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PAPER

The relation of body fat mass and distribution to markers of chronic inflammation

A Festa[1], R D'Agostino Jr[2], K Williams[1], AJ Karter[3], EJ Mayer-Davis[4], RP Tracy[5] and SM Haffner[1]*

[1]*Department of Medicine, Division of Clinical Epidemiology, University of Texas Health Science Center at San Antonio, Texas, USA;* [2]*Department of Public Health Sciences, Bowman Gray School of Medicine, Winston Salem, North Carolina, USA;* [3]*Division of Research, Kaiser Permanente, The Permanente Medical Group Inc, Oakland, California, USA;* [4]*Department of Epidemiology and Biostatistics, School of Public Health, University of South Carolina, Columbia, South Carolina, USA; and* [5]*Laboratory for Clinical Biochemistry Research, Department of Pathology, University of Vermont College of Medicine, Burlington, Vermont, USA*

OBJECTIVE: To study the relation of fibrinogen and C-reactive protein (CRP) to various measures of body fat and body fat distribution and to investigate whether these relations were explained by differences in insulin sensitivity.
DESIGN AND SUBJECTS: Cross-sectional analysis of the IRAS (Insulin Resistance Atherosclerosis Study), a large ($n = 1559$) tri-ethnic population (non-Hispanic whites, African-Americans and Mexican-Americans) across different states of glucose tolerance.
MEASUREMENTS: Glucose tolerance (oral glucose tolerance test), insulin sensitivity (frequently sampled intravenous glucose tolerance test and minimal model analysis), assessment of body fat mass and distribution (weight, girths, bioelectrical impedance), subclinical atherosclerosis (B-mode ultrasonography of carotid artery intima-media thickness, IMT), CRP (highly sensitive immunoassay), fibrinogen (standard assay).
RESULTS: Both CRP and fibrinogen were related to all measures of body fat. Strong correlations (correlation coefficient $r \geq 0.35$) were found between CRP and body mass index (BMI), waist circumference and adipose body mass, respectively. The associations were consistent in non-diabetic and type-2 diabetic subjects, were generally stronger in women, and were only moderately attenuated by the prevailing insulin sensitivity (S_I). In a multivariate linear regression model waist circumference explained 14.5% of the variability of circulating CRP levels ($P = 0.0001$), BMI 0.4% ($P = 0.0067$), and S_I 1.7% ($P = 0.0001$). Common carotid artery IMT was related to CRP and fibrinogen in men, but not in women, and was attenuated after adjusting for BMI or waist.
CONCLUSION: Our findings show that measures of body fat are strongly associated with circulating levels of CRP and fibrinogen. These associations were not explained by lower S_I in obese subjects. Chronic, subclinical inflammation may be one pathophysiological mechanism explaining the increased risk of atherosclerotic disease associated with adiposity.
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Keywords: inflammation; C-reactive protein; fibrinogen; body fat; waist circumference

Introduction

Obesity, in particular central adiposity, a disproportionate accumulation of visceral fat mass, is associated with an increased risk of cardiovascular disease.[1–4] More recently, knowledge on obesity has been further extended by a number of studies on adipose tissue-derived proteins, leading to the concept of the adipose tissue as an endocrine organ involved in a variety of metabolic pathways.[5] However, mechanisms linking adiposity and cardiovascular disease are complex and still incompletely understood. Two of these links might be alterations in hemostasis and fibrinolysis,[6] and chronic, subclinical inflammation.[7]

Fibrinogen, considered an integral part of the acute phase response as well as the hemostatic system, is a strong and independent predictor of myocardial infarction and stroke.[8] An association between C-reactive protein (CRP), a sensitive marker of inflammation, and atherosclerotic disease, has been shown more recently.[9–12] Various studies have reported a positive relation of measures of body fat with CRP[9,10,13–15] and fibrinogen.[8,16] However, the potential role of central adiposity and of insulin resistance has yet to be fully

***Correspondence: SM Haffner, The University of Texas Health Science Center at San Antonio, Mail Code 7873, 7703 Floyd Curl Drive, San Antonio, TX 78228-3900, USA.**
E-mail: haffner@uthscsa.edu
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elucidated. Finally, gender differences have been reported for levels of CRP,[17] and fibrinogen,[18,19] and gender differences have also been reported in the association of fibrinogen with measures of body fat.[20]

In the present study we investigated the relation of two inflammatory markers (CRP and fibrinogen) to measures of body fat and body fat distribution in a large tri-ethnic population (non-Hispanic white people, African-American people and Mexican-American people) across different states of glucose tolerance. Further, we studied whether these relations were explained by differences in insulin sensitivity, as assessed using a frequently sampled intravenous glucose tolerance test and minimal model analysis. Estimates of body fat included measures of overall body mass (body mass index, BMI), central body fat (waist circumference, waist-to-hip ratio, WHR), and fat-free mass (FFM) as well as adipose body mass (ABM), as assessed by bioelectrical impedance.

Study subjects and methods

The Insulin Resistance Atherosclerosis Study (IRAS) is a multicenter, epidemiological study aiming to explore relationships between insulin resistance, cardiovascular risk factors and disease across different ethnic groups and varying states of glucose tolerance. A full description of the design and methods of the IRAS has been published previously.[21] The IRAS protocol was approved by local institutional review committees, and all subjects gave informed consent.

A total of 1625 individuals participated in the IRAS. This report includes data on 1559 subjects, in whom CRP and fibrinogen were measured. The IRAS examination required two visits. Patients were asked prior to each visit to fast for 12 h, to abstain from heavy exercise and alcohol for 24 h, and to refrain from smoking the morning of the examination. Smoking was dichotomized into 'current' and 'past'/'never' by use of a standard questionnaire. Carotid artery intima-media thickness (IMT) was measured following a standard protocol to provide an index of subclinical atherosclerosis.[21] In brief, a bilateral assessment of the wall thickness was made in the internal carotid artery (ICA) and common carotid artery (CCA), using high-resolution B-mode ultrasonography with Toshiba SSA-270A imaging units (Toshiba America Medical Systems).

Assessment of glucose tolerance and insulin sensitivity

A standard 75 g oral glucose tolerance test was performed and glucose tolerance status was based on the World Health Organization criteria.[22] A frequently sampled intravenous glucose tolerance test (FSIGTT[23]) with minimal model analysis[24] was performed to assess insulin sensitivity. Two modifications of the original protocol were used. An injection of regular insulin, rather than tolbutamide, was used to ensure adequate plasma insulin levels for the accurate computation of insulin sensitivity across a broad range of glucose tolerance. In addition, the reduced sampling protocol (which required 12 rather than 30 plasma samples) were used because of the large number of subjects. Insulin sensitivity, expressed as the insulin sensitivity index (S_I), was calculated by mathematical modeling methods (MINMOD, version 3.0, 1994).

Measures of body fat and body composition

Height was recorded to the nearest 0.5 cm, weight was measured to the nearest 0.1 kg. BMI was calculated as weight/height^2 (kg/m^2), and was used as an estimate of overall adiposity. Girth measurements were estimated as the average of duplicate measures (taken to the nearest 0.5 cm using a steel tape). Waist circumference was measured on bare skin during mid-respiration at the natural indentation between the 10th rib and the iliacal crest (minimum waist). Hip girth was measured at the maximum circumference of the buttocks. Waist circumference and WHR were considered estimates of visceral fat mass. Resistance and reactance measures to estimate body composition were obtained using a bioelectrical impedance meter (RJL Systems, Clinton Township, MI). To estimate FFM, the following equation was used:[25] FFM (kg): $0.838 \times (\text{height}^2/\text{resistance}) + 4.179$. ABM was calulculated as body weight $-$ FFM, and percentage adipose mass as adipose mass/body weight $\times$ 100.

Laboratory measurements

Glucose and insulin levels to assess S_I were measured using standard methods as described previously.[16] CRP was measured by in-house ultra-sensitive competitive immunoassay (antibodies and antigens from Calbiochem, La Jolla, California) with an interassay CV of 8.9%.[26] Fibrinogen was measured in citrated plasma with a modified clot-rate assay using the Diagnostica STAGO ST4 instrument, as described previously.[27] This was based on the original method of Clauss[28] with a CV of 3.0%. Samples for fibrinogen and CRP were frozen and stored at -70°C at the centers not later than 90 min after blood drawing. Frozen samples were shipped on a monthly basis to the Laboratory for Clinical Biochemistry Research, University of Vermont (RPT), where measurements were performed.

Statistical analysis

Statistical analyses were performed using the SAS statistical software system (SAS, Cary, NC, USA). Table 1 shows descriptive data stratified by gender (percentage, *n*; mean values, range). Spearman rank correlations were performed. Because age, gender, smoking, ethnicity and diabetic status were related to the outcome variables in previous reports and/or the IRAS population partial correlation analyses were also performed. The analyses were also stratified by gender and glucose tolerance status (diabetic *vs* non-diabetic individuals). We also tested for possible interactions of gender,

Table 1 Descriptive data stratified by gender. The Insulin Resistance Atherosclerosis Study (IRAS)

	Women	*Men*	*P-value*
n	859	700	
Ethnicity (%, NHW, AA, H)	35/29/36	41/28/31	
Glucose tolerance (%, NGT/IGT/DM)	43/25/32	46/20/34	
Age (y)	55.6 (39–69)	55.7 (40–69)	NS
Weight (kg)	78.2 (43.0–156.4)	87.2 (43.7–186.4)	0.0001
Height (cm)	161.0 (136.9–182.0)	174.5 (151.1–199.3)	0.0001
BMI (kg/m^2)	30.2 (15.7–63.3)	28.6 (14.2–55.4)	0.0001
Waist circumference (cm)	90.5 (58.9–171.0)	97.2 (60.2–148.2)	0.0001
Waist-to-hip ratio (WHR)	0.83 (0.62–1.09)	0.94 (0.69–1.11)	0.0001
$S_I \times 10^{-4}$ ($min^{-1}\mu U^{-1}$ ml^{-1})	1.64 (0–14.1)	1.62 (0–14.2)	NS
ICA IMT (mm)	0.835 (0.40–4.32)	0.925 (0.43–3.32)	0.0001
CCA IMT (mm)	0.794 (0.43–2.11)	0.852 (0.36–2.19)	0.0001
Bioelectrical impedance			
Fat-free body mass (FFM, kg)	43.3 (28.7–79.1)	59.5 (38.3–101.0)	0.0001
Adipose body mass (ABM,kg)	34.9 (7.8–100.0)	27.7 (0.55–87.1)	0.0001
Percentage adipose mass (%)	43.3 (15.9–67.9)	30.9 (1.0–64.4)	0.0001
Fibrinogen (mg/dl)	292.3 (15–595)	270.1 (72–548)	0.0001
CRP (mg/l)	5.24 (0.13–67.8)	2.73 (0.10–36.6)	0.0001

NHW, non-Hispanic white people; AA, African-American people; H, Hispanic people; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, type 2 diabetes.
Data are percentage or mean values (range). Differences between groups were calculated by *t*-test.

ethnicity, and diabetic status, respectively, on the association of measures of body composition with the two outcome variables. We included in separate models interaction terms for gender × weight, gender × BMI, etc, and ethnicity × BMI, etc, and diabetic status × BMI etc. These models were adjusted for age, gender, ethnicity and clinic. The associations as shown were consistent across the three ethnic groups of the IRAS, therefore we pooled the data for all three ethnic groups for further analyses.

Further, multiple linear regression analyses were performed. For these analyses as well as the interaction models logarithmically transformed values of CRP were used because the distribution of the residuals from the fitted models became normally distributed after log transformation. First, to estimate the relative contribution of BMI (total adiposity) *vs* waist (visceral adiposity) to the two outcome variables we fit two analogous models (for CRP and fibrinogen as a dependent variable) by entering BMI and waist (stepwise) in addition to demographic variables, smoking and diabetic status (forced into the model). In a further model we entered BMI, waist and also S_I to estimate the impact of S_I on the associations. Second, we fit a model to estimate the individual contribution of single measures of body fat to the two outcome variables. In these models, in addition to demographic variables (age, gender, ethnic and clinic) and diabetic status (forced into the model), each of four measures of body fat (waist, BMI, ABM, WHR) were analyzed as independent variables separately. Finally, partial Spearman correlation analyses were performed to assess the relation of carotid artery IMT to CRP and fibrinogen, respectively.

P-values less than 0.05 (two-sided) were considered statistically significant.

Results

Women had higher BMI, adipose body mass, and fibrinogen and CRP levels, whereas body weight, height, waist circumference, WHR, FFM, and carotid artery IMT were higher in men (Table 1).

Spearman correlation analysis (overall population; Table 2)

Unadjusted (data not shown) and partial correlation analyses (adjusted for age, gender, ethnicity, clinic, smoking and diabetic status) showed that CRP and fibrinogen were related to all measures of body fat. CRP was also related to FFM

Table 2 Partial Spearman correlation analysis of measures of body fat and body fat distribution with CRP and fibrinogen

	CRP	*Fibrinogen*
(A) Adjusted for age, gender, ethnicity, clinic, smoking and diabetic status		
BMI	0.41	0.24
Waist circumference	0.42	0.29
WHR	0.27	0.20
FFM	0.13	0.02*
ABM	0.40	0.26
Percentage adipose mass	0.37	0.29
(B) Adjusted for A plus S_I		
BMI	0.28	0.17
Waist circumference	0.29	0.23
WHR	0.17	0.16
FFM	0.05*	−0.02*
ABM	0.28	0.20
Percentage adipose mass	0.26	0.23

All *P*-values 0.0001 except *P = NS
BMI, body mass index; WHR, waist-to-hip ratio; FFM, fat-free body mass; ABM, adipose body mass.

(Table 2A). The associations of measures of body composition were generally stronger with CRP than with fibrinogen. After further adjustment for S_I the relationships weakened, but remained highly significant, with the exception of the relation of CRP to FFM (Table 2B).

Multiple linear regression analyses (overall population; Table 3)

To estimate the relative contribution of overall (BMI) and visceral fat (waist circumference) to circulating levels of inflammatory markers we performed multiple linear regression models with log CRP and fibrinogen as dependent variables. In these models the variability of CRP levels explained by waist was considerably higher than the variability explained by BMI (15.0 *vs* 0.4%, Table 3A). The same pattern was found when analyzing fibrinogen as the dependent variable; the partial r^2 was 8.0% for waist, whereas BMI did not enter as a significant co-variate in this model.

Both measures of body fat (BMI and waist) remained significant determinants of log CRP after considering S_I as an additional co-variate (Table 3B). A similar pattern was found in the fibrinogen model, showing that the inclusion of S_I as an additional co-variate did not substantially change the effect of waist on fibrinogen levels.

Relative contribution of measures of body fat (partial r^2; overall population)

To assess the relative contribution of measures of body fat to circulating levels of inflammatory markers measures of body fat were considered separately in analogous multivariate regression models. Waist circumference explained 15.2%, BMI 13.9%, ABM 13.7% and WHR 6.8% of the variability of circulating CRP levels. For fibrinogen as the dependent variable the percentage of variability explained by waist circumference was 7.9%, by ABM 7.2%, by BMI 6.3% and by WHR 4.0%. Thus, as a single measure of body fat, waist circumference, ABM and BMI were comparable in predicting any of the two outcome variables, whereas WHR was a somewhat weaker predictor.

Analyses stratified by gender (Tables 4 and 5)

Partial Spearman correlation analysis (Table 4). The association of measures of body composition with CRP and fibrinogen were generally stronger in women than in men, except for the association of WHR and CRP, which was stronger in men (Table 4). FFM was related to CRP only in women, but not to fibrinogen. Interaction analyses showed stronger associations in women of CRP with ABM (P for interaction term < 0.05) and FFM ($P < 0.01$), respectively, as well as stronger associations of fibrinogen with BMI ($P < 0.05$), ABM ($P < 0.01$), and FFM ($P < 0.05$). In contrast,

Table 4 Partial Spearman correlation analysis (adjusted for age, ethnicity, clinic, smoking and diabetic status) stratified by gender

	CRP		*Fibrinogen*	
	Women	*Men*	*Women*	*Men*
BMI	0.45	0.34	0.31	0.15
Waist circumference	0.45	0.41	0.32	0.23
WHR	0.24	0.34	0.18	0.22
FFM	0.19	0.05*	0.07*	− 0.03*
ABM	0.44	0.36	0.32	0.20
Percentage adipose mass	0.41	0.34	0.33	0.22

All *P*-values 0.0001 except *P = NS.
BMI, body mass index; WHR, waist-to-hip ratio; FFM, fat-free body mass; ABM, adipose body mass.

Table 3 Multiple linear regression analysis with (1) log of CRP and (2) fibrinogen as the dependent variable (overall population)

Independent variable	*B*	P-*value*	*Partial* r^2 *(× 100)*	*Overall* r^2 *(× 100)*
(1) log of CRP				
Demographics and diabetic status only				13.2
(A) (+waist BMI)				
Waist	0.02	0.0001	15.0	28.3
BMI	0.03	0.0022	0.4	28.7
(B) (+waist, BMI and S_I)				
Waist	0.02	0.0001	14.5	27.7
S_I	−0.10	0.0001	1.7	29.4
BMI	0.03	0.0067	0.4	29.8
(2) Fibrinogen				
Demographics and diabetic status only				10.2
(A) (+waist, BMI)				
Waist	10.4	0.0001	8.0	18.1
(B) (+waist, BMI and S_I)				
Waist	10.3	0.0001	7.7	18.1

After forcing age, gender, clinic, ethnicity, smoking and diabetic status into the model (A) BMI and waist circumference, and (B) BMI, waist and S_I were entered as independent variables. Regression coefficients (B), *P*-values, partial r^2 for independent variables of interest (significantly contributing to the model) as well as the overall r^2 are shown on the table.

Table 5 Multiple linear regression analysis with log of CRP as the dependent variable stratified by gender

Independent variable	*B*	P-value	*Partial* r^2 *(×100)*	*Overall* r^2 *(×100)*
(A) Women				
Demographics and diabetic status				10.4
BMI	0.06	0.0001	16.5	26.9
S_I	−0.13	0.0001	2.7	29.6
(B) Men				
Demographics and diabetic status				8.4
Waist	0.03	0.0001	12.9	21.2
S_I	−0.09	0.0001	1.8	23.1

After forcing age, clinic, ethnicity, smoking and diabetic status into the model, BMI, waist and S_I were entered as independent variables.
Regression coefficients (*B*), *P*-values, partial r^2 for independent variables of interest (significantly contributing to the model) as well as the overall r^2 are shown on the table.

the association of CRP with WHR was stronger in men than in women ($P < 0.01$).

After adjustment for S_I the relationships generally weakened; strong associations ($r > 0.25$) remained those between CRP and waist ($r = 0.28$) in men, and between CRP and BMI ($r = 0.32$), waist ($r = 0.31$), ABM ($r = 0.32$), and percentage adipose mass ($r = 0.30$), respectively, in women (all *P*-values = 0.0001).

Multiple linear regression analysis (Table 5). In a multivariate regression model including waist, BMI and S_I as independent variables, BMI but not waist contributed significantly to circulating CRP levels in women, in contrast to men, where waist but not BMI was significantly related to CRP. When analyzing fibrinogen as the dependent variable in an analogous model, waist was the only significant determinant both in men and in women (data not shown).

Relative contribution of measures of body fat (partial r^2). The partial r^2 for single measures of body fat (except WHR) were higher in women for the two inflammatory markers (between 11.9 and 17.4% in women *vs* 8.0–15.1% in men for log of CRP, and between 5.9 and 9.8% in women *vs* 2.7–5.0% in men for fibrinogen), indicating that in women compared to men up to almost twice the variability of log of CRP and fibrinogen levels were explained by single measures of body fat. The association of WHR to log CRP showed an inverse pattern; WHR was a better predictor of log CRP levels in men (partial r^2 11.0%) than in women (partial r^2 4.6%).

Analyses stratified by glucose tolerance status

Partial Spearman correlation analysis. The associations as shown in the overall population were somewhat stronger in non-diabetic than in diabetic individuals. Correlation coefficients for CRP *vs* BMI, waist, WHR, ABM and percentage adipose mass were 0.43, 0.44, 0.28, 0.42 and 0.40 in non-diabetic subjects and 0.31, 0.33, 0.22, 0.30 and 0.28, respectively, in patients with type 2 diabetes (all *P*-values = 0.0001). FFM was related to CRP only in non-diabetic subjects ($r = 0.15$, $P = 0.0001$ *vs* $r = 0.08$, $P =$ NS in type 2 diabetes). Interaction terms reached statistical significance for the association of WHR with CRP ($P < 0.05$), being stronger in non-diabetic individuals. A similar pattern was observed for relationships of fibrinogen with measures of body fat (data not shown).

Multiple linear regression analysis (Table 6). Waist circumference but not BMI was consistently related to CRP in non-diabetic and diabetic individuals, explaining 16.2 and 10.5%, respectively, of the variability in circulating levels. A similar pattern was observed for fibrinogen as the dependent variable (data not shown).

Relation of inflammatory markers to subclinical atherosclerosis (carotid artery IMT; Table 7)

There was no significant relation of CRP or fibrinogen to ICA IMT (all correlation coefficients: $r < 0.07$, $P =$ NS). By contrast, we found a significant correlation between CCA IMT and CRP and fibrinogen, respectively (Table 7). This relation was attenuated after adjusting for BMI or waist, and, in stratified analyses, was significant only in men, but not in women.

Discussion

We found associations of fibrinogen and CRP with various measures of body fat across a large, tri-ethnic population with varying degrees of glucose tolerance, in line with previous work.[6,8–10,13–16,29] In addition, the present study yielded several novel findings: first, the associations were independent of the prevailing insulin resistance, as assessed by a direct measure of insulin sensitivity; second, we have shown significant gender differences in these associations; third, the associations were consistent across three ethnic

Table 6 Multiple linear regression analysis with log of CRP as the dependent variable stratified by diabetic status

Independent variable	*B*	P-value	*Partial* r^2 *(×100)*	*Overall* r^2 *(×100)*
(A) Non-diabetic subjects				
Demographics only				7.6
Waist	0.02	0.0001	16.2	23.8
S_I	−0.10	0.0001	2.2	26.1
BMI	0.04	0.0032	0.7	26.7
(B) Type 2 diabetes				
Demographics only				15.4
Waist	0.03	0.0001	10.5	25.9

After forcing age, gender, clinic, ethnicity and smoking into the model, BMI, waist and S_I were entered as independent variables. Regression coefficients (*B*), *P*-values, partial r^2 for independent variables of interest (significantly contributing to the model) as well as the overall r^2 are shown on the table.

Table 7 Partial Spearman correlation analysis of CCA IMT with inflammatory markers

	CRP	*Fibrinogen*
Overall (n = *1453*)		
(A) Demographics only	0.10	0.07
	(0.0001)	(0.01)
A + BMI	0.07	0.04
	(0.009)	(0.09)
A + waist	0.07	0.04
	(0.01)	(0.13)
Men (n = *652*)		
(A) Demographics only	0.15	0.14
	(0.0001)	(0.0003)
A + BMI	0.11	0.12
	(0.004)	(0.002)
A + waist	0.10	0.11
	(0.01)	(0.005)
Women (n = *801*)		
(A) Demographics only	0.06	0.01
	(0.10)	(0.7)
A + BMI	0.03	− 0.01
	(0.4)	(0.8)
A + waist	0.03	− 0.01
	(0.4)	(0.7)

Demographic co-variates include: age, gender (overall population only), ethnicity, clinic, smoking and diabetic status. *r*-values (*P*-values in parentheses).

groups and in type 2 diabetic as well as non-diabetic subjects, being somewhat stronger in the latter; and, finally, by comparing different measures of body fat, we found that waist circumference, BMI and adipose body mass (as determined by bioelectrical impedance) were more accurate in predicting CRP and fibrinogen levels, than WHR.

The association between inflammatory markers and markers of adiposity remained significant after adjusting for insulin sensitivity. From our data we can only speculate whether insulin resistance acts as a confounding or a mediating factor of this relation. Results of partial correlation analyses (Table 2) suggest an effect of S_I on the associations as shown; however, results of the multivariate regression analyses (Table 3) suggest that a modifying effect of insulin resistance, if any, seems to be modest. Previous reports, also from the IRAS cohort, have established an association of insulin resistane with obesity.[30,31] More recent reports have shown an association between inflammatory markers and features of the insulin resistance syndrome,[15] including insulin sensitivity in the IRAS.[32] Elevated circulating levels of inflammatory markers have been associated with incident cardiovascular disease,[9–12] and obesity may be one factor linking chronic, subclinical inflammation and atherosclerosis. There are several lines of evidence supporting this concept. Dietary fat (Ω-3 polyunsaturated fatty acids) seems to have a direct effect on the synthesis of pro-inflammatory cytokines by peripheral monocytes (TNFα, IL-1).[33] Pro-inflammatory cytokines, such as IL-6 and TNFα, are expressed in adipose tissue.[34,35] It has been shown that about 30% of total circulating concentrations of IL-6 originate from adipose tissue in healthy subjects,[35] and that its release is modulated by TNFα.[36] The role of TNFα is of particular interest, and it has been suggested that several effects of TNFα on lipid metabolism[37,38] or insulin signaling[34] may reflect autocrine or paracrine, rather endocrine effects.[15,35] One might therefore conclude that adipose tissue derived IL-6 but not TNFα exert significant systemic effects, thus providing a major link between chronic inflammation, adiposity and atherosclerotic disease. In fact, IL-6 has been shown to predict cardiovascular disease,[39] and, experimentally, to contribute to the development of early atherosclerotic lesions.[40]

In the present study, the associations between inflammatory markers and measures of body fat were stronger in women than in men, except for WHR. This finding is in accordance with recently published results from the Third National Health and Nutrition Examination Survey (NHANES III), showing higher odds ratios for elevated CRP concentrations by BMI classes in women than in men.[29] There are several possible explanations for this finding. First, body composition, as assessed by direct[41,42] and indirect measures,[41–43] (this study) is different in women compared to men. Men have a considerably larger proportion of total fat localized to the intra-abdominal deposits compared to women,[42,44] whereas total and subcutaneous fat depots are larger in women than in men.[41,42] It is therefore possible that body fat compartments (namely visceral *vs* subcutaneous fat), being differently distributed between the sexes, contribute differently to the associations as shown. In the present study, in a multivariate regression model BMI (total adiposity) and not waist was related to CRP levels in women, whereas in men it was waist (visceral adiposity) and not BMI (Table 5). Second, the strength of the associations between indirect measures of body fat (as assessed in the present study) and actual fat mass (as assessed by direct methods, such as densitometry or computed tomography) may differ between sexes. Third, higher levels of CRP and fibrinogen have been reported in women,[17–19] (present study), suggesting an impact of sex hormones on circulating levels of inflammatory proteins. Accordingly, postmenopausal hormone replacement treatment was associated with increased CRP concentrations.[45,46] Finally, the gender differences in the associations as shown in the present study are relatively small and the associations are in the same direction. Therefore, we cannot exclude the possibility that, albeit statistically significant, these differences may not be significant from a biological view.

Gender differences were also found in the relation of inflammatory markers to carotid artery IMT. Significant associations were found only in men, were generally weak, restricted to the CCA area, and partly mediated by BMI and waist. Previously, no association between CRP and ICA IMT was found in healthy, elderly persons,[14] and weak associations were found between CRP and CCA intima-media area in predialysis patients,[47] and between CRP and CCA IMT in ever-smoking women.[48] By contrast, a strong and consistent

association between CRP and clinically significant atherosclerosis has been shown cross-sectionally,[9,13] and prospectively.[9–12] Taken together, these data suggest a strong relation of serum levels of inflammatory markers to *advanced* atherosclerosis, but only a weak, if any, association with measures of *early* disease. The latter association may, in fact, be weak, or may be attributed to the inaccuracy of currently available methods to assess subclinical atherosclerosis. The gender differences as shown deserve further attention.

We also compared different measures of body fat. For clinical purposes, such as the assessment of cardiovascular risk and follow-up after therapeutic interventions, the currently most widely used measures are weight and BMI, and to a lesser degree WHR. In a previous study, waist circumference was correlated with more cardiovascular risk factors than WHR,[49] and the significance of increased waist circumference to predict general ill health has also been shown.[50] In the present study, waist circumference emerged as the best predictor of CRP and fibrinogen levels, explaining about 15% of circulating CRP levels as a single independent variable in the multivariate models. In previous studies, the explained total variability for circulating CRP levels (in various multivariate linear regression models) ranged from 15 to 42% (depending on the smoking status) in elderly persons[14] to 29.7% in women[48] approximating the 29.8% found in our full multivariate model (Table 3). The explained variability was lower for fibrinogen and for men, as well as for diabetic subjects. In these instances, additional factors that were not analyzed, were not measured or are still unidentified presumably contribute to a large extent to circulating levels of inflammatory proteins. In subjects with diabetes atherosclerosis prevails,[51] and atherosclerosis as well as factors indirectly attributable to chronic hyperglycemia, such as the formation of advanced glycation endproducts, endothelial dysfunction and oxidative stress[51] may contribute to the enhanced elaboration of acute-phase proteins.[7]

The results of the present study suggest that both visceral and subcutaneous adipose tissue seem to contribute to circulating levels of CRP and fibrinogen, as indicated by statistical associations with various measures of body fat. However, the finding that waist (visceral adiposity) was a stronger and more consistent predictor of inflammatory markers than BMI (general adiposity) suggests a dominant role of visceral adipose tissue, at least in men. On the other hand, it has to be acknowledged that many indirect measures of body fat (such as waist circumference, BMI, ABM) are highly interrelated and also related to both subcutaneous as well as visceral body fat mass (as assessed by direct measures). This makes it difficult to discern the relative contributions of subcutaneous *vs* visceral fat to the outcome variables from our data. Therefore, studies comparing direct measures of body fat (such as CT, densitometry or magnetic resonance imaging) with inflammatory markers are needed to resolve this issue.

We would also like to address potential weaknesses of the present study. We used indirect measures of body fat and body composition instead of direct measures such as CT or densitometry, mainly for logistical reasons due to the large number of study participants. Also, the correlation of indirect to direct measures of body fat are reasonably high.[41,42,44] Large sample size and heterogeneity of the study population (three ethnic groups, non-diabetic and diabetic subjects) together with the consistency of the results in each subgroup may be considered major strengths of this study.

In summary, our findings suggest that adiposity is strongly associated with circulating levels of CRP and fibrinogen and that visceral as well as subcutaneous adipose tissue contribute. The associations were somewhat stronger in women and in non-diabetic subjects and were only moderately attenuated by the prevailing insulin resistance. Chronic, subclinical inflammation may be one pathophysiological mechanism explaining the increased risk of atherosclerotic disease associated with adiposity.

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