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Free Fatty Acids are Associated with Metabolic Syndrome and Insulin Resistance, but not Inflammation in SLE Patients

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Abstract

Free fatty acids (FFAs) are implicated in the pathogenesis of insulin resistance and atherosclerosis. Inflammatory cytokines promote lipolysis and increase FFAs, a cause of endothelial dysfunction and increased atherosclerosis risk. We hypothesized that increased inflammation is associated with increased FFAs, resulting in insulin resistance and atherosclerosis in patients with systemic lupus erythematosus (SLE). We measured clinical variables, serum FFAs, homeostasis model assessment for insulin resistance (HOMA), inflammatory cytokines, markers of endothelial activation, cholesterol concentrations, and coronary artery calcium in 156 patients with SLE and 90 controls. We compared FFAs in patients with SLE and controls using Wilcoxon rank sum tests and further tested for the independent association between FFAs and disease status with adjustment for age, race, and sex using multivariable regression models. We assessed the relationship between FFA and continuous variables of interest using Spearman correlation and multivariable regression analysis. FFAs levels were higher in patients with SLE than controls (0.55 mmol/l [0.37- 0.71] vs. 0.44 mmol/l [0.32- 0.60], $P=0.02$). FFAs remained significantly higher among patients with SLE after adjustment for age, race, and sex ($P=0.03$), but not after further adjustment for BMI ($P=0.13$). FFA levels did not differ according to current immunosuppressive medication use in univariate and adjusted analysis (all $P>0.05$). Among patients with SLE, concentrations of FFAs were higher among those with metabolic syndrome compared to those without (0.66 mmol/l [0.46-0.81] vs. 0.52 mmol/l [0.35- 0.66], $P<0.001$). FFAs were positively correlated with insulin resistance (HOMA) ($\rho=0.23$, $P=0.004$, P adjusted=0.006) and triglyceride levels ($\rho=0.22$, $P=0.01$, P adjusted=0.004). FFAs were not associated with inflammatory cytokines (IL-6, TNF- α) (all $P>0.05$), but were positively associated with levels of E-selectin ($\rho=0.33$, $P<0.001$, P adjusted=0.001) and ICAM-1 ($\rho=0.35$, $P<0.001$, P adjusted=0.001). FFAs were correlated with coronary artery calcium score ($\rho=0.20$, $P=0.01$), but this was attenuated after adjustment for age, race and sex ($P=0.33$). FFAs are elevated in patients with SLE, particularly those with metabolic syndrome. FFAs in SLE are not associated with markers of generalized inflammation but are associated with insulin resistance and markers of endothelial activation.

Keywords

cardiovascular disease; systemic lupus erythematosus; free fatty acids; metabolic syndrome; endothelial activation; insulin resistance

Introduction

Patients with systemic lupus erythematosus (SLE) have an increased prevalence of premature atherosclerosis even after adjusting for traditional cardiovascular risk factors.^{1,2,3,4} We have previously reported that patients with SLE frequently meet criteria for metabolic syndrome and have a high prevalence of insulin resistance.⁵

Insulin resistance and the metabolic syndrome are associated with increased cardiovascular mortality in the general population,⁶ but the underlying mechanisms are unclear. The relationship between obesity, increased inflammation, insulin resistance and cardiovascular disease may be explained by free fatty acids (FFAs).⁷

FFAs are released from adipocytes through lipolysis, and are elevated in obesity due to increased adipose tissue.⁸ Insulin can down-regulate lipolysis, but in the setting of insulin resistance this regulatory mechanism is impaired, resulting in increased FFAs.^{9,10,11} Additionally, elevated FFAs promote peripheral insulin resistance, leading to further release of FFAs because insulin's anti-lipolytic action is impaired.¹²

Inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) stimulate lipolysis and increase FFAs.^{13,14} Conversely, experimental acute elevation of FFAs in rats increases hepatic expression of IL-6 and TNF- α .¹⁵ Similarly, in healthy humans an acute increase in FFAs induces oxidative stress and inflammatory changes.¹⁶ Therefore, FFAs not only promote inflammation, but also are increased by inflammation.

Elevated FFAs result in increased concentrations of soluble endothelial activation markers including E-selectin, vascular cellular adhesion molecule 1 (VCAM-1), and intracellular adhesion molecule 1 (ICAM-1).¹⁷ Furthermore, FFAs can decrease endothelial nitric oxide production and increase reactive oxygen species,¹⁸ predisposing to endothelial dysfunction,¹⁹ a potential mechanism underlying atherosclerosis and coronary artery disease.²⁰

SLE patients have chronic inflammation, and an increased prevalence of insulin resistance, endothelial dysfunction, and coronary atherosclerosis, but little is known about the contribution of FFAs to these. We therefore addressed the hypothesis that a unifying mechanism underlying accelerated atherosclerosis in SLE is that inflammation increases FFAs, resulting in insulin resistance and atherosclerosis.

Materials and Methods

Study population

We studied 156 patients with SLE without diagnosed coronary artery disease and 90 control subjects in whom serum FFAs were measured and the relationships between inflammation and atherosclerosis have been well defined.^{1,4,5,21,22} Recruitment and study procedures have been described in detail.¹ Subjects were 18 years of age or older and patients with SLE fulfilled the 1982 classification criteria.²³ Control subjects did not have SLE or other inflammatory disease. Patient and control groups were frequency matched for age, race and

sex. The study was approved by the Vanderbilt University Institutional Review Board and all subjects gave written informed consent.

Clinical Data

Clinical information, laboratory data, and coronary calcium scores were obtained as described previously.^{1,4,5} Disease activity and damage were evaluated with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)²⁴ and the Systemic Lupus International Collaborating Clinics / American College of Rheumatology damage index (SLICC), respectively.²⁵ The Framingham risk score was calculated using blood pressure, smoking status, serum lipid concentrations, age and sex, but not diabetes.²⁶ Body mass index (BMI) was calculated and expressed as kg/m². Insulin resistance was measured using the homeostasis model assessment of insulin resistance (HOMA) index and calculated as [serum insulin (μU/ml) × glucose (mmol/l)]/ 22.5.²⁷

The modified World Health Organization Criteria was used to classify a patient as having metabolic syndrome, which requires the presence of insulin resistance defined by any of the following criteria: HOMA in the top quartile of a population without diabetes, impaired fasting glucose (≥ 110 mg/dl) or diabetes. In addition, two of the following three criteria are also required: (1) central obesity: waist > 94 cm in men and > 88 cm in women; (2) dyslipidemia: triglycerides ≥ 150mg/dl, or HDL <40 mg/dl in women or <35 mg/dl in men; and (3) high blood pressure: ≥ 140/90 mmHg or use of drugs for hypertension.²⁸ Based on the Study of Inherited Risk of Coronary Atherosclerosis data we defined a HOMA index >2.114 as representing the top quartile of a population without diabetes.²⁸

FFAs were measured using an enzymatic assay (HR series NEFA-HR(2) assay, Wako Diagnostics, Richmond, VA, USA) from serum samples obtained after an overnight fast and expressed as mmol/L. The expected normal range for serum FFAs for fasting patients is 0.1-0.6 mmol/L. The range of linearity of this method is up to 2.0 mmol/L. The inter-assay coefficient of variation is 2.7% for levels in the normal range and 1.1% for elevated levels. Glucose, total cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL) were measured by the Vanderbilt University Medical Center Clinical Laboratory. IL-6, TNF-α, insulin, soluble E-selectin, VCAM-1, and ICAM-1 were measured by multiplex ELISA (Lincoplex® Multiplex Immunoassay Kit, Millipore Corp., Billerica MA, USA) in 115 SLE and 83 control patients.

Coronary artery calcium was measured by electron beam computed tomography (EBCT) scanning with an Imatron C-150 scanner (GE/Imatron, South San Francisco, CA, USA) in 108 SLE patients and 80 controls, and a 64-row multidetector CT (Light-Speed VCT, General Electric) in 29 SLE patients and 5 controls. Coronary artery calcium scores were quantified in Agatston units.²⁹ For logistic reasons, scans were not performed in 18 SLE and 5 control patients.

Statistical Analysis

Descriptive statistics were calculated as median with the interquartile range (median [IQR]) for continuous variables, and frequency and proportion for categorical variables. Wilcoxon's rank sum tests were used to compare continuous variables between SLE patients and control subjects, and Pearson's chi-square test to compare categorical variables. We first assessed for the independent association of FFAs and disease status by adjusting for age, sex and race, and in a separate model, with further adjustment for BMI.

To assess the correlation between FFAs and continuous clinical variables (including the coronary artery calcium score), Spearman's rank correlation coefficients (rho) were calculated separately among patients with SLE and control subjects.

The independent associations between FFAs and components of the lipid profile (total cholesterol, LDL, HDL and triglycerides), markers of insulin resistance (HOMA, fasting glucose and insulin), inflammation (IL-6, TNF- α), markers of endothelial activation (E-selectin, ICAM-1 and VCAM-1), Framingham risk score, current use of medications, and SLE disease activity (SLEDAI and SLICC) were assessed using multivariable linear regression models with adjustment for age, race and sex.

Additionally, we tested whether the association of FFAs with HOMA and metabolic syndrome were independent of BMI by further including BMI to the above model.

Similar regression analysis was performed for markers of endothelial activation. To assess the association between FFAs and markers of endothelial activation (E-selectin, ICAM-1 and VCAM-1) independent of inflammation, we further adjusted for IL-6 and TNF- α separately.

The association between FFAs and coronary artery calcium score was examined with a proportional odds logistic regression model adjusting for age, race and sex.

Concentrations of FFAs, triglycerides, HOMA, VCAM-1, ICAM-1, E-selectin, IL-6 and TNF- α were natural logarithm-transformed to improve normality. Statistical analyses were performed using R version 2.10.0 (<http://www.r-project.org>) and 2-sided P values less than 0.05 were considered statistically significant.

Results

Demographics

Demographic, clinical and laboratory characteristics of SLE and control cohorts have been reported previously (Table 1).^{1,4,5} Patient and control groups were similar with regard to age (40 years [30-48] vs. 42 years [30-49], $P=0.72$), sex (12% vs. 13% male, $P=0.68$), and race (67% vs. 73% Caucasian, $P=0.32$). However, BMI (27.3 kg/m² [23.4-32.7] vs. 25.2 kg/m² [22.5-30.1], $P=0.04$), as well as the prevalence of the metabolic syndrome (25.6% vs. 8.9%, $P=0.001$), was higher in SLE patients.

Association of FFAs with SLE status and inflammation

Concentrations of FFAs in patients with SLE were higher than in controls (0.55 mmol/L [0.37-0.71] vs. 0.44 mmol/L [0.32-0.60], $P=0.02$) (Figure 1); this remained significant after adjustment for age, race and sex ($P=0.03$). FFAs were positively correlated with BMI in SLE ($\rho=0.35$, $P<0.001$), so further adjustment for BMI was done, and the association between FFA and SLE status was no longer significant ($P=0.13$).

FFAs were not associated with inflammatory mediators, IL-6 or TNF- α , in patients with SLE (Table 2) in univariate or adjusted analysis. There was a trend towards an inverse association between FFAs and SLEDAI, a measure of disease activity in univariate analysis ($\rho=-0.19$, $P=0.02$, P adjusted=0.08), but not between FFAs and SLICC, a measure of disease damage ($\rho=0.01$, $P=0.88$, P adjusted=0.47) (Table 2).

Association of FFAs with insulin resistance and metabolic syndrome

In patients with SLE, FFAs were positively correlated with HOMA ($\rho=0.23$, $P=0.004$) (Table 2). FFAs were higher in those with metabolic syndrome compared to those without (0.66 mmol/L [0.46-0.81] vs. 0.52 mmol/L [0.35-0.66], $P<0.001$) (Figure 2). These associations remained significant after adjustment for age, race and sex ($P=0.006$ for HOMA and $P=0.002$ for metabolic syndrome), but were attenuated after further adjustment for BMI

($P=0.40$ for HOMA and $P=0.11$ for metabolic syndrome. FFAs were associated with triglycerides ($\rho=0.22$, $P=0.005$, P adjusted= 0.004).

Association of FFAs with cardiovascular risk and endothelial activation

FFAs were positively correlated with cardiovascular risk as determined by the Framingham risk score ($\rho=0.25$, $P=0.002$) and with coronary artery calcium score in univariate analysis ($\rho=0.20$, $P=0.01$), but not after adjustment for age, race and sex ($P=0.25$ for Framingham risk score and $P=0.33$ for coronary artery calcium score) (Table 2).

FFAs were positively correlated with endothelial activation markers, E-selectin and ICAM-1 ($\rho=0.33$, and $\rho=0.35$ respectively, both $P<0.001$). The association remained significant after adjustment for age, race and sex (both $P=0.001$) (Table 2), and with additional adjustment for BMI ($P=0.001$ for E-selectin and $P=0.01$ for ICAM-1). This association persisted after further adjustment for IL-6 ($P=0.003$ for E-selectin and $P=0.02$ for ICAM-1) and TNF- α ($P=0.01$ for E-selectin and $P=0.03$ for ICAM-1). FFAs were not associated with VCAM-1 in univariate and adjusted analysis.

FFAs and medications

Concentrations of FFAs did not differ significantly among patients with SLE currently using or not using methotrexate, hydroxychloroquine, prednisone, azathioprine, or mycophenolate mofetil (all adjusted P values >0.05) (Table 3). Similarly, statin use was not associated with concentrations of FFAs (P adjusted= 0.65).

Discussion

The major new findings of this study are that FFAs are elevated in patients with SLE, particularly in those with metabolic syndrome, and that these FFAs may contribute to endothelial activation, and insulin resistance.

To our knowledge, elevation of FFAs in SLE patients has not been described. However, it appears that the major contribution to the elevation of FFAs in SLE patients is related to increased BMI rather than inflammation, contrary to our hypothesis.

Inflammatory cytokines can increase FFAs. Several *in vitro* studies in which IL-6 or TNF- α were added to isolated adipocytes resulted in increased lipolysis.^{30,31} Moreover, infusions of IL-6 or TNF- α in healthy subjects resulted in an acute increase in circulating FFAs.^{32,33} However, these studies examined acute changes. In our study, SLE patients had chronically increased IL-6 and TNF- α concentrations, and there was no correlation between these cytokines and FFAs. Thus, duration of exposure to inflammatory cytokines may alter their lipolytic activity.

Insulin resistance and FFAs may have a cause and effect relationship. For example, insulin resistance developed when FFAs were increased in healthy subjects through lipid infusion.³⁴ Conversely, insulin sensitivity improved with decreasing FFAs concentration in obese patients.³⁵ We found a correlation between FFAs and insulin resistance, as determined by HOMA, as well as with the presence of metabolic syndrome. However, the relationship was lost after statistical adjustment for BMI. Thus, contrary to our hypothesis that inflammation drives lipolysis, the major driving factor of elevated FFAs in SLE was BMI, suggesting that simply the presence of more adipose tissue provided more FFAs. In contrast, we recently reported FFAs in rheumatoid arthritis patients are associated with insulin resistance (HOMA) independent of BMI.³⁶ These differences between SLE and rheumatoid arthritis are interesting in that BMI appears to contribute more to insulin resistance in SLE patients, than in rheumatoid arthritis patients.³⁷

FFAs have been linked to atherosclerosis. FFAs were correlated with carotid intimal media thickness (IMT) in diabetic patients, but not in control subjects.³⁸ Similarly, FFAs and carotid IMT were significantly correlated in renal transplant recipients; a population in whom increased FFAs, chronic inflammation, and insulin resistance are prevalent.³⁹ We found that FFAs were associated with coronary calcium score in SLE patients, but the significance was attenuated after adjustment for age, race, and sex.

Although, there was no independent association between FFAs and coronary artery calcium score, our findings suggest that FFAs may promote endothelial dysfunction in SLE patients. The evidence linking FFAs and atherosclerosis is based in part on their known effects on vascular endothelium.⁴⁰ A transient increase in plasma FFAs to the range seen in obesity and type 2 diabetes mellitus increased circulating ICAM-1, VCAM-1, and E-selectin concentrations by 13 to 35% in healthy subjects.¹⁷ ICAM-1 is expressed on endothelial cells and lymphocytes, and its soluble form correlates with degree of atherosclerosis as determined by carotid artery intimal-medial thickness in hypertensive, diabetic patients.⁴¹ E-selectin is expressed on activated endothelial cells and is responsible for adhesion and migration of leukocytes,⁴² and its soluble form is an independent predictor of carotid plaque in SLE.⁴³ Thus, our finding of an independent association between ICAM-1 and E-selectin with FFAs in SLE patients is of interest.

Limitations of our study include the cross-sectional design that precludes the inference of cause and effect. Also, we did not measure non-calcified coronary atherosclerotic plaque and, thus, cannot exclude the possibility that such plaques are associated with elevated FFAs. Additionally, we only studied fasting circulating FFAs, and not rate of appearance, or postprandial concentrations; specific studies to address those responses in patients with SLE and controls will be of interest. The assay used to measure FFAs does not detect oxidized fatty acids. This would be of interest in future studies.

In conclusion, concentrations of FFAs are higher in patients with SLE than controls, largely due to elevated BMI and not inflammation. FFAs are associated with the presence of insulin resistance and metabolic syndrome. Additionally, FFAs are independently associated with markers of endothelial activation but not with coronary artery calcium.

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Figure 1.

Free fatty acids (mmol/L) in SLE and control subjects. FFAs were higher in SLE than controls (0.55 mmol/L [0.37-0.71] vs 0.44 mmol/L [0.32-0.60], $P=0.019$). This remained significant after adjustment for age, race and sex (adjusted $P=0.033$). After additional adjustment for BMI there was no statistical difference (adjusted $P=0.132$). Data are presented as box plot where boxes represent the interquartile range [IQR], the lines within boxes represent the median, and the lines outside the boxes represent the lower quartile minus 1.5 times the IQR or the upper quartile plus 1.5 times the IQR.

Figure 2.

Free fatty acids concentrations (mmol/L) in SLE patients without (No) and with (Yes) metabolic syndrome as defined by WHO criteria. FFAs were higher in those with metabolic syndrome compared to those without (0.660 mmol/L [0.455-0.808] vs. 0.515 mmol/L [0.350-0.660]), $P < 0.001$). This remained significant after adjustment for age, race and sex (adjusted $P < 0.001$), but not after additional adjustment for BMI (adjusted $P = 0.11$). Data are presented as box plot where boxes represent the interquartile range [IQR], the lines within boxes represent the median, and the lines outside the boxes represent the lower quartile minus 1.5 times the IQR or the upper quartile plus 1.5 times the IQR.

Table 1
Characteristics of SLE and control patients

	SLE n = 156	Controls n = 90	P value
Age (Years)	40 [30-48]	42 [30-49]	0.72
Sex (% Male)	12%	13%	0.68
Race (% Caucasian)	67%	73%	0.32
BMI (kg/m ²)	27.3 [23.4-32.7]	25.2 [22.5-30.1]	0.04
Current Smoker	21%	18%	0.60
Hypertension	43%	17%	<0.001
Systolic BP (mmHg)	116 [106-127]	116 [107-127]	0.85
Diastolic BP (mmHg)	72 [65-79]	70 [64-78]	0.40
Diabetes mellitus	3.9%	1.1%	0.21
Metabolic syndrome	25.6%	8.9%	0.001
HOMA	1.47 [0.69-2.52]	1.08 [0.66-2.08]	0.085
Fasting glucose (mg/dl)	82 [76-91]	84.5 [80-89.8]	0.12
Waist Hip ratio ^a	0.846 [0.787-0.908]	0.805 [0.75-0.866]	0.003
Statin Use	12.8%	7.8%	0.22
Total cholesterol (mg/dl)	166 [139-203]	181 [160-205]	0.04
HDL cholesterol (mg/dl)	48 [36-55]	47 [38-61]	0.47
LDL cholesterol (mg/dl)	94 [76-125]	110 [89-134]	0.004
Triglycerides (mg/dl)	104 [76-149]	82 [63-117]	0.002
IL-6 (pg/ml) ^b	5.83 [2.27-26.81]	1.77 [0.87-4.73]	<0.001
TNF- α (pg/ml) ^c	4.8 [3.0-8.0]	2.4 [1.8-3.0]	<0.001
E-selectin (ng/ml) ^c	23.9 [17.7-28.6]	18.4 [13.8-23.0]	<0.001
ICAM-1 (ng/ml) ^c	175.9 [135.5-226.5]	142.9 [118.2-176.0]	<0.001
VCAM-1 (ng/ml) ^c	1,077.4 [887.9-1,203.3]	984.4 [810.2-1,105.4]	0.04
Disease duration (Years)	6 [3-11]	-	-
SLEDAI	4 [0-6]	-	-
SLICC	1 [0-1]	-	-
Framingham risk score	5 [1-10]	7 [0-11]	0.77
CAC (Agatston units) ^{*d}	0.0 [0.0-0.0] (66.7 $\pm$ 340.0)	0.0 [0.0-0.0] (3.5 $\pm$ 26.5)	0.002

Data presented as median [interquartile range] or percentage (%).

* For CAC, data is also presented as (mean $\pm$ SD). P-values for Wilcoxon rank sum test for continuous variables and Pearson's chi-square test for categorical variables.

^aData available for n=224

^bdata available for n=186

^cdata available for n=192

^ddata available for n=238. BMI=body mass index, BP=blood pressure, HOMA= homeostasis model assessment of insulin resistance, IL-6= interleukin 6, TNF α = tumor necrosis factor- α , ICAM-1= intracellular adhesion molecule 1, VCAM-1= vascular cell adhesion molecule 1,

SLEDAI= systemic lupus erythematosus disease activity index, SLICC= systemic lupus International Collaborating Clinics/American College of Rheumatology damage index, CAC= Agatston coronary artery calcium score.

Table 2
Correlation between free fatty acids and measures of inflammation, insulin resistance, coronary atherosclerosis in SLE

	Rho ^a	p-value	adjusted ^b p-value
Lipids			
Total cholesterol	0.11	0.16	0.47
LDL cholesterol	0.08	0.31	0.55
HDL cholesterol	-0.07	0.41	0.06
Triglycerides	0.22	0.005 *	0.004 *
Insulin resistance			
HOMA	0.23	0.004 *	0.006 *
Inflammatory markers			
IL-6	0.06	0.54	0.31
TNFα	-0.002	0.98	0.46
Endothelial activation			
E-selectin	0.33	<0.001 *	0.001 *
VCAM-1	0.02	0.80	0.61
ICAM-1	0.35	<0.001 *	0.001 *
Coronary artery disease			
FRS	0.25	0.002 *	0.25
CAC	0.20	0.01 *	0.33 ^c
Other			
BMI	0.35	<0.001 *	<0.001 *
SLEDAI	-0.19	0.02 *	0.08
SLICC	0.01	0.88	0.47

HOMA= homeostasis model assessment of insulin resistance, IL-6= interleukin 6, TNFα= tumor necrosis factor-α, VCAM-1= vascular cell adhesion molecule 1, ICAM-1= intracellular adhesion molecule 1, FRS= Framingham risk score, CAC= Agatston coronary artery calcium score, BMI= body mass index, SLEDAI= systemic lupus erythematosus disease activity index, SLICC= systemic lupus International Collaborating Clinics/American College of Rheumatology damage index.

^a Spearman's correlation coefficient.

^b Multivariable linear regression model with adjustment for age, race and sex.

^c Proportional logistic regression model with adjustment for age, race and sex.

* p<0.05

Table 3
Effect of Medication use on FFAs in SLE patients

Medication	FFAs median		p-value	adjusted ^a p-value
	Current use	No Current use		
Methotrexate	0.54 mmol/l [0.38-0.59]	0.56 mmol/l [0.37-0.72]	0.58	0.55
Hydroxychloroquine	0.58 mmol/l [0.37-0.73]	0.53 mmol/l [0.40-0.66]	0.55	0.60
Prednisone	0.55 mmol/l [0.37-0.72]	0.55 mmol/l [0.37-0.69]	0.73	0.95
Azathioprine	0.42 mmol/l [0.32-0.56]	0.56 mmol/l [0.38-0.72]	0.07	0.20
Mycophenolate	0.56 mmol/l [0.49-0.69]	0.55 mmol/l [0.37-0.71]	0.57	0.23
Statin class	0.51 mmol/l [0.35-0.71]	0.55 mmol/l [0.37-0.71]	0.73	0.65

Data presented as median [interquartile range].

^a adjusted for age, race and sex.