interaction on the additive scale was evaluated by the interaction contrast ratio (22).

RESULTS — In the cohort random sample, plasma FFA levels were positively and statistically significantly associated with female sex, heart rate, BMI, waist circumference, waist-to-hip ratio, plasma triglycerides, and inflammation score and inversely associated with height (Table 1). No significant associations were found at baseline between FFAs and fasting glucose, insulin, or HOMA-IR, although insulin levels were somewhat lower at higher levels of FFAs.

In the basic model adjusting for age, sex, race, and center (model 1), hazard ratios for diabetes increased monotonically across quartiles of FFAs (Table 2). When compared with estimates from the basic model, HRs were strengthened when further adjusted for fasting glucose and insulin (model 2). By contrast, associations between FFAs and diabetes were attenuated when adjusted for BMI and waist circumference (model 3), the inflammation score (model 4), or plasma triglyceride level (model 5). In a fully adjusted model including all of the above variables (model 6), the HR for highest quartile of FFA level remained statistically significant (HR 1.63, 95% CI 1.04–2.57). Similar results were found when FFA level was modeled as a continuous variable (Table 2), when HOMA-IR was substituted for fasting insulin, and when waist-to-hip ratio was substituted for waist circumference (data not shown). Using model 1 and FFA level as a continuous variable, the HR per interquartile range of FFAs was 1.35 (1.14–1.59) for cases diagnosed within 3 years of the baseline exam, 1.11 (0.88–1.39) for cases diagnosed 3–6 years after the baseline exam, and 1.21 (0.97–1.51) for cases diagnosed more than 6 years after the baseline exam.

Adjustment for education level, alcohol intake, cigarette smoking status or pack-years, sport index, hypertension, parental history of diabetes, height, HDL cholesterol, or heart rate did not materially change the associations shown in Table 2. For example, the HR comparing the highest quartile of FFA level to the lowest quartile was 1.66 (1.08–2.55) in a model with all these additional covariates.

Relative risks for diabetes among those above the median FFA level compared with those below the median were similar in men (HR 1.34, 95% CI 0.92–1.96) and women (1.51, 1.12–2.02), African Americans (1.36, 0.98–1.88) and

Table 2—*Adjusted HRs and 95% CIs for incident diabetes according to baseline FFAs modeled in quartiles or continuously*

	Model 1 (basic)		Model 2		Model 3		Model 4		Model 5		Model 6	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
FFA in quartiles (range in g/l)												
1 (0.04–0.15)	1.00	(ref.)	1.00	(ref.)	1.00	(ref.)	1.00	(ref.)	1.00	(ref.)	1.00	(ref.)
2 (0.16–0.20)	1.19	0.85–1.67	1.52	0.98–2.36	1.21	0.84–1.73	1.18	0.83–1.69	1.13	0.80–1.60	1.46	0.94–2.25
3 (0.21–0.29)	1.48	1.06–2.06	1.64	1.01–2.66	1.47	1.04–2.10	1.34	0.95–1.90	1.40	1.00–1.97	1.59	0.99–2.55
4 (0.30–0.67)	1.68	1.20–2.34	1.96	1.26–3.06	1.42	0.99–2.05	1.47	1.03–2.08	1.26	0.88–1.80	1.63	1.04–2.57
FFA as a continuous variable												
Per 0.14 g/l	1.26	1.09–1.45	1.37	1.17–1.62	1.17	1.00–1.37	1.18	1.02–1.37	1.09	0.94–1.27	1.25	1.05–1.49

Model 1: adjusted for age, sex, race, and center; model 2: adjusted for model 1 variables + fasting glucose and (log) fasting insulin; model 3: adjusted for model 1 variables + BMI and waist circumference; model 4: adjusted for model 1 variables + inflammation score; model 5: adjusted for model 1 variables + (log) plasma triglycerides; model 6: adjusted for model 1 variables + BMI, waist circumference, inflammation score, (log) plasma triglycerides, fasting glucose, and (log) fasting insulin.

whites (1.47, 1.08–2.01), subjects with (1.19, 0.77–1.85) and without (1.53, 1.16–2.03) a parental history of diabetes, subjects with impaired (1.38, 0.80–2.39) and normal baseline fasting glucose (1.52, 1.14–2.03), and subjects with elevated (1.18, 0.75–1.84) and normal (1.44, 1.09–1.89) fasting plasma triglyceride level. Relative risks for subjects above the median FFA level compared with those below the median were lowest for those of normal weight and highest for the obese: BMI <25 (1.10, 0.63–1.92), 25–29 (1.29, 0.88–1.90), 30–34 (1.40, 0.83–2.36); and ≥35 kg/m^2 (1.69, 0.91–3.15). However, formal tests for interaction between FFAs and sex, race, parental history of diabetes, baseline fasting glucose, triglycerides, or BMI were not statistically significant ($P > 0.05$) on either the additive or multiplicative scales.

CONCLUSIONS — Using a case-cohort design, we found a modest association between fasting plasma FFA level and incident diabetes in middle-aged African-American and white subjects from four U.S. communities. This association was strengthened by adjustment for baseline fasting glucose and insulin and attenuated by adjustment for plasma triglycerides or measures of adiposity and systemic inflammation; however, elevated FFAs were an independent predictor of diabetes in models adjusting for all of these variables.

FFAs have long been recognized as an adipose-derived signal and potential mediator of insulin sensitivity, insulin secretion, and hepatic glucose regulation in obesity (23). Experimental elevation of plasma FFAs results in greater insulin resistance in skeletal muscle and is accompanied by activation of nuclear factor-κB, part of a major proinflammatory pathway (24). It has been hypothesized that the insulin-sensitizing effects of thiazolidinediones may occur, at least in part, through decreased lipolysis of adipose tissue and subsequent reductions in circulating FFAs (25).

Recently there has been increasing interest in the active role of adipose tissue in the regulation of metabolism. Adipocytes secrete signaling molecules such as leptin, adiponectin, and proinflammatory cytokines that have important effects on lipid and carbohydrate metabolism (10,26). Within this context, elevated FFAs may be an important mechanism through which deranged adipocyte metabolism produces insulin resistance, decreased β-cell secretion of insulin (4), and thus type 2 diabetes.

There have been few prospective epidemiologic studies to evaluate whether FFA levels predict the development of diabetes and whether these associations are independent of established risk factors for diabetes such as obesity. In the Pima study (17), subjects with plasma FFA levels in the highest decile had a risk of diabetes that was 2.3-fold higher than that of subjects in the lowest decile, after adjusting for sex, percent body fat, insulin sensitivity, waist-to-thigh ratio, and triglycerides. In the Paris Prospective Study (16), higher FFA levels were associated with deterioration of glucose tolerance (i.e., progression to diabetes among those with normal glucose tolerance at baseline), with a relative risk of 1.3 per SD (0.12 mmol/l) change in FFAs in a model adjusted for age, baseline fasting and 2-h glucose, and iliac-to-thigh ratio. By contrast, baseline FFA levels in the Ely Study were slightly lower in those who progressed to diabetes compared with those who did not (18). Relative risks were not presented in that study. Unlike our study, all of the earlier prospective studies diagnosed diabetes on the basis of an oral glucose tolerance test. In at least two studies (17,18), cross-sectional associations between fasting FFA levels and 2-h glucose levels were larger than those with fasting glucose levels, possibly indicating that FFAs may be a stronger predictor of postprandial than fasting hyperglycemia.

If the effect of FFAs is modified by other factors such as obesity, then differences in study populations may at least partially explain variability in the strength of association across studies. Although participants in the Ely Study (18) were of similar mean age and BMI to those in the ARIC study, those in the Pima study (17) were nearly 30 years younger, on average, and considerably more obese, with an average BMI of 34 kg/m^2, while those in the Paris Prospective Study (16) were exclusively male, somewhat less obese than ARIC participants, and possibly more physically active due to police employment. It is possible that elevated FFAs may serve as a marker to differentiate those with greater derangements in adipocyte metabolism from those with relatively normal adipocyte function primarily in populations such as the Pima

Indians with a substantial burden of obesity. In support of this hypothesis, the association between FFAs and diabetes increased across categories of overweight and obesity in our study. However, a formal test of interaction between FFA level and BMI was not statistically significant.

The interpretation of these and other epidemiologic studies is dependent on the role, if any, that FFAs are hypothesized to play in the etiology of type 2 diabetes. If FFAs are intermediate on the causal pathway between obesity or low-grade inflammation and the development of type 2 diabetes, then associations between FFAs and diabetes would be expected to become weaker, as they did in the present study, upon statistical adjustment for measures of adiposity or chronic inflammation. The strong cross-sectional association between fasting triglyceride and FFA levels observed in our study likely reflects a complex metabolic cycle in which the direction of association is unclear. Significant associations between FFAs and triglycerides have been reported in some (16,27) but not all (17) studies. High plasma concentrations of triglyceride-rich lipoproteins lead to an increase in the release of FFAs into the circulation through lipolysis by lipoprotein lipase (28). However, FFAs also provide the substrate for production of triglyceride-rich lipoproteins (29) and stimulate production of these lipoproteins in the liver (30). Thus, elevated fasting triglycerides and FFAs may both serve as markers of increased FFA flux and disordered fat storage (31). Statistical adjustment of one analyte for the other takes on uncertain meaning in this context and may represent a form of overadjustment.

An interesting finding in the present study is the lack of a cross-sectional association between FFA levels and fasting insulin in the cohort random sample. Other studies have reported similar findings (27,32). If elevated FFAs are causally related to diabetes, one might expect that these effects would be mediated by greater insulin resistance, indirectly measured by hyperinsulinemia due to upregulated insulin secretion. However, fasting insulin levels also reflect insulin clearance and pancreatic β-cell function, both of which may be impaired by chronically elevated FFA levels. Additionally, insulin suppresses adipocyte release of FFAs (33). One cross-sectional study that utilized a euglycemic-hyperinsulinemic clamp found strong inverse associations between fasting FFA concentrations and whole-body glucose disposal rate but virtually no association between FFAs and fasting plasma insulin (34), possibly because higher fasting insulin reflects a mixture of insulin resistance and β-cell function. That the addition of fasting insulin as a covariate produced a stronger association between FFA level and diabetes (i.e., negative confounding) may indicate that insulin, in this context, is more reflective of better β-cell function than worsened insulin resistance.

Some studies have shown a cross-sectional association between visceral obesity or insulin resistance and sympathetic nervous system activation (35). Catecholamines activate hormone-sensitive lipase, which leads to lipolysis in fat depots and release of FFAs (8). Catecholamines may induce insulin resistance (36), but it is unclear whether this is mediated by FFAs. Heart rate, a marker of autonomic dysfunction, elevated sympathetic tone, neurohormonal factors, and physical fitness, was positively associated with incident diabetes in the ARIC study (37). In the present analysis, heart rate was cross-sectionally associated with FFA level but did not materially change the estimate of the strength of association between FFAs and diabetes when included as a covariate in multivariate models.

In addition to a possible interaction between FFAs and obesity described earlier, several other risk factors for diabetes may also modify the association between FFAs and diabetes. Because elevated FFAs impair normal β-cell function, FFAs may accelerate the progression to diabetes in those who are genetically predisposed (38) or already have some degree of glucose intolerance at baseline (39). However, our data do not support this hypothesis, as relative risks for diabetes associated with elevated FFAs were somewhat lower in subjects with a parental history of diabetes and in those with impaired fasting glucose.

It is possible that a single measure at the baseline exam may not adequately reflect usual FFA level over the period at risk and that total peripheral FFAs may not capture those aspects of FFA flux that are most relevant to diabetes risk. Release of FFAs from stored triglycerides is higher in visceral fat and is directed to the liver via the portal vein, and chronically elevated portal FFAs may contribute to hepatic insulin resistance (25). Because of hepatic extraction of FFAs from the circulation, FFA levels in peripheral blood may be a poor indicator of regional differences in adipose tissue lipolysis. Furthermore, the relative composition of fatty acids, in part reflecting dietary fat intake, may be more important than total circulating FFAs in determining risk of diabetes. A separate investigation of Minneapolis participants in the ARIC study (40) found that a high proportion of saturated fatty acids in plasma cholesterol esters and phospholipids was associated with increased incidence of type 2 diabetes, while total monounsaturated and polyunsaturated fatty acids did not show consistent relations to diabetes incidence. Unfortunately the overlap of participants in that study with participants in our study is not large enough to permit a joint analysis of FFA level and fatty acid composition.

In conclusion, we found that fasting plasma FFA level was positively associated with incident diabetes in the ARIC study after accounting for measures of glucose metabolism, adiposity, chronic inflammation, and plasma triglycerides. Associations were modest but somewhat stronger in obese individuals. These population-based prospective data lend further support for an etiologic role for FFAs in the development of diabetes.

Acknowledgments— Support for this study was provided by NIDDK grant R01-DK56918 and NHLBI contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022. B.B.D. and M.I.S. received support from a Centers of Excellence Grant of CNPq (Brazilian National Research Council).

The authors thank the staff and participants in the ARIC study for their important contributions. The authors thank David Kwan for assistance with statistical programming.

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