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Alkaline phosphatase and percentage body fat predict circulating C-reactive protein in premenopausal women

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Abstract

C-reactive protein (CRP) is considered a marker of inflammation, which is a risk factor for many chronic diseases. However, determinants of CRP remain unclear and were studied in a strictly defined cohort of healthy premenopausal women (n=233) using multiple regression models. Independent predictors of serum CRP (model R^2=0.59) were percentage body fat, serum alkaline phosphatase (ALP), sex hormone-binding globulin and white blood cell count. The close association between CRP and ALP suggests that enzymatic activity of ALP may be important for the anti-inflammatory effects of CRP, which should be confirmed with additional studies.

Key words: *Obesity; SHBG; insulin; liver enzymes; white blood cells*

Introduction

Chronic low-grade inflammation that is associated with obesity is considered to influence the development of the metabolic syndrome, type 2 diabetes, cardiovascular disease (Hotamisligil 2006) and various malignancies (Coussens &Werb 2002). In response to inflammation and infection, the liver increases synthesis of many acute-phase reactant proteins, including C-reactive protein (CRP). CRP was discovered as an acute-phase reactant in humans by Tillet and Francis in 1930 (Tillet &Francis 1930). It belongs to the phylogenetically ancient and highly conserved family of pentraxins. Circulating levels of CRP increase dramatically in response to infection and other inflammatory processes (Black et al. 2004). CRP is believed to play an important role in innate immunity by binding phosphocholine (PC) on microbes and on injured or apoptotic cells, thereby initiating the process of phagocytosis by macrophages (Casey et al. 2008).

Increased CRP levels are also associated with obesity (Visser et al. 1999). CRP levels have been associated with the number of manifestations of the metabolic syndrome (Zambon et al. 2005, Florez et al. 2006) and are predictive for the development of this syndrome and type 2 diabetes (Freeman et al. 2002, Ridker 2007). CRP is therefore considered to be a potentially important predictor of cardiovascular disease risk (de Ferranti &Rifai, 2007). CRP may also increase with development of some human malignancies (McSorley et al. 2007, Polterauer et al. 2007, Gockel et al. 2006). However, although CRP has been associated with several adverse health outcomes, the independent predictors of CRP remain unclear.

Many liver proteins are routinely measured in serum to diagnose liver dysfunction. For example, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are useful diagnostic markers for liver diseases, including non-alcoholic fatty liver disease (NAFLD), which is a clinically important manifestation of the metabolic syndrome. Serum CRP levels are also increased in NAFLD (Kerner et al. 2005, Booth et al. 2008). This is not surprising, because this is an inflammatory condition,

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and the synthesis of CRP occurs primarily in the liver, where it is regulated by proinflammatory cytokines, including, interleukin (IL)-1, IL-6 and tumour necrosis factor-α, which are produced by adipose tissue (Yudkin et al. 1999, Zhang et al. 1995). White blood cell (WBC) counts also increase in obesity and inflammatory conditions (Dixon &O'Brien 2006) and these cells are an important source of proinflammatory cytokines. Thus, in response to stress, the liver makes an array of proteins including CRP, and adiposity may disturb the homeostasis of these liver proteins. Surprisingly little is known about the influence of obesity on serum levels of various proteins synthesized by the liver and their inter-relationships. This was investigated in a strictly defined cohort of healthy premenopausal women from whom blood samples were obtained during the luteal phase of the menstrual cycle, in order to minimize the influences of ovulation and changing hormone levels, and to study the roles of luteal phase hormones.

Methods

Study design

A defined group of premenopausal women (n=233), which included all major racial and ethnic groups found in the US population, was studied during the luteal phase of two separate menstrual cycles. Subjects were recruited from within an 80 km radius of Galveston, Texas, using flyers and advertisements that were posted or sent by web or postal mail. The study protocol was approved by the Institutional Review Board of the University of Texas Medical Branch (UTMB) and by the Human Research Protection Office of the US Army Medical Research and Materiel Command. Written informed consents were obtained from all subjects.

Enrollment was restricted to healthy 30–40-year-old premenopausal women with regular menstrual cycles. The subjects were enrolled in an extended dietary intervention study of biomarkers for breast cancer risk. Exclusion criteria were: breast cancer; serious health conditions such as cardiovascular diseases; current pregnancy or breast feeding; recent use of contraceptive medications (oral, injection or patch); and menopause. Inclusion of women with regular menstrual cycles (every 24–35 days) provided for blood collection during the luteal phase of the cycle, when progesterone levels are increased. This allowed us to study associations with levels of both 17β-estradiol and progesterone. Three study visits were scheduled during the luteal phase of two separate menstrual cycles (a total of six visits), usually between cycle days 20 and 24. The samples collected during the baseline visits of these subjects were the source for these analyses. In order to exclude effects of acute inflammation, study visits were rescheduled if a subject reported a concurrent acute illness or infection.

Anthropometrics, body composition and reproductive factors

At each study visit, body weight and height, waist circumference at the umbilicus, and hip circumference at the widest point around the buttocks were measured. At one study visit, total, lean and fat body mass were measured in duplicate (before and after repositioning) with the subject supine, by dual energy X-ray absorptiometry (DXA) (Model Discovery A; ModelQDR4500A; Hologic, Waltham, MA, USA). Coefficients of variation (CV) for the two readings of body composition by DXA were <5% and the mean of the two measurements was used for statistical analyses. Reproductive and general health histories were obtained once using a self-administered standard clinic questionnaire.

Hormone assays and blood chemistries

Fasting (~12 h) venous blood samples were drawn between 08:00 and 10:00 a.m. on all study visits. Aliquots of serum and plasma were stored at -80°C until analysed. Due to limited resources, three plasma samples (all from one menstrual cycle) were analysed in duplicate for 17β-estradiol, testosterone and progesterone. A radioactive immunoassay (RIA) kit was used to measure plasma progesterone concentrations (sensitivity, 0.1 ng ml^{-1}). Enzyme-linked immunosorbent assay (ELISA) kits were used for measuring plasma testosterone concentrations (sensitivity, 0.04 ng ml^{-1}) and plasma 17β-estradiol concentrations (sensitivity, 7 pg ml^{-1}). Serum samples from the first and fifth study visits were assayed for CRP (sensitivity, 0.0016 mg l^{-1}) by a high-sensitivity ELISA, and for sex hormone-binding globulin (SHBG) (sensitivity, 0.61 nmol l^{-1}) by ELISA, and for insulin by direct RIA (sensitivity, 1.3 μIU ml^{-1}). All immunoassays were performed using commercially available kits (Diagnostic System Laboratories, Inc., Webster, TX, USA). All hormone measurements were in duplicate. Duplicate serum concentrations were used to determine intra-assay CV. Quality controls (low and high level) provided with the kits were run with each assay and these levels were used to determine interassay CV (n>30). The intra- and interassay CV for all analytes were <10%.

Fasting serum glucose, total and high-density lipoprotein cholesterol (HDL-C), triglycerides, albumin, ALT, AST and ALP were measured in serum samples from the first and sixth study visits by a certified hospital clinical laboratory using VITROS® 5.1 FS (Ortho-Clinical Diagnostics, Rochester, NY, USA). White blood cell counts in blood samples from the first and sixth study visits were

obtained with an automated Sysmex, HST-N system (Sysmex America Inc., Mundelein, IL, USA).

Statistical analyses

Data are presented as means and standard deviations (SD) for continuous variables and as frequencies for categorical variables. The distributions of concentrations of serum proteins, hormones and enzymes were checked for normality. Values of CRP, ALT and AST were log-transformed prior to statistical analyses due to skewed distribution. The distribution of CRP, ALT and AST among the study subjects is reported in raw form. Pearson's correlation coefficients were estimated to assess the linear relationship between log CRP, ALP and the predictor variables. Ethnicity and parity were treated as categorical variables. Subjects were divided into three groups: normal weight, overweight and obese, according to World Health Organization (WHO) guidelines (WHO 2000), and multiple comparisons across groups were made using the MULTTEST Procedure in SAS®.

The outcome variables for this study were serum CRP, ALP and various measures of adiposity. Means of two measurements of serum CRP, SHBG, lipids and liver enzymes, and blood WBC count, using samples that were collected from each woman at least 1 month apart, were the predictor variables. Means of three measurements of steroid hormones, 17β-estradiol, testosterone and progesterone (all from one menstrual cycle) were used in statistical analyses. Independent predictors of CRP, ALP and various measures of adiposity were determined by multiple ordinary least square (OLS) regression analysis (REG Procedure in SAS®, version 9.1, SAS Institute Inc., Cary, NC, USA).

Results

Based on WHO guidelines, 30% of the subjects in this study were in the healthy weight range (body mass index, BMI=18.5–24.9 kg m^{-2}), 33% were overweight (BMI= 25–29.9 kg m^{-2}), and 37% were obese (BMI≥30 kg m^{-2}) (Table 1). The ethnic composition of the study population, which was 51% non-Hispanic white, 30% Hispanic, 14% African American, 2% Asian, 3% unspecified ethnicity and one Native American, did not differ across the three BMI categories (Table 1). Fasting serum concentrations of glucose, triglycerides, HDL-C, albumin, ALP, CRP, SHBG, insulin and blood WBC counts exhibited significant BMI-dependent differences. The WBC counts across the three BMI groups were all within the normal range (3.9–10 x10^3 μl^{-1}), which is evidence against acute infection. There were no BMI-dependent differences ($p \leq 0.01$) in fasting concentrations of ALT, AST, cholesterol, 17β-estradiol, progesterone and testosterone.

For CRP, ALT, AST, ALP, triglycerides, glucose, HDL-C, insulin, SHBG and WBC count there were high correlations between two measurements in the same subjects at two different time points, with Pearson's correlation coefficients ranging from 0.53 to 0.89 (results not shown). Therefore, for these variables, the means of two measurements at least 1 month apart were used for analyses of correlations between various other study variables of interest.

Pearson's correlation coefficients showed that log CRP correlated strongly and positively with all anthropometric variables (r ranging from 0.29 to 0.70, all $p<0.0001$), but negatively with height ($p=0.001$) and age of menarche ($p=0.02$) (Table 2). Log CRP was not associated with age in this group, which had a narrow age range (30–40 years). Log CRP also correlated strongly and positively with serum insulin, triglycerides, glucose, ALP and WBC count (all $p<0.0001$), and negatively with serum SHBG, HDL-C (both $p<0.0001$) and albumin ($p=0.0003$). Log ALT, log AST, cholesterol and luteal phase estradiol, progesterone and testosterone levels were not associated with serum CRP in these subjects.

Similar to CRP, fasting serum levels of ALP correlated with anthropometric variables, namely, body weight, BMI, fat body mass, percentage body fat, waist and hip circumference ($r=0.30$–0.39, all $p<0.0001$; Table 2) and lean body mass ($p=0.04$). Serum ALP had a weak inverse correlation with height ($p=0.01$) and did not correlate with age. Fasting serum ALP also correlated positively with fasting serum levels of insulin, glucose and triglycerides, and blood WBC count, and negatively with SHBG, HDL-C and albumin (p-values between 0.01 and 0.0001, Table 2). ALP was also not associated with log ALT, log AST or luteal phase estradiol, progesterone and testosterone in univariate analysis. Unlike CRP, ALP did not correlate with age of menarche.

Twenty-nine of 200 subjects (14.5%) were smokers. Smoking status was not available for 33 subjects. The incidence of smoking was low in our study subjects, and due to a lack of any significant differences in predictor variables between smokers and non-smokers (TTEST Procedure in SAS®), no adjustment was made for smoking in the final regression analysis. Alcohol intake of the study subjects, as the mean intake determined from three different food records was extremely low (2.6 ± 6.5 g daily, median intake 0.03 g daily) and was not included in the regression analysis.

Independent predictors of CRP, ALP or anthropometrics

The predictors of CRP (or ALP) were studied using multiple OLS regression models and biomarkers related to the metabolic syndrome, such as SHBG, insulin, ALP (or log CRP when ALP was the dependent variable), log ALT, log

Table 1. General characteristics of the study subjects (30–40-year-old premenopausal women from within an 80 km radius of Galveston, TX, *n*=233 unless otherwise indicated).

	All	Normal weight[a]	Overweight	Obese
		(18.5–24.9 kg m^{-2})	(25–29.9 kg m^{-2})	(≥30 kg m^{-2})
	n = 233	*n* = 69	*n* = 78	*n* = 86
Demographic and anthropometric				
BMI (kg m^{-2})	28.4 (5.5)[b]	22.3 (1.7)	27.5 (1.5)[‡]	34.2 (3.5)[‡¶]
Age (years)	36.3 (2.7)	36.1 (2.4)	36.3 (2.6)	36.4 (3.0)
Body weight (kg)	74.4 (14.5)	59.9 (6.6)	71.7 (6.7)[‡]	88.4 (11.1)[‡¶]
Height (cm)	161.9 (6.7)	163.8 (6.2)	161.5 (6.8)	160.6 (6.8)[§]
Waist circumference (cm)	87.6 (11.4)	75.8 (5.9)	85.5 (5.5)[‡]	98.9 (7.4)[‡¶]
Hip circumference (cm)	109.2 (12.0)	97.7 (6.1)	107.1 (5.0)[‡]	120.3 (10.3)[‡¶]
Waist to hip ratio	0.80 (0.06)	0.78 (0.05)	0.80 (0.05)[†]	0.83 (0.07)[¶]
Lean body mass (kg)	46.4 (6.2)	42.0 (4.6)	45.3 (4.5)[‡]	50.9 (5.6)[‡¶]
Fat body mass (kg)	28.2 (9.8)	18.2 (4.2)	26.6 (4.2)[‡]	37.7 (7.7)[‡¶]
Percentage body fat	36.9 (6.7)	30.1 (5.2)	37.0 (4.0)[‡]	42.3 (4.5)[‡¶]
Race/ethnicity				
Non-Hispanic white, *n* (%)	119 (51.1)	46 (66.7)	38 (48.7)	35 (40.7)
Hispanic, *n* (%)	70 (30.0)	12 (17.4)	23 (29.5)	35 (40.7)
African American, *n* (%)	33 (14.2)	6 (8.7)	13 (16.7)	14 (16.3)
Other[c], *n* (%)	11 (4.7)	5 (7.2)	4 (5.2)	2 (2.3)
Reproductive				
Parity				
Yes, *n* (%)	203 (87)	60 (87.0)	66 (84.6)	77 (90.6)
No, *n* (%)	29[d] (13)	9 (13.0)	12 (15.4)	8[d] (9.4)
Age of menarche (years), *n*=227	12.6 (1.5)	12.8 (1.5)	12.7 (1.5)	12.5 (1.6)
Number of complete pregnancies	2.2 (1.3)	2.1 (1.3)	2.2 (1.3)	2.2 (1.4)
Blood chemistry (fasting)				
Glucose (mmol l^{-1})	4.8 (0.6)	4.6 (0.3)	4.7 (0.4)	5.0 (0.8)[‡§]
Triglycerides (mmol l^{-1})	1.2 (0.7)	0.8 (0.3)	1.1 (0.6)	1.4 (0.8)[†¶]
Cholesterol (mmol l^{-1})	4.7 (0.8)	4.5 (0.8)	4.7 (0.8)	4.8 (0.8)
HDL-C (mmol l^{-1})	1.4 (0.3)	1.6 (0.3)	1.4 (0.3)[‡]	1.3 (0.2)[‡]
ALP (u l^{-1})	70.3 (16.0)	63.4 (15.7)	68.3 (13.7)	77.3 (15.7)[†¶]
ALT (u l^{-1})	3.2 (0.3)	3.2 (0.2)	3.2 (0.3)	3.3 (0.3)
AST (u l^{-1})	20.8 (4.9)	20.8 (4.5)	21.3 (5.1)	20.3 (5.0)
Albumin (g l^{-1})	40.9 (3.2)	42.2 (2.8)	40.9 (3.5)	39.7 (2.8)[‡]
WBC count (x10^3 µl^{-1})	6.2 (1.5)	5.4 (1.1)	6.1 (1.4)	6.9 (1.6)[‡¶]
Hormones (fasting)				
CRP (mg l^{-1})	6.5 (6.9)	1.8 (1.9)	5.4 (4.4)[†]	11.2 (8.2)[‡¶]
SHBG (nmol l^{-1})	101.0 (39.4)	126.2 (39.1)	97.9 (31.3)[‡]	83.5 (35.8)[‡]
Insulin (pmol l^{-1})	80.5 (53.8)	43.5 (27.2)	70.6 (45.5)[†]	119.2 (52.1)[‡¶]
Testosterone (pmol l^{-1})	25.8 (12.2)	24.1 (13.0)	24.9 (10.0)	28.1 (13.1)
17β-Estradiol (pmol l^{-1}), *n*=201	293.2 (136.3)	281.7 (99.4)	302.9 (149.1)	294.3 (152.5)
Progesterone (nmol l^{-1})	33.8 (16.2)	34.1 (16.3)	36.6 (15.2)	31.0 (16.8)

ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; SHBG, sex hormone-binding globulin; WBC, white blood cells.[a]Based on WHO BMI criteria;[b]mean (SD), [c]other, non-specified, Asian and American Indian ethnicities combined;[d]missing frequency=1; [†]p<0.05 vs normal-weight group, [‡]p<0.005 vs normal-weight group, [§]p<0.05 vs overweight group, [¶]p<0.005 vs overweight group.

AST, glucose, triglycerides and HDL-C, WBC count and anthropometrics as the independent variables (Table 3). Because luteal phase estradiol, progesterone and testosterone were not associated with log CRP or ALP in univariate and in exploratory multiple regression analyses (all p≥0.10; results not shown), final multivariate regression models did not include sex steroids as predictor variables.

For log CRP as the dependent variable, serum ALP (β=0.15, p =0.003), WBC count (β=0.15, p=0.003) and percentage body fat (β=0.49, p<0.0001) were the strongest positive independent predictors of CRP, while serum SHBG (β=-0.12, p=0.02) was a negative predictor of CRP. These four predictor variables could explain 59% of the variance in serum CRP levels (Table 3). HDL-C,

Table 2. Pearson's correlation coefficients for log C-reactive protein (CRP) and serum alkaline phosphatase(ALP) with various predictor variables among study subjects, n =233 unless otherwise indicated.

	Log CRP		ALP	
	r	*p*-Value	*r*	*p*-Value
Demographic and anthropometrics				
Height	-0.21	0.001	-0.17	0.01
Weight	0.58	<0.0001	0.31	<0.0001
BMI	0.67	<0.0001	0.39	<0.0001
Lean body mass	0.29	<0.0001	0.14	0.04
Fat body mass	0.66	<0.0001	0.38	<0.0001
Percentage body fat	0.70	<0.0001	0.39	<0.0001
Waist circumference	0.65	<0.0001	0.34	<0.0001
Hip circumference	0.57	<0.0001	0.30	<0.0001
Age of menarche	-0.15	0.02	-0.05	n.s.
Hormones and blood chemistry				
Insulin	0.49	<0.0001	0.36	<0.0001
SHBG	-0.42	<0.0001	-0.19	0.004
Testosterone	0.10	n.s	0.08	n.s
17β-Estradiol, *n*=201	0.11	n.s	-0.01	n.s
Progesterone	-0.06	n.s	-0.05	n.s
Cholesterol	0.12	n.s	0.06	n.s
HDL-C	-0.41	<0.0001	-0.17	0.01
Triglycerides	0.34	<0.0001	0.19	0.004
Glucose	0.25	<0.0001	0.19	0.004
Albumin	-0.24	0.0003	-0.17	0.01
ALP	0.44	<0.0001	1.00	
Log ALT	0.1	n.s	0.11	n.s
Log AST	-0.04	n.s	0.05	n.s
WBC count	0.45	<0.0001	0.29	<0.0001

ALT, alanine amino transferase; AST, aspartate amino transferase; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; SHBG, sex hormone-binding globulin; WBC, white blood cells.

triglycerides, insulin, log ALT or log AST (all p >0.1, Table 3), while correlated with CRP in univariate analyses, were not independent predictors of CRP. BMI, waist circumference and body fat, but not lean body mass, could replace percentage body fat as independent predictors of CRP only in the regression models omitting percentage body fat as a predictor variable.

The close association between ALP and CRP suggested the need to investigate the predictors of ALP. The results of multiple OLS regression models with ALP as the dependent variable are listed in Table 3. Serum CRP was the strongest positive predictor of serum ALP (β=0.27, p<0.003), followed by serum insulin (β=0.18, p=0.03), and these two predictors could explain 25% of the variance in serum ALP concentrations. However, serum ALT, AST, SHBG, glucose, HDL-C and triglycerides, WBC count and body fat (and other anthropometric variables) were not independent predictors of ALP (Table 3).

Table 3. Multiple OLS **r**egression models with log C-reactive protein (CRP) and alkaline phosphatase (ALP) as the dependent variables in study subjects (n=233).

	CRP	ALP
Percentage body fat	0.49 (<0.0001)*	0.12 (0.18)
Log CRP (mg l^{-1})	.	0.27 (0.003)
ALP (u l^{-1})	0.15 (0.003)	.
Log ALT (u l^{-1})	0.03 (0.54)	0.05 (0.50)
Log AST (u l^{-1})	0.002 (0.97)	0.06 (0.44)
SHBG (nmol l^{-1})	-0.12 (0.02)	0.04 (0.54)
Insulin (pmol l^{-1})	0.05 (0.41)	0.18 (0.03)
Glucose (mmol l^{-1})	-0.02 (0.61)	02 (0.76)
Triglycerides (mmol l^{-1})	0.04 (0.49)	-0.03 (0.66)
HDL-C (mmol l^{-1})	-0.07 (0.20)	0.06 (0.44)
WBC count (x10^3 μl^{-1})	0.15 (0.004)	0.10 (0.15)
Model R^2	0.59	0.25

ALT, alanine amino transferase; AST, aspartate amino transferase; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; SHBG, sex hormone-binding globulin; WBC, white blood cells.*Standardized β-coefficients (p value).

Data in the literature suggest that ALT and AST are useful surrogate markers for obesity (Kerner et al. 2005, Booth et al. 2008). We used multiple OLS regression models to explore the predictors of all anthropometric variables, but only results for BMI, waist circumference, percentage body fat and lean body mass are included herein (Table 4). Log CRP was the strongest positive predictor of all measures of obesity (all p<0.0001; β coefficients ranging from 0.39 to 0.55), followed by insulin (positive) and albumin (negative). However, serum CRP was not a predictor of lean body mass (p=0.26). WBC count was an independent predictor of waist circumference (p=0.04). SHBG was a negative predictor for BMI, waist circumference and lean body mass but not for percentage body fat (or total body fat, results not shown). Log ALT was a significant predictor of waist circumference and lean body mass. Log AST was a predictor of waist circumference. In contrast, ALP was not a predictor for any of the anthropometric variables. Serum CRP, together with serum insulin, SHBG and albumin, could explain 60% of the variance in BMI. Serum CRP, together with serum insulin, SHBG, ALT, AST, albumin and WBC count could explain 58% of the variance in waist circumference. Serum CRP, together with serum insulin and albumin could explain 55% of the variance in percentage body fat.

Discussion

The primary finding of this study was that obesity, CRP and ALP were closely correlated in a defined cohort of apparently healthy, ethnically diverse 30–40-year-old women, who were not postmenopausal and not on any exogenous estrogens and with BMIs ranging from

Table 4. Multiple OLS regression models showing associations between various measures of adiposity and predictor variables in study subjects (n=233).

	BMI	Waist circumference	Percentage body fat	Lean body mass
Log CRP (mg l^{-1})	0.39 (<0.0001)*	0.36 (<0.0001)	0.55 (<0.0001)	0.09 (0.26)
ALP (u l^{-1})	0.04 (0.42)	-0.02 (0.66)	0.06 (0.27)	-0.05 (0.40)
Log ALT (u l^{-1})	0.09 (0.09)	0.17 (0.004)	-0.02 (0.77)	0.19 (0.02)
Log AST (u l^{-1})	-0.08(0.11)	-0.14 (0.01)	-0.07 (0.22)	-0.09 (0.25)
SHBG (nmol l^{-1})	-0.1 (0.04)	-0.12 (0.02)	-0.06 (0.26)	-0.13 (0.07)
Insulin (pmol l^{-1})	0.26 (<0.0001)	0.27 (<0.0001)	0.14 (0.02)	0.21 (0.007)
Albumin (g l^{-1})	-0.21 (<0.0001)	-0.15 (0.001)	-0.14 (0.004)	-0.13 (0.03)
WBC count (x10^3 μl^{-1})	0.04 (0.38)	0.1 (0.04)	0.002 (0.97)	0.04 (0.61)
Model R^2	0.60	0.58	0.55	0.20

ALT, alanine amino transferase; ALP, alkaline phosphatase; AST, aspartate amino transferase; BMI, body mass index; CRP, C-reactive protein; SHBG, sex hormone-binding globulin; WBC, white blood cells.*Standardized β-coefficients (p value).

normal to obese. Log CRP had a strong correlation with serum ALP in univariate (r=0.44, Table 2) and in multivariate analyses (Table 3), and CRP was the strongest independent predictor of ALP. These observations coupled with literature reports (cited below) are consistent with a biologically plausible hypothesis that ALP may contribute importantly to the role of CRP in innate immunity.

CRP was first identified more than 75 years ago through its calcium-dependent binding to phosphocholine (PC) groups in C-polysaccharides of the cell wall of pneumococcal bacteria. Although the crystal structure of CRP is known, its physiological and pathophysiological functions remain unclear. PC is a constituent of most biological membranes and was recently defined as the principal ligand of CRP (Marnell et al. 2005). Gram-negative bacteria express PC in a phase-dependent manner, and the binding of CRP to the PC components has been reported for a variety of such pathogens, including *Leishmania donovani, Haemophilus influenza, and Neisseria meningitidis* (Casey et al. 2008, Weiser et al. 1998). By binding to the PC component of polysaccharides, CRP opsonizes invading microbes and facilitates their uptake by macrophages and neutrophils, thus playing a key role in the innate protective response to pathogenic infections.

ALPs are a group of enzymes in humans and other species that hydrolyse phosphate esters at an alkaline pH (McComb et al. 1979). In healthy subjects, liver and bone are the major sources of serum ALP, but it is also present in lung, kidneys, leukocytes, intestine and placenta (Giannini et al. 2005). Serum ALP is measured as a clinically useful marker for liver and bone disease. ALP can, at physiological pH, remove one of the two core phosphate groups from the carbohydrate moiety of lipopolysaccharide (LPS or endotoxin) in Gram-negative bacteria (Bates et al. 2007). LPS is pivotal in eliciting an inflammatory response and toxicity (Verweij et al. 2004), and the activity of ALP can render LPS non-toxic and thereby protect the host against infectious agents (Bentala et al. 2002). Therefore, it has been suggested that ALP has a role complementary to that of CRP in combating pathogens.

More recently, binding of CRP to the PC moiety of oxidized phosphatidylcholine (oxPC) on the surface of apoptotic or necrotic cells and oxidized LDL was recognized (Chang et al. 2002). Although PC is widely expressed in cell walls, calcium-dependent binding of CRP is limited to PC in bacterial and apoptotic cell walls. CRP does not bind to PC on viable cells in which the PC head groups of phospholipids are not exposed. Binding of CRP to PC is crucial for the removal of bacteria or apoptotic cells. ALP activity may be critically important in exposing PC in the cell walls and making it available for CRP binding, analogous to a role attributed to phospholipase A_2 (Hack et al. 1997). Furthermore, CRP may limit the atherogenic oxidation of LDL. For example, Rufail et al. found that physiological amounts of CRP strongly inhibit copper-mediated oxidation of LDL in a dose-dependent manner (Rufail et al. 2006).

Reference ranges for serum CRP concentrations in healthy premenopausal women are not well established and may vary between laboratories, not all of which use the high-sensitivity method utilized in this study. The overall mean serum CRP concentration of 6.5 mg l^{-1} in this study, measured with a high-sensitivity assay, was higher than levels reported for 30–39-year-old US women (Ford et al. 2004). This difference could be attributed to a higher incidence of obesity in our study population. It is noteworthy that, unlike Ford et al. (2004), we did not exclude subjects with CRP levels >10 mg l^{-1} from the analyses, because all such subjects were in the overweight and obese categories (Table 1). Because all our subjects had no complaints and had normal WBC counts, bacterial infections were unlikely. Serum ALP concentrations in our subjects were all also within the normal range defined for this age group (Eastman & Bixler 1977).

Adipose tissue produces inflammatory cytokines that regulate production of many acute-phase reactants including CRP, a liver protein and an important marker of inflammation. Our observations that percentage body fat

(and also body fat, results not shown) was the strongest independent predictor of CRP and that WBC, another important source of proinflammatory cytokines, was also an independent predictor of CRP, are consistent with a regulatory role of cytokines in the production of CRP (Yudkin et al. 1999).

Total serum ALP is often elevated in NAFLD and is used clinically as marker for this condition. However, body fat was not an independent predictor of ALP, nor was ALP an independent predictor of body fat in this defined group of premenopausal women with a wide range of BMI. Additionally, WBC, another important source of proinflammatory cytokines, was not an independent predictor of ALP. These results argue against a role of proinflammatory cytokines in influencing ALP concentrations, although both CRP and ALP are often elevated in inflammatory conditions (Kerner et al. 2005, Cheung et al. 2009). In univariate analyses, measures of adiposity, CRP and ALP correlated with each other. However in multivariate analyses, an independent association was observed only for measures of adiposity and CRP, and for CRP and ALP, but not for ALP and obesity. These results suggest that ALP is probably important for the biological function, but not for increasing concentrations of CRP.

Some reports suggested that ALT and AST may be useful surrogate biomarkers for obesity, and these may also be elevated in NAFLD. In this defined cohort of apparently healthy, ethnically diverse 30–40-year-old premenopausal women, we found that CRP was the strongest positive independent predictor of all indices of excess weight (Table 4), followed by insulin and albumin. ALT and AST were additional positive independent predictors but only for waist circumference. ALP was not an independent predictor for any one of the anthropometric measurements. SHBG was a negative independent predictor of BMI, waist circumference, lean body mass and CRP, consistent with prior studies that SHBG correlates strongly with insulin, insulin resistance and waist circumference (Nayeem et al. 2009, Akin et al. 2009). Taken together, of the liver proteins studied, CRP appears to be more closely associated with fatty tissue as a body component, while SHBG, ALT and AST are more closely linked to the amount of lean tissue. Insulin and albumin are associated with both fat and lean body mass.

A small study had shown that the association of CRP and ALP is gender specific and limited to females, implicating the involvement of sex steroids (Cheung et al. 2008). We showed that in a strictly defined group of premenopausal women with a wide range of body weight, estrogen, progesterone and testosterone were not predictors of CRP and ALP. Our finding of a BMI-dependent increase in the WBC count, although within the normal range, is consistent with previous findings that the WBC count is associated with obesity and the metabolic syndrome; the mechanisms are unknown but may include fat deposition in the liver (Nakanishi et al. 2002, Dixon & O'Brien 2006).

The major strengths of our study are (1) the inclusion of a defined group of apparently healthy young women not taking exogenous hormone preparations, (2) the measurement of CRP by an ultrasensitive method, and (3) the measurement of ALT, AST, ALP, lipids, CRP, SHBG, insulin and glucose on at least two separate occasions. Weaknesses of the study include the limited age range of the subjects and the cross-sectional rather than longitudinal nature of the study. We also did not separately measure liver-specific ALP isoenzyme in this study. However, prior studies investigating inflammation and liver enzymes reported total serum ALP (Kerner et al. 2005) which mostly represents liver ALP in adult humans (Young 1967).

In summary, we have demonstrated a strong interrelationship among circulating CRP, ALP and body fat. Of the liver proteins we studied, CRP was the strongest independent predictor of adiposity, followed by AST or ALT, and SHBG. Although not an independent predictor of obesity/adiposity, ALP activity may contribute importantly to the anti-inflammatory effects of CRP. Furthermore, factors produced by adipose tissue and WBC (e.g. proinflammatory cytokines) may influence circulating concentrations of CRP. The critical question is whether an imbalance in the ratio of CRP to ALP, such as may occur with obesity, may contribute to pathological features of the metabolic syndrome.

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Declaration of interest

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