Original Article

Obesity Is a Major Determinant of the Association of C-Reactive Protein Levels and the Metabolic Syndrome in Type 2 Diabetes

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The inflammatory factor C-reactive protein (CRP) and the fibrinolytic variables fibrinogen and plasminogen activator-1 (PAI-1) are associated with long-term cardiovascular morbidity. To determine the contribution of body adiposity (BMI), insulin sensitivity (homeostasis model assessment of insulin resistance [HOMA-IR], and glycemia (HbA_{1c} [A1C]) to the levels of these inflammatory and fibrinolytic variables in recently diagnosed (≤3 years), drug-naive, type 2 diabetic subjects (fasting plasma glucose ≤10 mmol/l), we examined a representative subgroup (n = 921) of the U.S. cohort in ADOPT (A Diabetes Outcome Progression Trial). The relationship between levels of CRP, fibrinogen, PAI-1 antigen and PAI-1 activity, and baseline variables including National Cholesterol Education Program Adult Treatment Panel III metabolic syndrome phenotype were explored. All four factors increased significantly with increasing numbers of metabolic syndrome components (P = 0.0136 to P < 0.0001). BMI (P < 0.0001) and HOMA-IR (P < 0.0001) but not A1C (P = 0.65) increased with increasing numbers of metabolic syndrome components. Adjustment of CRP levels for BMI eliminated the association between CRP and the number of metabolic syndrome components, while adjusting for HOMA-IR did not (P = 0.0028). The relationships of PAI-1 antigen and PAI-1 activity with the number of metabolic syndrome components were maintained after adjusting for BMI (P = 0.0002 and P = <0.0001, respectively) or HOMA-IR (P = 0.0008 and P = <0.0001, respectively), whereas that with fibrinogen was eliminated after adjusting for BMI but not after adjusting for HOMA-IR (P = 0.013). Adjustment for A1C had no effect on any of the relationships between the inflammatory and fibrinolytic factors and the metabolic syndrome. We conclude that in recently diagnosed, drug-naive type 2 diabetic subjects, markers of inflammation and fibrinolysis are strongly related to the number of metabolic syndrome components. Further, for CRP and fibrinogen this relationship is determined by body adiposity and not by insulin sensitivity or glucose control. *Diabetes* 55:2357–2364, 2006

Increased levels of the nontraditional cardiovascular disease (CVD) risk factors C-reactive protein (CRP), plasminogen activator-1 (PAI-1), and fibrinogen have been associated with increased CVD in the general population (1–4). Type 2 diabetes is also associated with increased levels of these nontraditional CVD risk factors (5,6). In the nondiabetic population, insulin resistance and obesity are important determinants of increases in these inflammatory and fibrinolytic variables (5–9). However, the role of obesity and insulin resistance relative to worsening glycemia as determinants of these risk factors has not been extensively explored in subjects with type 2 diabetes.

The metabolic syndrome (10), which comprises the more traditional CVD risk factors such as blood pressure, lipids, and central obesity, has been shown to be a risk factor for the development of both CVD and diabetes (11–13). The vast majority of subjects with type 2 diabetes have the metabolic syndrome, and these individuals are at higher risk of CVD than subjects with type 2 diabetes who do not have the metabolic syndrome (12,13). Furthermore, in nondiabetic subjects the metabolic syndrome has been associated with higher levels of CRP as well as PAI-1 and fibrinogen (7,11). However, the relationship between the presence or absence of the metabolic syndrome and concentrations of CRP, PAI-1, and fibrinogen has not been explored in a large cohort of type 2 diabetic subjects who are drug naive.

The interpretation of analyses of nontraditional CVD risk factors in subjects with type 2 diabetes has been complicated by the fact that many subjects with the disease have been treated with glucose-lowering pharmacological therapies at the time of investigation, and these

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ADOPT, A Diabetes Outcome Progression Trial; CRP, C-reactive protein; CVD, cardiovascular disease; HOMA-IR, homeostasis model assessment of insulin resistance; PAI-1, plasminogen activator-1.

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TABLE 1
Demographic and metabolic variables in all subjects and subdivided based on the presence or absence of the metabolic syndrome

	All	Metabolic syndrome yes	Metabolic syndrome no	*P* value* (metabolic syndrome yes vesus no)
n (% male)†	921 (51)	803 (49)	118 (66)	<0.0001
Age (years)	55.3 ± 0.3	55.6 ± 0.4	52.9 ± 1.0	0.011
GAD positive (%)	43 (4.7)	32 (4.0)	11 (9.3)	0.011
BMI (kg/m^2)	33.7 ± 0.2	34.5 ± 0.2	28.1 ± 0.5	<0.0001
Waist circumference (cm)	108 ± 0.5	110 ± 0.5	93 ± 1.2	N/A
Systolic blood pressure (mmHg)	129 ± 0.5	131 ± 0.5	119 ± 1.3	N/A
Diastolic blood pressure (mmHg)	79 ± 0.3	79 ± 0.3	75 ± 0.8	N/A
Fasting plasma glucose (mmol/l)	8.4 ± 0.05	8.4 ± 0.05	8.3 ± 0.14	N/A
A1C (%)	7.5 ± 0.03	7.5 ± 0.03	7.6 ± 0.10	0.079
HOMA-IR (μU/ml per mmol/l) *	3.4 (3.2–3.5)	3.6 (3.5–3.8)	2.0 (1.8–2.3)	<0.0001
Non-HDL cholesterol (mmol/l) *	3.8 (3.8–3.9)	3.9 (3.8–4.0)	3.6 (3.4–3.8)	0.003
LDL cholesterol (mmol/l) *	2.9 (2.8–2.9)	2.8 (2.8–2.9)	3.0 (2.8–3.2)	0.037
HDL cholesterol (mmol/l) *	1.1 (1.1–1.1)	1.1 (1.1–1.1)	1.3 (1.3–1.4)	N/A
Triglycerides (mmol/l) *	1.9 (1.9–2.0)	2.1 (2.0–2.2)	1.1 (1.0–1.2)	N/A

Data are means ± SE, *n* (%), or geometric means (95% CI). **P* value based on Wilcoxon's rank-sum test for continuum variables and χ^2 test for dichotomous variables. †Nine patients did not have adequate data to assign metabolic syndrome category. N/A, not determined as used to categorize metabolic syndrome yes/no.

agents may affect the levels of these markers. For example, thiazolidinediones have been shown to lower levels of nontraditional CVD risk factors in individuals with type 2 diabetes (14–16). Therefore, to minimize the possible confounding effects of glucose-lowering pharmacological treatment, we examined the determinants of CRP and PAI-1 activity and antigen as well as fibrinogen in a large cohort of participants in the ADOPT (A Diabetes Outcome Progression Trial) study residing in the U.S. (17). These individuals were recently diagnosed with type 2 diabetes and were drug naive, thus allowing us to compare the effects of obesity, insulin resistance, and glycemia without the possible confounding effects of diabetes treatment.

RESEARCH DESIGN AND METHODS

The ADOPT study recruited 4,357 individuals with diabetes diagnosed within 3 years who remained drug naive for glucose-lowering therapy. Of these subjects, 921 individuals in the U.S. underwent further testing, which included measurement of inflammatory and fibrinolytic markers. Their baseline data form the basis of this report. The study protocol was reviewed and approved by institutional review boards for each center, and participants gave written informed consent before participating in the study.

The study is a randomized, double-blind, parallel-group trial, the details of which have been described previously (17). Subjects eligible to participate in ADOPT were 30–75 years of age and had a fasting plasma glucose concentration between 7 and 10 mmol/l despite diet and exercise intervention.

At baseline, subjects had anthropometric measurements (weight, height, and waist circumference) and seated blood pressure measured using standardized procedures across all study centers (17). Specifically, waist circumference was measured at the level of the umbilicus. Fasting blood samples were drawn for measurement of metabolic variables including lipids, plasma glucose, HbA_{1c} (A1C), and immunoreactive insulin levels.

Assays and calculations. All assays were performed at a central laboratory (17). Plasma glucose was measured using a hexokinase assay method (Olympus America, Melville, NY). A1C was determined using the Biorad Variant Hemoglobin A1C assay (Hercules, CA). Serum immunoreactive insulin was quantified using a double antibody radioimmunoassay (Linco, St. Louis, MO). Total cholesterol and triglycerides were measured by enzymatic methods (Olympus America). HDL cholesterol was determined using a precipitation method (Olympus America). Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. GAD antibodies were measured using a radioimmunoassay that recognizes autoantibodies to GAD65 (RSR; Cardiff, Wales, U.K.).

Highly sensitive CRP was measured by fixed time nephelometry (reporting range 0.2 mg/l to 10 g/l, coefficient of variation [CV] <7%) (Dade Behring, Deerfield, IL). PAI-1 antigen was quantified using an enzyme immunoassay (limit of detection <4 ng/ml, CV 5–13%) (Biopool TintElize; Trinity Biotech, Carlsbad, CA) and PAI-1 activity by bioimmunoassay (limit of detection 6 IU/ml, CV 2–5%) (Chromolize PAI-1; diaPharma, West Chester, OH). Fibrinogen was measured using photo-optical clot detection (reporting range 0.04–1.4 g/dl, CV 5–10%; MLA Electra 1000C).

Insulin sensitivity was estimated with HOMA2 IR (18). Software for calculating this measure was obtained from http://www.dtu.ox.ac.uk/homa.

The National Cholesterol Education Program Adult Treatment Panel III criteria (10) were used to determine whether individuals had the metabolic syndrome. As all subjects in the study by definition met the glucose criteria (≥5.5 mmol/l; 100 mg/dl), they only had to meet two of the remaining four criteria to be classified as having the metabolic syndrome. Individuals with a waist circumference ≥102 cm in men and ≥88 cm in women were considered to have met the criterion for waist circumference. Subjects with a blood pressure ≥130/85 mmHg or a history of hypertension (n = 505) were considered to have met the blood pressure criterion. Individuals with triglycerides ≥1.695 mmol/l (150 mg/dl) or who were taking fibrates (n = 38) were considered to have met the criterion for triglycerides, while those with a HDL cholesterol level ≤1.036 mmol/l (40 mg/dl) in men and ≤1.295 mmol/l (50 mg/dl) in women or who were taking niacin (n = 15) were considered to have met the criterion for HDL cholesterol. This information was obtained by self-report, examination of medical records, and/or examination of individual subjects' medications.

Statistical methods. Where appropriate, data were log transformed to achieve a normal distribution. Wilcoxon's rank-sum tests were performed to compare variables between groups with and without the metabolic syndrome. General linear models assuming normal errors (19) were used to summarize CRP, PAI-1 antigen, PAI-1 activity, and fibrinogen among subjects grouped according to the number of metabolic syndrome criteria met. Linear trend tests were performed as a contrast within the general linear models. Partial correlation analyses were performed using the Spearman method. Data are presented as mean ± SE, except for log-transformed data where geometric means with 95% CIs are shown. A two-sided $P \leq 0.05$ was considered statistically significant.

RESULTS

Demographic and metabolic variables. Baseline demographic and metabolic variables for all 921 subjects and those with and without the metabolic syndrome are listed in Table 1. A total of 87.2% of subjects met criteria for the metabolic syndrome. Subjects with the metabolic syndrome had a markedly greater BMI than those without the metabolic syndrome. In addition, their non-HDL cholesterol was higher, and they were more insulin resistant than those without the metabolic syndrome. As expected, subjects with the metabolic syndrome also had higher mean systolic and diastolic blood pressures, higher triglycerides and waist circumference, and lower HDL cholesterol. However, there was no significant difference in fasting

TABLE 2
Measures of fibrinolytic and inflammatory factors in all subjects and subdivided based on sex and the presence or absence of the metabolic syndrome

	All	Metabolic syndrome yes	Metabolic syndrome no	*P* value* (metabolic syndrome yes versus no)
n	921	803	118	
Fibrinogen (mg/dl)	320 (314–327)	323 (317–330)	300 (284–318)	0.006
Men	313 (305–321)	317 (308–325)	296 (278–314)	0.049
Women	328 (318–338)	330 (320–340)	310 (274–350)	0.257
P value (men versus women)	$P = 0.001$	$P = 0.006$	$P = 0.331$	
PAI-1 antigen (ng/ml)	40.3 (38.7–41.9)	42.0 (40.3–43.7)	30.4 (26.6–34.8)	<0.0001
Men	38.2 (36.1–40.3)	40.3 (38.0–42.6)	28.9 (24.8–33.6)	<0.0001
Women	42.5 (40.1–45.0)	43.7 (41.3–46.3)	33.5 (25.4–44.2)	0.0096
P value (men versus women)	$P = 0.007$	$P = 0.073$	$P = 0.633$	
PAI-1 activity (IU/ml)	20.7 (19.5–22.0)	23.2 (21.9–24.7)	9.6 (7.9–11.7)	<0.0001
Men	19.6 (18.0–21.4)	22.8 (20.9–24.8)	9.8 (7.8–12.4)	<0.0001
Women	21.7 (19.9–23.6)	23.7 (21.9–25.7)	9.2 (6.2–13.7)	<0.0001
P value (men versus women)	$P = 0.105$	$P = 0.493$	$P = 0.442$	
CRP (mg/l)	3.8 (3.5–4.1)	4.1 (3.8–4.4)	2.2 (1.8–2.7)	<0.0001
Men	2.6 (2.4–2.8)	2.8 (2.5–3.1)	1.8 (1.4–2.3)	0.0009
Women	5.6 (5.1–6.2)	5.9 (5.4–6.5)	3.2 (2.1–5.1)	0.0005
P value (men versus women)	$P < 0.0001$	$P < 0.0001$	$P = 0.008$	

Data are geometric means (95% CI). *P value based on Wilcoxon's rank-sum test.

plasma glucose or A1C in subjects with or without the metabolic syndrome.

BMI correlated significantly with HOMA-IR ($r = 0.53$, $P < 0.0001$) but not A1C ($r = -0.01$, $P = 0.72$), while HOMA-IR and A1C were not related ($r = -0.05$, $P = 0.130$).

Fibrinolytic and inflammatory factors. Table 2 lists the results of fibrinogen, PAI-1 antigen and activity, and CRP measurements within sex and among those with and without the metabolic syndrome, overall and within subgroups classified by the other factor, e.g., by metabolic syndrome group separately for men and women. Fibrinogen, PAI-1 antigen, and CRP levels were significantly higher in women, while levels of PAI-1 activity did not differ by sex. Individuals with the metabolic syndrome had higher fibrinogen, PAI-1 antigen, PAI-1 activity, and CRP levels than those without the metabolic syndrome. With the exception of fibrinogen in women, in both sexes subjects with the metabolic syndrome had higher levels of inflammatory and fibrinolytic factors. There were no significant interactions between sex and metabolic syndrome status for any of these four biomarkers.

Relationship of glucose control, insulin sensitivity, and body size to the fibrinolytic and inflammatory factors. The associations of A1C, HOMA-IR, and BMI with fibrinogen, PAI-1 antigen, PAI-1 activity, and CRP are listed in Table 3. A1C was weakly associated with fibrinogen, while BMI was more strongly associated. In contrast, HOMA-IR was not associated with fibrinogen. A1C was not associated with PAI-1 activity or antigen, while HOMA-IR and BMI were both strongly associated with PAI-1 antigen and PAI-1 activity. A1C, HOMA-IR, and BMI were all associated with CRP levels, although the strength of these associations differed.

Relationship of glucose control, insulin sensitivity, and body size to the metabolic syndrome and the number of its components. The numbers of subjects meeting different numbers of metabolic syndrome components is listed in Table 4. Only 3% of subjects met only one criterion (glucose), while 25.6% of subjects met all five criteria. Interestingly, in this population, A1C did not differ regardless of the number of components of the metabolic syndrome, while HOMA-IR and BMI increased progressively with increasing numbers of the metabolic syndrome criteria.

Figure 1 shows the relationship between numbers of metabolic syndrome components and inflammatory and fibrinolytic factors. As expected from data presented earlier, there was a positive and significant association between the number of metabolic syndrome components and these factors, although this relationship was only modest for fibrinogen. After adjustment for BMI, the relationship between PAI-1 antigen and PAI-1 activity and the number of metabolic syndrome components was virtually unchanged. In contrast, the positive and significant relationship between the number of metabolic syndrome components and fibrinogen and CRP was eliminated after adjustment for BMI.

In Fig. 2, the associations between the number of meta-

TABLE 3
Relationship of A1C, HOMA-IR, and BMI to fibrinolytic and inflammatory factors

	Fibrinogen (mg/dl)		PAI-1 antigen (ng/ml)		PAI-1 activity (IU/ml)		CRP (mg/l)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
A1C (%)	0.09	0.010	−0.01	0.658	−0.05	0.166	0.09	0.005
HOMA-IR (μU/ml per mmol/l)	0.03	0.328	0.36	<0.0001	0.44	<0.0001	0.29	<0.0001
BMI (kg/m^2)	0.17	<0.0001	0.25	<0.0001	0.26	<0.0001	0.43	<0.0001

Each cell represents a partial correlation coefficient r and P value adjusted for age, sex, and ethnicity.

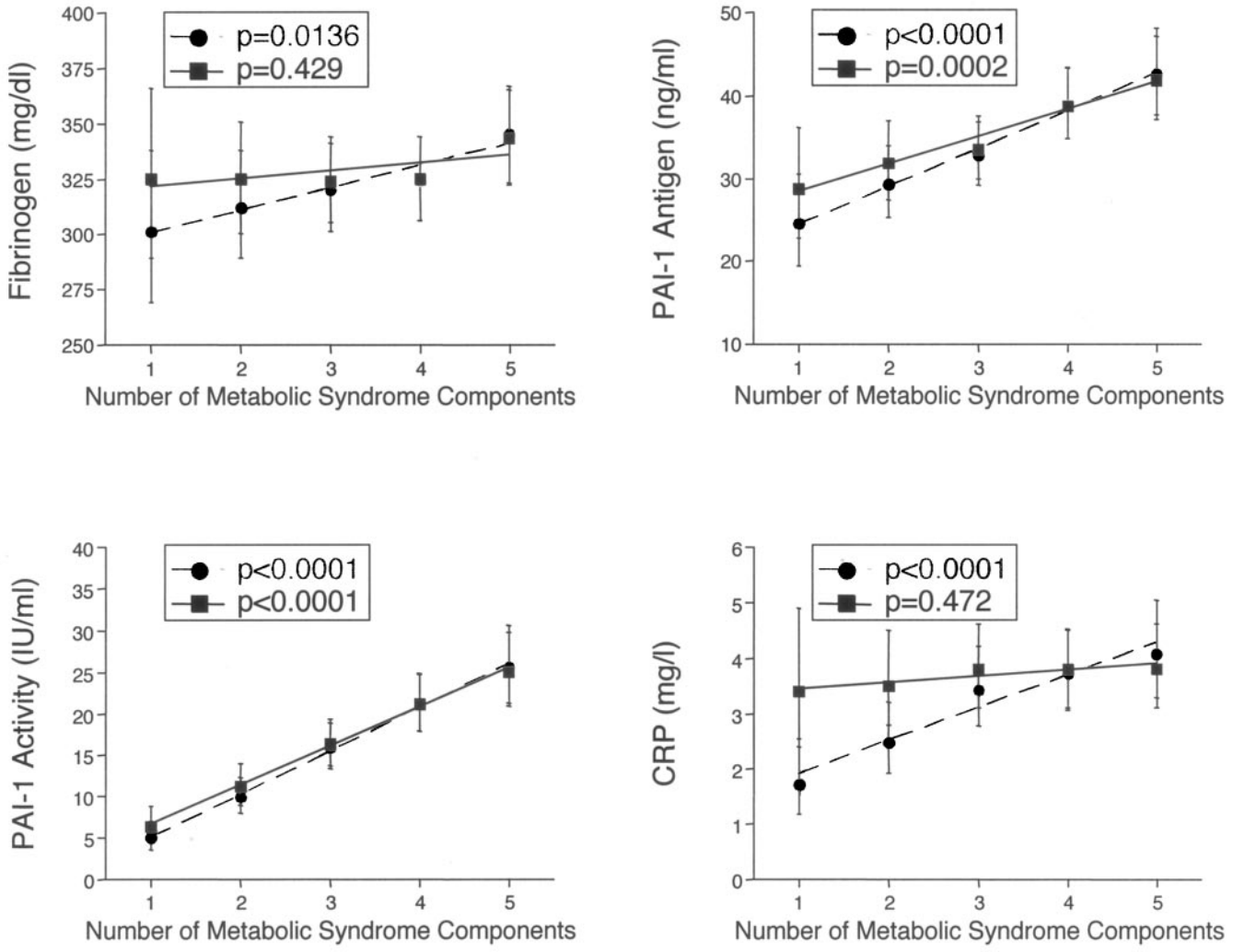

FIG. 1. Relationship of number of metabolic syndrome components and inflammation and fibrinolytic factors adjusted for BMI as well as age, sex, and ethnicity. The trend line describing the relationships adjusted for age, sex, and ethnicity is illustrated as the broken line, while that adjusted for BMI, age, sex, and ethnicity is shown as the solid black line. Data are geometric means (95% CI).

bolic syndrome components and inflammatory and fibrinolytic factors before and after adjustment for HOMA-IR are illustrated. This figure is similar to Fig. 1 except that the adjustment is made for HOMA-IR rather than BMI. Adjustment for HOMA-IR did not abolish the relationship between fibrinogen and the number of metabolic syndrome components. After adjustment for HOMA-IR, both PAI-1 activity and antigen also remained significantly associated with the number of metabolic syndrome components. In contrast to the relationship depicted in Fig. 1, after adjustment for HOMA-IR, CRP remained highly significantly associated with the number of metabolic syndrome components.

The relationships between the number of metabolic syndrome components and inflammatory and fibrinolytic factors before and after adjustment for A1C are illustrated in Fig. 3. As shown in this figure, adjusting for A1C failed to change any of the relationships between the different inflammatory and fibrinolytic factors and the number of metabolic syndrome components.

DISCUSSION

In this analysis of drug-naive subjects with recently diagnosed type 2 diabetes, we have demonstrated that obesity and insulin resistance are strongly associated with both

TABLE 4
Relationship of A1C, HOMA-IR, and BMI to the number of metabolic syndrome components

Number of metabolic syndrome components	1	2	3	4	5	*P* value for trend test
n (%)	28 (3)	90 (9.8)	227 (24.7)	340 (36.9)	236 (25.6)	
A1C (%)	7.5 (7.2–7.9)	7.8 (7.5–8.0)	7.6 (7.4–7.8)	7.7 (7.5–7.8)	7.5 (7.3–7.7)	0.650
HOMA-IR (μU/ml per mmol/l)	1.3 (1.1–1.6)	2.3 (2.0–2.6)	3.1 (2.8–3.4)	3.6 (3.3–4.0)	4.2 (3.7–4.7)	<0.0001
BMI (kg/m^2)	23.9 (21.4–26.3)	28.3 (26.7–29.9)	32.2 (31.0–33.4)	33.6 (32.5–34.8)	34.6 (33.3–35.9)	<0.0001

Data are model-adjusted geometric means (95% CI). ANCOVA for all models adjusted for age, sex, and ethnicity.

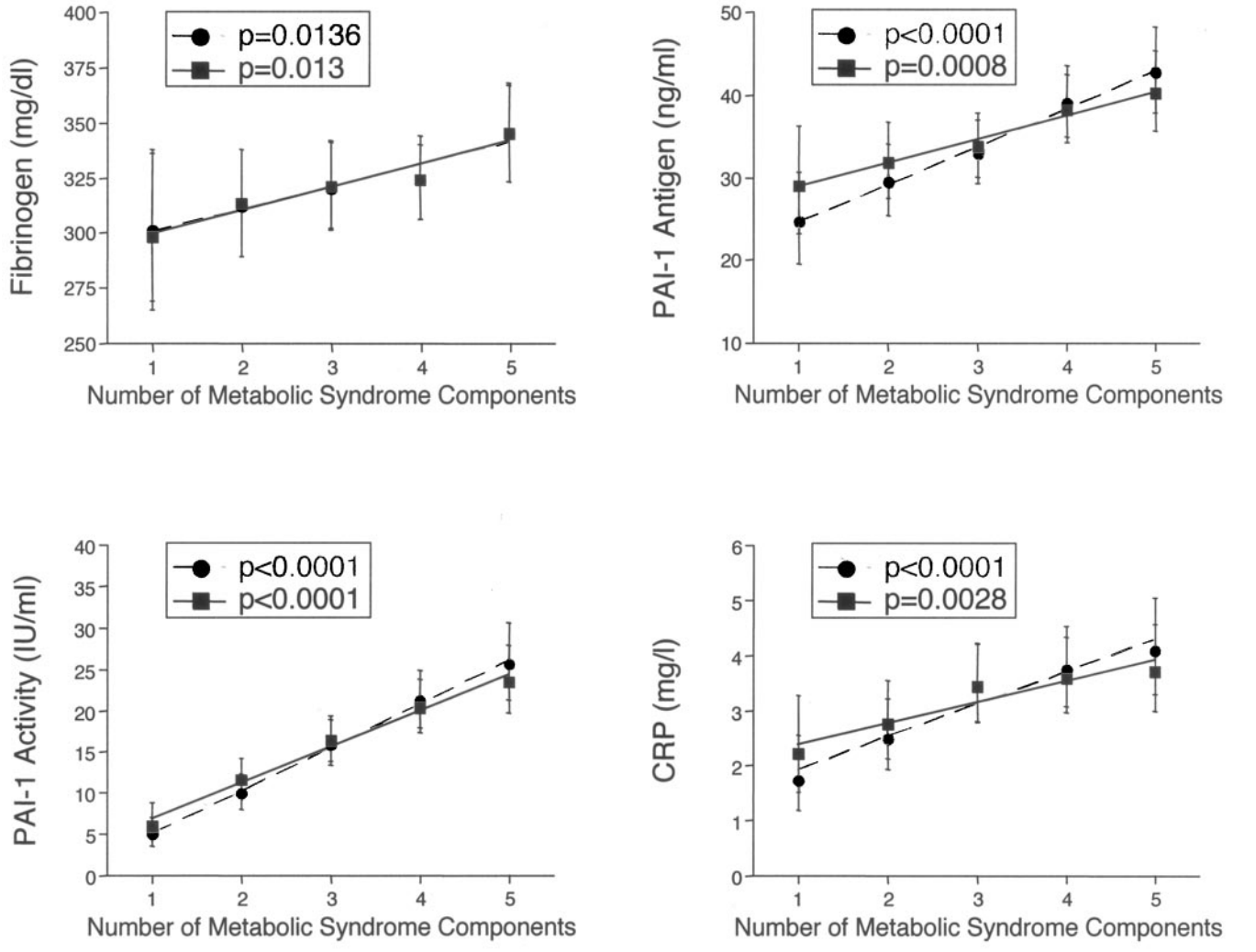

FIG. 2. Relationship of number of metabolic syndrome components and inflammation and fibrinolytic factors adjusted for HOMA-IR as well as age, sex, and ethnicity. The trend line describing the relationships adjusted for age, sex, and ethnicity is illustrated as the broken line, while that adjusted for HOMA-IR, age, sex, and ethnicity is shown as the solid black line. Data are geometric means (95% CI).

inflammatory and fibrinolytic variables. These data extend previous reports done predominantly in nondiabetic subjects (5–9) and show that even in diabetic subjects with a fasting glucose ≤10 mmol/l, these relationships still hold. In contrast, we have shown for the first time in this large cohort of diabetic subjects not on glucose-lowering pharmacological therapy that A1C was not an important determinant of the levels of fibrinogen, PAI-1 activity, PAI-1 antigen, or CRP.

We also examined the relationship between the presence of the metabolic syndrome in recently diagnosed diabetic subjects and these inflammatory and fibrinolytic variables. In this cohort of 921 subjects, the prevalence of the metabolic syndrome was high (87.2%), a finding similar to that in National Health and Nutrition Examination Survey III (13). Not surprisingly, subjects with the metabolic syndrome were more obese than those without the syndrome, and a greater proportion of individuals with the metabolic syndrome had been diagnosed with hypertension (52.3 vs. 34.4%); however, only a small proportion of metabolic syndrome patients were receiving fibrates (4.5%) or niacin (1.7%) as was the case for both fibrate (1.7%) and niacin (0.9%) use in those without the metabolic syndrome. As individuals with a diagnosis of hypertension were also more likely to be receiving antihypertensive agents, the phenotypic differences in blood pressure and lipids between those with and without the metabolic syndrome were likely underestimated. In addition to these phenotypic findings, those with the metabolic syndrome had higher levels of CRP, fibrinogen, PAI-1 activity, and PAI-1 antigen, a finding in keeping with what has been reported in nondiabetic subjects (5–9). These findings are relevant as there is now ongoing debate about the relevance of the term "metabolic syndrome" (20,21), and in the course of this discourse it has been strongly suggested that further research is necessary and that some of this should focus on the potential role of inflammatory and fibrinolytic factors.

There was a strong relationship between the number of metabolic syndrome components and these inflammatory and fibrinolytic variables. However, the relationship between the number of metabolic syndrome components and CRP appeared to be largely the result of obesity as it was markedly attenuated, becoming nonsignificant after adjusting for BMI. This observation strongly supports the concept that CRP in diabetic subjects primarily reflects the magnitude of adiposity. Our findings are very consistent with those recently reported by Timpson et al. (22) who,

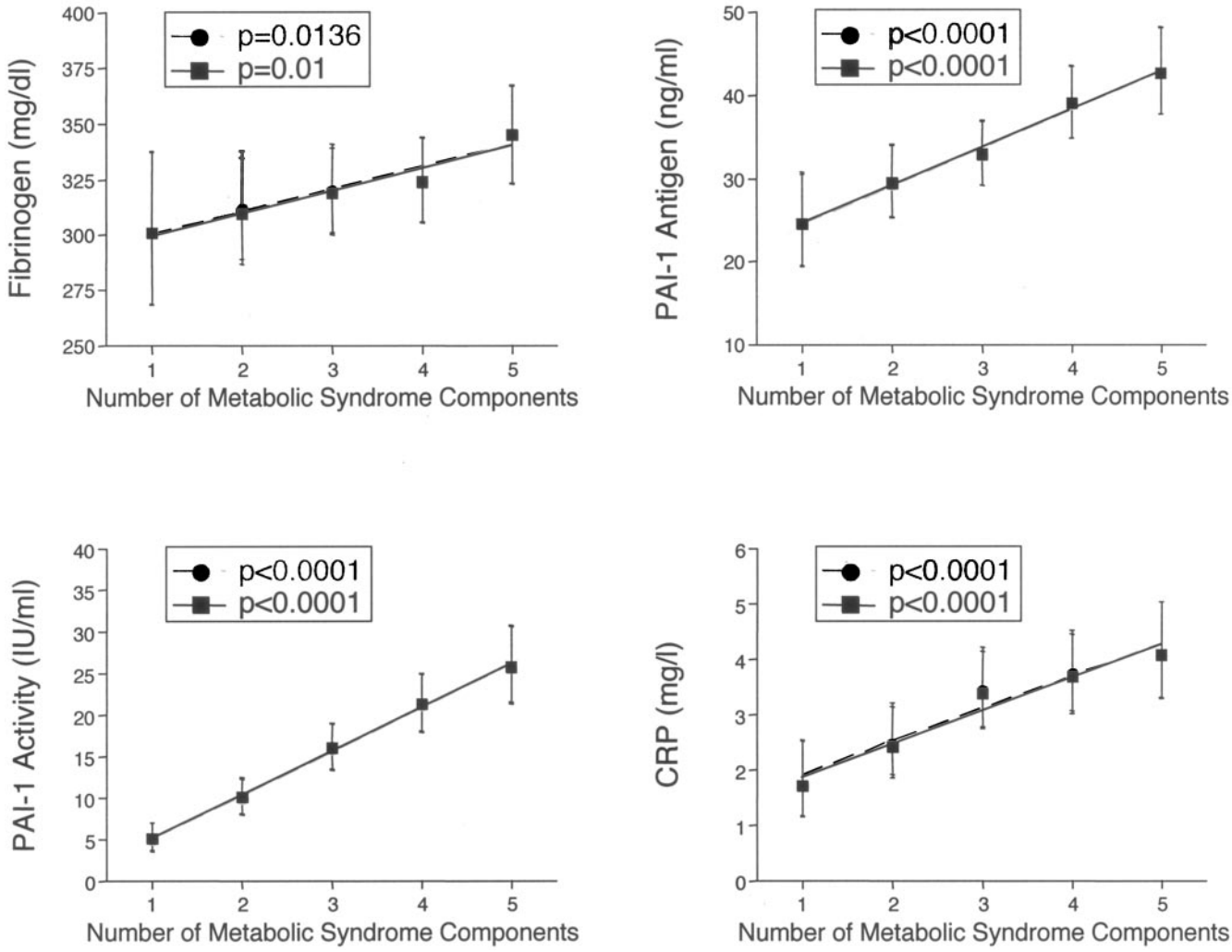

FIG. 3. Relationship of number of metabolic syndrome components and inflammation and fibrinolytic factors adjusted for A1C as well as age, sex, and ethnicity. The trend line describing the relationships adjusted for age, sex, and ethnicity is illustrated as the broken line, while that adjusted for A1C, age, sex, and ethnicity is shown as the solid black line. Data are geometric means (95% CI).

using different methodology, demonstrated in women participating in the British Women's Heart and Health Study that obesity and not CRP haplotype was associated with components of the metabolic syndrome. Thus, these observations are in keeping with adipose tissue and its cellular components being an important site for the production of this proinflammatory cytokine (23). In contrast, neither HOMA-IR nor A1C was related to CRP levels suggesting that neither insulin sensitivity nor glucose control per se have an independent effect on systemic inflammation in individuals with diabetes diagnosed within 3 years and who are not receiving glucose-lowering therapy.

In the context of fibrinolytic variables measured in this study, it is of interest to note that both BMI and HOMA-IR were strongly related to both PAI-1 activity and antigen, but only BMI was related to fibrinogen. These observations once again underscore the importance of obesity and insulin sensitivity in determining PAI-1 activity and antigen. As adjustment for BMI did not fully account for the relationship between these variables and the metabolic syndrome; this suggests that there are other important determinants of PAI-1 activity and antigen. This may be explained by the fact that a variety of tissues, in addition to adipose tissue and its cellular content such as macrophages, have been suggested to be sites for the production and activation of PAI-1 (24–27).

It is well recognized that increased systemic inflammation, impaired fibrinolysis, and increased coagulation are risk factors for the development of CVD (1–4). Therefore, the results of the current analyses have potentially interesting implications for the treatment of individuals with pre-diabetes and recent-onset diabetes who are known to be at increased risk of CVD. Behavioral changes resulting in weight loss have been shown in both diabetic subjects (28,29) and individuals with impaired glucose tolerance (30) to markedly decrease CRP levels but have a more modest effect on fibrinogen. These observations are in keeping with our finding of obesity being a major determinant of CRP levels.

In addition to lifestyle changes, some glucose-lowering agents appear to have independent effects on CRP. The two most commonly used classes of oral agents, metformin and the sulfonylureas, have different effects. Metformin, which is commonly associated with little weight gain or even weight loss, results in a small decrease in CRP (16,30), while sulfonylureas, which commonly result in weight gain, have little effect on CRP (31), despite produc-

ing similar reductions in A1C as metformin. Interestingly, the thiazolidinediones, which typically result in weight gain, have the greatest effect to decrease CRP levels (14–16). This effect may be related to the distribution of fat within the central fat compartments (subcutaneous and intra-abdominal), a factor known to be a major determinant of insulin sensitivity (32,33), and the redistribution of body fat that commonly occurs with these agents (34,35). However, many of these studies have tended to be small and have not directly compared the effect of these three major classes of oral agents on inflammatory and fibrinolytic variables. The longitudinal follow-up data from the current ADOPT cohort will provide such an opportunity.

In the current study, we examined individuals with diabetes and found that obesity was a major contributor to the relationship between the metabolic syndrome, inflammation, and fibrinolysis. While we did not study obese subjects with normal glucose tolerance, we believe it is quite probable that obesity will have a similar effect in such a cohort based on findings in the Insulin Resistance Atherosclerosis Study (IRAS) (36). We also recognize that the entry criteria for the study meant that the diabetic cohort we studied excluded individuals with a fasting plasma glucose $\geq$10 mmol/l and individuals in whom the disease had been diagnosed more than 3 years prior. Thus, it is possible that our conclusions regarding the role of glycemia may have been different if we had examined individuals with even higher glucose levels or if we had studied subjects who had had the disease for a longer duration and were not receiving agents that may affect CRP and fibrinogen. However, we do not feel it is very likely that this would be the case, as type 2 diabetes is typically associated with obesity independent of the duration of the disease and even when hyperglycemia is greater than in the cohort we studied. Rather, it is quite possible that studies that examined levels of these factors without accounting for the degree of obesity may have reached different conclusions to ours.

In conclusion, we have demonstrated that in recently diagnosed diabetic subjects in whom there is no confounding effect of treatment with glucose-lowering agents, the metabolic syndrome is highly prevalent and is associated with increased inflammation and impaired fibrinolysis. Obesity plays a key role in mediating these effects, and insulin resistance may have an added effect. However, glucose control does not appear to be a determining factor. These findings would suggest that therapies targeting obesity and/or insulin resistance that improve metabolic syndrome risk factors might have the potential to reduce CVD events, a hypothesis that is being tested in the Look AHEAD (Action for Health in Diabetes) study (37).

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REFERENCES

1. Jager A, van Hinsbergh VW, Kostense PJ, Emeis JJ, Yudkin JS, Nijpels G, Dekker JM, Heine RJ, Bouter LM, Stehouwer CD: von Willebrand factor, C-reactive protein, and 5-year mortality in diabetic and nondiabetic subjects: the Hoorn Study. *Arterioscler Thromb Vasc Biol* 19:3071–3078, 1999
2. Ridker PM: Inflammation, infection, and cardiovascular risk: how good is the clinical evidence? *Circulation* 97:1671–1674, 1998
3. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR: Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 347:1557–1565, 2002
4. Hamsten A, Wiman B, de Faire U, Blomback M: Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *N Engl J Med* 313:1557–1563, 1985
5. Festa A, D'Agostino R Jr, Mykkanen L, Tracy RP, Zaccaro DJ, Hales CN, Haffner SM: Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of glucose tolerance: the Insulin Resistance Atherosclerosis Study (IRAS). *Arterioscler Thromb Vasc Biol* 19:562–568, 1999
6. Festa A, D'Agostino R Jr, Tracy RP, Haffner SM: Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 51:1131–1137, 2002
7. Festa A, D'Agostino R Jr, Howard G, Mykkanen L, Tracy RP, Haffner SM: Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* 102:42–47, 2000
8. Juhan-Vague I, Alessi MC, Mavri A, Morange PE: Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. *J Thromb Haemost* 1:1575–1579, 2003
9. 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
10. Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol In Adults: Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 285:2486–2497, 2001
11. Ridker PM, Buring JE, Cook NR, Rifai N: C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14,719 initially healthy American women. *Circulation* 107:391–397, 2003
12. Hunt KJ, Resendez RG, Williams K, Haffner SM, Stern MP: National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. *Circulation* 110:1251–1257, 2004
13. Alexander CM, Landsman PB, Teutsch SM, Haffner SM: NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes* 52:1210–1214, 2003
14. Fonseca VA, Reynolds T, Hemphill D, Randolph C, Wall J, Valiquet TR, Graveline J, Fink LM: Effect of troglitazone on fibrinolysis and activated coagulation in patients with non-insulin-dependent diabetes mellitus. *J Diabetes Complications* 12:181–186, 1998
15. Haffner SM, Greenberg AS, Weston WM, Chen H, Williams K, Freed MI: Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. *Circulation* 106:679–684, 2002
16. Chu NV, Kong AP, Kim DD, Armstrong D, Baxi S, Deutsch R, Caulfield M, Mudaliar SR, Reitz R, Henry RR, Reaven PD: Differential effects of metformin and troglitazone on cardiovascular risk factors in patients with type 2 diabetes. *Diabetes Care* 25:542–549, 2002
17. Viberti G, Kahn SE, Greene DA, Herman WH, Zinman B, Holman RR, Haffner SM, Levy D, Lachin JM, Berry RA, Heise MA, Jones NP, Freed MI: A Diabetes Outcome Progression Trial (ADOPT): an international multicenter study of the comparative efficacy of rosiglitazone, glyburide, and metformin in recently diagnosed type 2 diabetes. *Diabetes Care* 25:1737–1743, 2002
18. Wallace TM, Levy JC, Matthews DR: Use and abuse of HOMA modeling (Review). *Diabetes Care* 27:1487–1495, 2004
19. Neter J, Kutner MH, Nachtsheim CJ, Wasserman W: *Applied Linear Statistical Models.* Chicago, Irwin, 1996
20. Kahn R, Buse J, Ferrannini E, Stern M: The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 28:2289–2304, 2005

21. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F: Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 112:2735–2752, 2005
22. Timpson NJ, Lawlor DA, Harbord RM, Gaunt TR, Day IN, Palmer LJ, Hattersley AT, Ebrahim S, Lowe GD, Rumley A, Davey Smith G: C-reactive protein and its role in metabolic syndrome: mendelian randomisation study. *Lancet* 366:1954–1959, 2005
23. Wellen KE, Hotamisligil GS: Inflammation, stress, and diabetes. *J Clin Invest* 115:1111–1119, 2005
24. Simpson AJ, Booth NA, Moore NR, Bennett B: Distribution of plasminogen activator inhibitor (PAI-1) in tissues. *J Clin Pathol* 44:139–143, 1991
25. Lyon CJ, Hsueh WA: Effect of plasminogen activator inhibitor-1 in diabetes mellitus and cardiovascular disease. *Am J Med* 115 (Suppl. 8A):62S–68S, 2003
26. Pandolfi A, Iacoviello L, Capani F, Vitacolonna E, Donati MB, Consoli A: Glucose and insulin independently reduce the fibrinolytic potential of human vascular smooth muscle cells in culture. *Diabetologia* 39:1425–1431, 1996
27. Ma LJ, Mao SL, Taylor KL, Kanjanabuch T, Guan Y, Zhang Y, Brown NJ, Swift LL, McGuinness OP, Wasserman DH, Vaughan DE, Fogo AB: Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1. *Diabetes* 53:336–346, 2004
28. Brinkworth GD, Noakes M, Parker B, Foster P, Clifton PM: Long-term effects of advice to consume a high-protein, low-fat diet, rather than a conventional weight-loss diet, in obese adults with type 2 diabetes: one-year follow-up of a randomised trial. *Diabetologia* 47:1677–1686, 2004
29. Pereira MA, Swain J, Goldfine AB, Rifai N, Ludwig DS: Effects of a low-glycemic load diet on resting energy expenditure and heart disease risk factors during weight loss. *JAMA* 292:2482–2490, 2004
30. The Diabetes Prevention Program Research Group: Intensive lifestyle intervention or metformin on inflammation and coagulation in participants with impaired glucose tolerance. *Diabetes* 54:1566–1572, 2005
31. Yudkin JS, Panahloo A, Stehouwer C, Emeis JJ, Bulmer K, Mohamed-Ali V, Denver AE: The influence of improved glycaemic control with insulin and sulphonylureas on acute phase and endothelial markers in type II diabetic subjects. *Diabetologia* 43:1099–1106, 2000
32. Lemieux I, Pascot A, Couillard C, Lamarche B, Tchernof A, Almeras N, Bergeron J, Gaudet D, Tremblay G, Prud'homme D, Nadeau A, Despres JP: Hypertriglyceridemic waist: a marker of the atherogenic metabolic triad (hyperinsulinemia; hyperapolipoprotein B; small, dense LDL) in men? *Circulation* 102:179–184, 2000
33. Cnop M, Landchild MJ, Vidal J, Havel PJ, Knowles NG, Carr DR, Wang F, Hull RL, Retzlaff BM, Walden CE, Knopp RH, Kahn SE: The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations: distinct metabolic effects of two fat compartments. *Diabetes* 51:1005–1015, 2002
34. Carey DG, Cowin GJ, Galloway GJ, Jones NP, Richards JC, Biswas N, Doddrell DM: Effect of rosiglitazone on insulin sensitivity and body composition in type 2 diabetic patients [corrected]. *Obes Res* 10:1008–1015, 2002
35. Miyazaki Y, Mahankali A, Matsuda M, Mahankali S, Hardies J, Cusi K, Mandarino LJ, DeFronzo RA: Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. *J Clin Endocrinol Metab* 87:2784–2791, 2002
36. Festa A, Hanley AJ, Tracy RP, D'Agostino R Jr, Haffner SM: Inflammation in the prediabetic state is related to increased insulin resistance rather than decreased insulin secretion. *Circulation* 108:1822–1830, 2003
37. The Look AHEAD Research Group: Look AHEAD (Action for Health in Diabetes): design and methods for a clinical trial of weight loss for the prevention of cardiovascular disease in type 2 diabetes. *Control Clin Trials* 24:610–628, 2003