Body fat percentage is more strongly associated with biomarkers of low-grade inflammation than traditional cardiometabolic risk factors in healthy young adults – the Lifestyle, Biomarkers, and Atherosclerosis study

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ABSTRACT
The primary aim was to appraise the relationship between body fat percentage and the inflammatory markers C-reactive protein (CRP) and orosomucoid in a population of young, non-smoking, healthy, Swedish adults, without any chronic diseases. A secondary aim was to compare whether these associations differed between the women using estrogen contraceptives and those who did not. We assessed the association in linear regression models between body fat percentage based on a bio-impedance measurement and plasma concentrations of CRP and orosomucoid in men and women aged 18–26 years, n = 834. Statistically significant associations were found between body fat percentage and both biomarkers of inflammation, with β coefficients of 0.30 (95% CI 0.24–0.37) and 0.28 (0.22–0.35) for CRP and orosomucoid, respectively (p < .001). Adjustment for established risk factors marginally lowered the effects sizes (partial betas, 0.28 and 0.20, respectively), while the strong statistically significant associations remained. In the female cohort, estrogen and non-estrogen using subpopulations did not significantly differ in the correlations between body fat percentage and the inflammatory biomarkers, even adjusted for established cardiometabolic risk factors. In conclusion, in healthy young adults, higher levels of body fat percentage are associated with elevations in plasma biomarkers of inflammation, suggesting that a systemic inflammatory process, promoting atherosclerosis, may commence already at this early stage in life. CRP and orosomucoid plasma concentrations differed between users and non-users of estrogen contraceptives, but both subgroups showed similar correlations between increasing body fat percentage and increasing plasma concentrations of the biomarkers of inflammation.

Introduction
Chronic inflammation is a well-established feature of cardiovascular disease, and an essential component in the pathogenesis of its associated tissue and organ dysfunction [1]. Early studies on cardiovascular risk employed biomarkers such as erythrocyte sedimentation rate, discovered by Robin Fährus in 1921 [2], a non-specific marker of inflammation, and established its association with mortality among patients admitted for acute myocardial infarction [3]. Over time, new biomarkers were introduced giving more specific information about the type and origin of the inflammatory activity. C-reactive protein (CRP) has been shown to be useful as a predictor of future cardiovascular events [4] and has a possible role as a mediator in the pathogenesis of atherosclerosis [5]. To complement established risk stratification scores, such as the Framingham risk score and systematic coronary evaluation (SCORE), an addition of CRP to the calculation of risk has been proposed as a means to provide a dynamic marker of atherosclerotic burden [4]. However, a lack of a general consensus on its optimal clinical use, as well as its tendency to react in a non-specific manner to any insult, in particular bacterial infection, detracts from its clinical usefulness as a predictor of atherosclerosis. In patients with already manifest cardiovascular disease (CVD) it identifies groups of individuals at risk of recurring events [4]. The search for drivers of low-grade chronic vascular inflammation resulted in the identification of adipocytes as an active secretory organ releasing both anti- and proinflammatory cytokines, capable of promoting a sustained inflammatory activity in target cells, for instance in the liver [6]. Obesity is a cardiovascular risk factor that has been referred to as a pandemic because of its increasing prevalence world-wide [7]. It entails a dysregulation of the endocrine function of adipocytes [8], and is associated with dyslipidemia and glucose intolerance [9]. Recently, orosomucoid, also known as α-1-acid glycoprotein, another established biomarker of inflammation [10], was shown to be selectively expressed in adipocytes under certain conditions.
where it acts immunomodulatory under inflammatory load [11]. An orosomucoid elevation in males aged 28–61 was associated with an increased risk of CVD events in individuals without traditional cardiovascular risk factors [12], and individuals who underwent Roux-en-y gastric bypass to reduce obesity had decreasing orosomucoid levels postoperatively [13].

Currently, biomarkers in plasma as well as imaging modalities mainly address late symptomatic stages of CVD [14]. Novel methods of early risk detection are desirable in order to be able to devise preventive measures and timely therapeutic intervention. In this study we examined the relationship between low-grade systemic inflammation, traditional cardiovascular risk factors and adiposity. The established biomarkers CRP, measured by a high-sensitivity assay (hsCRP), and orosomucoid were used to assess inflammation. We hypothesized that a low-grade systemic inflammation may present early in the atherosclerotic process in young, healthy, non-smoking individuals with an increased body fat mass. The aim was to examine possible differences in the associations of body fat percentage and traditional cardiovascular risk factors with plasma concentrations of hsCRP and orosomucoid, respectively. A secondary aim was to examine whether estrogen-containing contraceptives, which are known to reduce orosomucoid concentrations [15,16] and has been shown to increase CRP concentrations [17], would affect any such associations.

Materials and methods

Study population

The cross-sectional Lifestyle, Biomarkers and Atherosclerosis study (the LBA study) constitutes the study population (n = 834, healthy adults between 18 and 26 years, 69% females) [18]. Subjects were recruited by advertising at the Örebro University, and in a local newspaper. Signed consent was obtained from all subjects. A questionnaire was used to assess the lifestyle habits of the subjects and served as verification that they met the inclusion criteria of being nonsmokers not suffering from any chronic disease. The questionnaire contained a field where the participants were asked to fill in whether they regularly took any contraceptives or other medication. The study was approved by the Regional Ethics Review Board, Uppsala, ref 2014/224.

Body composition measurements

Upon enrollment in the study anthropometric measurements were recorded during the first of two visits. Height was measured without shoes with a stadiometer to the nearest 0.5 cm. A Tanita scale (Tanita BC-418 MA; Tanita Europe B.V., Amsterdam, The Netherlands) was used to measure weight and body fat percentage. Body mass index (BMI) was calculated. Bioelectric impedance analysis, considered superior to BMI as an estimation of body fat [19], was chosen as the main surrogate marker of body fat percentage. In previous studies its performance has been validated in comparison with dual X-ray absorptiometry [20].

Laboratory investigations

Samples were collected after an overnight fast into sodium citrate fluoride vacutainer tubes for glucose analysis and serum and plasma vacutainer tubes for the rest of the analyses (BD Vacutainer; BD AB, Stockholm, Sweden). Serum was left to clot for at least 30 min before centrifugation and subsequent analysis. HsCRP, Orosomucoid, Apolipoprotein A-1 (Apo A-1) and Apolipoprotein B (Apo B) were analyzed on a Siemens ADVIA 1800 Chemistry instrument with a coefficient of variation (CV) of 5% at 0.74 mg/L with the Siemens High Sensitivity CRP Assay (ADVIA 1800 Chemistry System, Upplands Väsby, Sweden). The Apo A-1 assay had a CV of 4% at 0.9 g/L and the Apo B assay a CV of 5% at 1.5 g/L (Apolipoprotein A-1 (Apo A-1), Apolipoprotein B (Apo B) (Siemens Healthcare Inc., Upplands Väsby, Sweden). Orosomucoid had a CV of 4% at 0.47 g/L using the DAKO orosomucoid immunoturbidimetry assay (Agilent, Santa Clara, CA). The reagent consists of polyclonal rabbit antibodies to pooled human sera, and cross-reacts with both human orosomucoid-1 and orosomucoid-2. Total cholesterol (CHOL), Triglycerides (TG), high-density lipoprotein (HDL) and glucose were assayed colorimetrically with Vitros MicroSlide technology (5.1TM FS; Clinical Chemistry Instruments, Raritan, NJ). Direct low-density lipoprotein (direct LDL) was assayed by a two-step colorimetric assay with Vitros MicroWell technology. CHOL (3% CV at 3.9 mmol/L), TG (CV of 4% at 1.3 g/L), HDL (6% CV at 1.0 mmol/L), LDL (5% CV at 2.4 mmol/L), and glucose (4% CV at 4.6 mmol/L) were analyzed on a Vitros 5.1 system (Vitros 5.1TM FS, Clinical Chemistry Instruments, Raritan, NJ). Insulin was analyzed with the Abbott Architect Insulin Assay, a sandwich immunoassay using chemiluminescence detection with a CV of 7% at 8 mIU/L on an Architect i2000SR unit (Abbott, Abbot Park, IL).

Statistical analysis

Statistical analyses were performed with statistical software SPSS version 22 (IBM, Armonk, NY). Normal distribution was appraised by assessment of the size of the standard deviation (SD) in comparison with the mean, as well as graphically displayed in a histogram and visually evaluated. Non-normally distributed variables were natural log (ln) transformed before inclusion in statistical analysis. Association between the inflammatory markers as dependent variables were tested against body fat percentage and traditional plasma risk factors using linear univariate and multivariate regression models. Scatter plots were made to evaluate whether there was a linear relationship between the independent and dependent variables in the models. Additionally, for each model the residuals were plotted for normality as a control of the quality of the model. All
variables that were entered in the regression models were z-score transformed for men and women separately and then merged. As a precautionary measure, addition of sex as a factor was tested in all models to exclude any systematic differences in the associations between men and women. The regression models were repeated excluding individuals whose CRP concentrations were above 5 mg/L, \( n = 49 \), which could be thought to correspond to a viral upper respiratory tract infection rather than a low-grade inflammation reflective of an increased cardiovascular risk. Because a number of study participants dropped out before the second visit, or for technical reasons, there are missing values in the data set. The following number is missing for each variable: insulin 19 subjects, orosomucoid 14 subjects, hsCRP 12 subjects, apo B/apo A-1 ratio 10 subjects, LDL 6 subjects, CHOL 5 subjects, HDL 5 subjects, TG 4 subjects. In all multivariate models, as a conservative measure, missing values were replaced by the sex specific mean of the variable (corresponding to 0, as all variables were z-score transformed for use in these models). Statistical significance of differences in the associations between men and women. The factor was tested in all models to exclude any systematic differences in the associations between men and women. The beta coefficients of the association were similar: \( \beta \approx 0.46 \) before, and \( \beta ≈ 0.46 \) after exclusion.

Results

Population characteristics of the LBA study population is shown in Table 1. There are some significant differences between men and women in body fat percentage, BMI, HDL, CHOL, apo B/apo A-1 ratio, hsCRP, and orosomucoid. When stratified in females by use of estrogen-containing contraceptives, significant differences in mean concentrations of orosomucoid, hsCRP, LDL, CHOL, and TG were found (Table 2).

Univariate associations

Body fat percentage was positively associated with the plasma concentrations of both the inflammatory biomarkers CRP and the orosomucoid (Table 3). The significant associations remained when stratified for men and women (data not shown). The relationship between percent body fat percentage in the population and the inflammatory markers is visualized in Figure 1. After exclusion of individuals with CRP concentrations above 5 mg/L, the correlation between CRP and body fat percentage remained statistically significant. The beta coefficients of the association were similar: \( \beta = 0.30 [0.24–0.37] \) before, and \( \beta = 0.38 [0.30–0.46] \) after exclusion. Similarly, the association between orosomucoid and body fat percentage remained significant upon exclusion of individuals with a CRP above 5 mg/L.

Multivariate associations

In multivariate regression analyses, associations between body fat percentage and the biomarkers of inflammation were adjusted for fasting serum insulin in Table 3, Model 1, and both fasting serum insulin and lipoprotein apo B/apo A-1 ratio in model 2. After adjustment, the associations of body fat percentage with the plasma biomarkers remained statistically significant (Table 3). Further adjusting for CHOL, LDL, HDL, TG, and fasting glucose did not affect the \( \beta \) coefficients for the association between body fat percentage and either CRP or orosomucoid (data not shown).

Table 1. Baseline characteristics.

| Variable                  | Male, \( n = 259 \) | Female, \( n = 575 \) | \( p \) Value |
|---------------------------|---------------------|-----------------------|--------------|
| Age                       | 22 ± 2.0            | 22 ± 1.9              | n.s.         |
| Bioimpedance fat percentage (%) | 15 ± 5.6           | 28 ± 6.6              | <.001        |
| BMI (kg/m²)               | 23 ± 3.1            | 22 ± 3.6              | <.001        |
| LDL (mmol/L)              | 2.3 ± 0.69          | 2.3 ± 0.71            | n.s.         |
| HDL (mmol/L)              | 1.2 ± 0.28          | 1.4 ± 0.36            | <.001        |
| Total cholesterol (mmol/L)| 4.0 ± 0.79          | 4.3 ± 0.77            | <.001        |
| Triglycerides (mmol/L)    | 0.79 ± 0.35         | 0.81 ± 0.36           | n.s.         |
| Fasting serum insulin (mIU/L)| 7.5 ± 3.7      | 8.0 ± 4.5             | n.s.         |
| apo B/apo A1 ratio       | 0.56 ± 0.14         | 0.51 ± 0.14           | <.001        |
| hsCRP (mg/L)              | 1.3 ± 2.7           | 1.9 ± 4.0             | .027         |
| Orosomucoid (g/L)         | 0.72 ± 0.16         | 0.68 ± 0.17           | <.001        |

Mean ± SD (standard deviation). n.s.: non-significant.

Table 2. Estrogen contraceptive use and mean differences in population characteristics, body composition and laboratory investigations among women in the LBA study.

| Variable                  | Non-estrogen using women, \( n = 415 \) | Estrogen contraceptive using women, \( n = 162 \) | \( p \) Value |
|---------------------------|-----------------------------------------|-----------------------------------------------|--------------|
| Age                       | 22 ± 1.9                                | 22 ± 1.9                                      | n.s.         |
| Bioimpedance fat percentage (%) | 28 ± 6.7                               | 28 ± 6.5                                      | n.s.         |
| BMI (kg/m²)               | 22 ± 3.8                                | 22 ± 3.2                                      | n.s.         |
| LDL (mmol/L)              | 2.2 ± 0.64                              | 2.6 ± 0.82                                    | <.001        |
| HDL (mmol/L)              | 1.4 ± 0.36                              | 1.5 ± 0.38                                    | n.s.         |
| Total cholesterol (mmol/L)| 4.2 ± 0.74                              | 4.5 ± 0.80                                    | <.001        |
| Triglycerides (mmol/L)    | 0.75 ± 0.32                             | 0.98 ± 0.41                                   | <.001        |
| Fasting serum insulin (mIU/L)| 7.9 ± 4.8                           | 8.1 ± 4.0                                      | n.s.         |
| apo B/apo A1 ratio       | 0.51 ± 0.14                             | 0.52 ± 0.15                                   | n.s.         |
| hsCRP (mg/L)              | 1.3 ± 2.2                               | 3.4 ± 6.4                                     | <.001        |
| Orosomucoid (g/L)         | 0.70 ± 0.17                             | 0.62 ± 0.16                                   | <.001        |

Mean ± SD (standard deviation). n.s.: non-significant.
In women, stratifying for use and non-use of estrogen containing contraceptives (Table 4) showed similar statistically significant correlations between body fat percentage and the inflammatory markers in the two subgroups, despite the markedly differing mean concentrations (Table 2).

**Discussion**

This study found a significant association between body fat percentage and two biomarkers of low-grade inflammation in a population of young, healthy adults. The association remained significant after adjustment for the established biochemical risk factors of atherosclerosis. A separate adjustment for apo B/apo A-1 ratio (Table 2, Model 2) was performed because the accuracy of LDL cholesterol (LDL-C) concentration as a marker of cardiovascular risk has recently been called into question; discordances have been found in some conditions, such as metabolic syndrome, diabetes and hypertriglyceridemia, between LDL particle concentration and LDL-C [21]. However, no differences in associations between body fat percentage and either CRP or orosomucoid was seen regardless of whether apo B/apo A-1 ratio or LDL-C was included in the adjusted models.

Insulin resistance is thought to be implicated in the pathophysiology of the increased cardiometabolic risk in obese children, promoting the inflammation and endothelial dysfunction that precedes increased stiffness measurements [22]. An increased insulin resistance promotes inflammation in adipose tissue [23]. The complicated interplay between obesity and insulin resistance is a classical 'chicken or the egg' problem which remains an open issue. We, therefore, adjusted our regression models for fasting serum insulin, but this explained only a minor part of the association between the inflammatory markers and an increased body fat percentage (Table 3, Model 1). The analyses were performed on z-score transformed variables on men and women together, but adding a term for sex in the models, or stratifying, did not alter the significance of the associations, consistent with our interpretation that an increased body fat percentage results in a similar increase in biomarkers of low-grade inflammation in both sexes. The inflammatory markers increase with increasing body fat in a similar manner for both men and women, despite a higher mean body fat percentage in women compared with men.

Orosomucoid is known to decrease with increasing estrogen levels [15, 16]. By stratifying the female cohort for estrogen-based contraceptive use, the associations between body fat percentage and either orosomucoid or CRP did not significantly differ between the two groups, confirming the important contribution of body fat percentage to plasma concentrations of these biomarkers, irrespective of estrogen levels (Table 4). The mechanism of the effect of estrogen on CRP is not fully elucidated, but it has been suggested to be mediated by a modulation of transcription in hepatocytes [24].

While both CRP and orosomucoid are acute-phase reactants produced by the liver and increase in response to inflammatory signaling, their respective functions differ: CRP is a complement activator binding to bacteria and host cells, and is considered a possible mediator in atherosclerotic pathophysiology [5]. In contrast to CRP, orosomucoid increases more slowly in inflammatory states, but remains elevated for a prolonged period [10, 25]. CRP may thus be a

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**Table 3.** Associations of the inflammatory biomarkers CRP and orosomucoid with body fat percentage. All variables were ln transformed and sex-specific z scores were used in the general linear models.

|                      | CRP              | Orosomucoid       |
|----------------------|------------------|-------------------|
|                      | $\beta_{F\%}$   | $R^2$ adj         | $\beta_{F\%}$   | $R^2$ adj         |
| Univariate           | 0.30 (0.24–0.37) | <.001             | 0.28 (0.22–0.35) | <.001             |
| Model 1              | 0.28 (0.21–0.35) | <.001             | 0.22 (0.15–0.29) | <.001             |
| Model 2              | 0.28 (0.20–0.35) | <.001             | 0.20 (0.13–0.27) | <.001             |

CI: confidence interval; $\beta_{F\%}$: standardized $\beta$ coefficient for body fat percentage. Model 1 includes, in addition to body fat percentage, fasting serum insulin. Model 2 includes, in addition to Model 1, apo B/apo A1 ratio.
more straightforward marker of acute inflammatory load, whereas elevations in orosomucoid, which is synthesized by both and adipocytes and hepatocytes under different conditions [11], may reflect both adipocyte immunomodulatory signaling induced by metabolic dysregulation in adiposity, and cytokine driven hepatic synthesis in response to inflammation [11]. CRP and orosomucoid are both independent predictors of future cardiovascular risk [26, 27], a relationship consistent with evidence indicating inflammation as a key regulatory process uniting multiple risk factors, including obesity, to the alterations of vascular function seen in atherosclerosis [28]. The detrimental effects of obesity may begin early in life, and childhood obesity is known to be associated with an increased cardiovascular risk in adulthood [29]. Previously, an increase in circulating inflammatory chemokines was found to correlate with preatherosclerotic vascular alterations in young, healthy children [30]. Evidence indicates inflammation as the common, mechanistic link between obesity and atherosclerosis [31]. Adipokine release from adipose tissue is associated with endothelial dysfunction and systemic inflammation, which are both features characteristic of atherosclerosis [32]. Similar patterns of inflammatory cell accumulation, in particular a recruitment of a heterogeneous macrophage population associated with cell death and T-cell activation are prominent features of both obesity and atherosclerosis [28]. Such relationships between adiposity and inflammation provide an explanation for the strong association seen in our population between an increased body fat and inflammatory biomarkers.

A limitation to take into account is the cross-sectional study design employed, precluding inferences of causality in the relationship between body composition and markers of inflammation. Males were underrepresented and well-educated health-aware subjects overrepresented. Outcome studies are needed to further elucidate the significance of body fat related inflammation. Among the strengths of the study are the fairly large sample size, the low number of missing values in most variables, the exclusion of subjects with chronic diseases, and our ability to assess the possible impact of estrogen-containing contraceptive use.

In conclusion, higher levels of bioimpedance-measured body fat percentage in healthy young adults is associated with modest but significant elevations in plasma biomarkers of inflammation, in both sexes. In females, oral contraceptive use does not affect this association. The strong association between an increased body fat and increased concentration of inflammatory markers suggest that a systemic inflammatory process, promoting atherosclerosis, may commence already at this early stage in life in individuals with an increased body fat.

Disclosure statement

None of the authors have a conflict of interest or financial or other relationships that might lead to a conflict of interest.

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Table 4. Associations of the inflammatory biomarkers CRP and orosomucoid with body fat percentage, stratified for estrogen contraceptive use in the female cohort.

| CRP | Non-estrogen using women (n = 415) | Estrogen using women (n = 162) |
|-----|----------------------------------|--------------------------------|
| βb (95% CI) | p | R² adj | βb (95% CI) | p | R² adj |
| **Univariate** | | | **Univariate** | | |
| Univariate | 0.38 (0.26–0.42) | <.001 | 0.25 (0.10–0.43) | .001 | 0.056 |
| Model 1 | 0.36 (0.23–0.41) | <.001 | 0.24 (0.072–0.43) | .006 | 0.050 |
| Model 2 | 0.36 (0.23–0.41) | <.001 | 0.25 (0.085–0.45) | .004 | 0.055 |
| **Orosomucoid** | | | **Orosomucoid** | | |
| Non-estrogen using women (n = 415) | | | Estrogen using women (n = 162) | | |
| βb (95% CI) | p | R² adj | βb (95% CI) | p | R² adj |
| **Univariate** | | | **Univariate** | | |
| Univariate | 0.29 (0.21–0.38) | <.001 | 0.35 (0.19–0.51) | <.001 | 0.10 |
| Model 1 | 0.22 (0.13–0.31) | <.001 | 0.26 (0.086–0.42) | .004 | 0.13 |
| Model 2 | 0.19 (0.099–0.28) | <.001 | 0.27 (0.097–0.44) | .002 | 0.13 |

CI: confidence interval; βb: standardized β coefficient for body fat percentage. Model 1 includes, in addition to body fat percentage, fasting serum insulin. Model 2 includes, in addition to Model 1, apo B/apo A1 ratio.
