Relationships between serum fatty acid composition and multiple markers of inflammation and endothelial function in an elderly population

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Abstract

Background: Fatty acid (FA) composition in serum has been associated with C-reactive protein (CRP), but associations with other markers of inflammation and endothelial function, e.g. adhesion molecules are unknown. We recently suggested a possible role of the lipogenic enzyme stearoyl coenzymeA desaturase-1 (SCD-1) in inflammation. This study investigates the associations between serum FA composition, including SCD-1 index, and various inflammatory and endothelial function markers.

Methods: 264 Swedish men and women aged 70 years participated in this cross-sectional population-based study. FA composition was measured in serum cholesteryl esters and was correlated to inflammatory markers (CRP, interleukin [IL]-2, IL-6, IL-8, tumor necrosis factor [TNF]-α, vascular cellular adhesion molecule [VCAM]-1, intercellular adhesion molecule [ICAM]-1, E-selectin, P-selectin, L-selectin, interferon-γ, and monocyte chemotactic protein [MCP]-1), using linear regression analysis. SCD-1 activity was estimated by FA product-to-precursor ratio (16:1/16:0).

Results: Serum FA composition was significantly associated with CRP and E-selectin but not with other inflammatory markers. After adjusting for BMI, smoking, physical activity, alcohol consumption and lipid-lowering therapy, the proportion of palmitoleic acid and SCD-1 index were positively correlated with CRP concentrations (P = 0.003 and P = 0.001, respectively).

Conclusion: A FA composition reflecting high intake of saturated fat and a high SCD-1 index is independently related to CRP concentrations, but not to other markers of inflammation and endothelial function in this population of elderly men and women. Given the absent association between FA composition and the other markers, CRP may be the preferable marker to use when investigating potential relationships between FAs and low-grade inflammation.

Keywords: Fatty acids; Inflammation; Endothelial function; SCD-1; C-reactive protein; Adhesion molecules

1. Introduction

Vascular inflammation and endothelial dysfunction may participate in the pathogenesis of atherosclerosis. Markers of low-grade inflammation and endothelial dysfunction have been associated with cardiovascular disease (CVD) [1,2]. The underlying cause of this low-grade inflammation is unknown but one contributing factor may be dietary fatty acids (FAs) [3]. Serum FA composition can be used as an objective biomarker of dietary fat quality [4–6]. FA composition is however also affected by endogenous elongation and desaturation catalyzed by enzymes such as stearoyl coenzymeA desaturase-1 (SCD-1). SCD-1 converts saturated FAs

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to monounsaturated FAs [7]. Recently, we observed that a high SCD-1 index, and decreased proportion of linoleic acid (18:2n-6) was independently associated with increased CRP concentrations in elderly men [8], but studies investigating the link to other various inflammation markers and adhesion molecules are lacking.

If the previously observed associations are present in women and also involve endothelial dysfunction and other inflammatory markers beyond CRP are unknown. We therefore aimed to investigate if serum FA composition was related to different markers of inflammation and endothelial function in a cross-sectional study consisting of both men and women at age 70. Further, we examined the potential relationship between estimated SCD-1 activity and inflammatory and endothelial function markers, also taking several potential confounders into account. This is to our knowledge the first study associating FA composition to endothelial function markers such as adhesion molecules as well as to other novel inflammatory markers.

2. Methods

2.1. Subjects

The subjects participated in the PIVUS (Prospective Investigation of the Vasculature in Uppsala Seniors) study. All persons aged 70 and living in the community of Uppsala, Sweden, were eligible. Between April 2001 and June 2004, 2025 subjects were randomly invited within 1 month of their 70th birthday in order to standardize for age, and 1016 (50.1%) participated. FA composition in serum cholesteryl esters (CEs) was assessed in 273 randomly selected participants. Those with CRP levels >10 mg/l (n = 9) were excluded and 264 subjects constituted the study population. The study was approved by the Ethics Committee of the University of Uppsala. The participants gave informed consent before entering the study.

2.2. Baseline investigation

Subjects were investigated after an overnight fast as previously described [9]. No medication or smoking was allowed after midnight. Medical history, smoking habits and regular medication were assessed by a questionnaire. BMI was calculated as weight (kg) divided by height (m) squared. After recordings of height and weight, an arterial cannula was inserted in the brachial artery for blood sampling. Lipid variables and fasting blood glucose were measured by standard laboratory techniques. FAs were measured in serum CE and are presented as the relative sum of the FAs analysed. Methanol was added to serum for lipid extraction and CE was separated from the other lipid esters by thin-layer chromatography. CE was transmethylated by addition of acidic methanol. The percentage composition of methylated FAs was determined by gas-liquid chromatography. SCD-1 activity was estimated by calculation of an SCD-1 index using FA product-to-precursor ratio (16:1n-7)/16:0. Physical activity was divided into light and hard exercise and classified as number of activities for at least 30 min per week. This was assessed by asking the participant how many times per week he/she performed light (e.g. walking, gardening) respectively hard exercise (e.g. running, swimming) for at least 30 min.

As the participation rate in the PIVUS cohort was only 50%, an evaluation of cardiovascular disorders and medications was carried out in 100 consecutive non-participants. The prevalence of cardiovascular drug intake, ischemic heart disease, statin use and insulin treatment were similar to those in the investigated sample, while the prevalence of diabetes, congestive heart failure and stroke tended to be higher among the non-participants [9].

2.3. Inflammatory and endothelial function markers

High sensitive CRP was measured in serum by an ultrasensitive particle enhanced immunoturbidimetric assay (Orion Diagnostica, Espoo, Finland) on a Konelab 20 autoanlyser (Thermo Clinical Labsystems, Espoo, Finland). The inter-assay coefficient of variation was 3.2%. Cytokines, chemokines and adhesion molecules were analysed on the Evidence® array biochip analyser (Randox Laboratories, Ltd., Crumlin, UK) [10]. The functional sensitivity for the different inflammatory markers was as follows; CRP: 0.1 mg/l, IL-2: 4.1 pg/ml, IL-6: 0.3 pg/ml, IL-8: 1.5 pg/ml, TNF-α: 1.8 pg/ml, ICAM-1: 18.6 ng/ml, VCAM-1: 3.1 ng/ml, E-selectin: 3.1 ng/ml, P-selectin: 11.2 ng/ml, L-selectin: 32.8 ng/ml, interferon-γ: 1.8 pg/ml and MCP-1: 19.4 pg/ml. Also IL-1α, IL-1β, IL-4 and IL-10 were included in the Evidence® array biochip cytokine panel, but were found to have insufficient sensitivity for measurements in the present sample and were therefore not evaluated.

2.4. Statistical analyses

The distribution of the variables was examined by the Shapiro–Wilk W test. To attain normal distribution, 16:1n-7, 18:0, 18:3n-6, 20:5n-3, SCD-1, alcohol as well as all inflammatory markers, except for P-selectin, were logarithmically transformed. IL-2, IL-6, TNF-α, and interferon-γ were not normally distributed and therefore analysed with Spearman’s rank test. The correlation between FA composition and inflammatory and endothelial function markers was investigated by Pearson’s correlation analysis and linear regression analysis. Multivariate analysis adjusting for BMI, smoking habits, alcohol consumption, physical activity and usage of lipid-lowering medicine was also made. To avoid type-1 errors due to multiple testing a P-value <0.01 was considered as statistically significant. A JMP software was used for statistics (SAS Institute, Cary, NC).
3. Results

Population characteristics are presented in Table 1. Seventeen subjects (6%) were current smokers and 35 (13%) used lipid-lowering medicine, of which 94% were statins. Table 2 shows the relative percentage of the measured FAs.

In univariate analysis, FA composition was associated with concentrations of CRP and E-selectin (see Table 3). No associations were found between FAs and IL-2, IL-6, IL-8, TNF-α, VCAM-1, ICAM-1, P-selectin, L-selectin, interferon-γ or MCP-1.

In the multivariate model (n = 211) adjusting for relevant covariates (BMI, smoking, physical activity, alcohol consumption and lipid-lowering therapy), palmitoleic acid (P = 0.003) and SCD-1 index (P = 0.001) remained positively related to CRP concentrations (Table 3). None of the other inflammatory markers were significantly associated with FA composition in multivariate analysis. The abolished correlation between FAs and E-selectin and between 18:1, 18:2n-6, 20:3n-6 and CRP were mainly explained by BMI and alcohol consumption. Thus, these factors seem to be the most important confounders concerning the associations between FAs, CRP and E-selectin (data not shown).

Further, if excluding subjects with CVD or diabetes (n = 204), CRP were correlated with palmitoleic (r = 0.23, P = 0.001), linoleic (r = −0.19, P = 0.008), DHGLA (r = 0.19, P = 0.007), and SCD-1 index (r = 0.23, P = 0.0008). Linoleic acid (r = −0.18, P = 0.01) and DHGLA (r = 0.21, P = 0.003) were related to E-selectin. Borderline significance was observed for the association between oleic acid (P = 0.015) and CRP, and for palmitoleic acid, GLA, and E-selectin (P = 0.025 and P = 0.029, respectively). Palmitoleic acid and SCD-1 index remained significantly related to CRP when adjusting for BMI, smoking, physical activity, alcohol consumption, and use of lipid-lowering drugs (n = 167).

4. Discussion

In univariate analyses, FA composition in serum CE was correlated with CRP and E-selectin whereas no associations were observed for the other markers of inflammation and endothelial function. CRP was positively associated with monounsaturated FAs, 20:3n-6 and SCD-1 index, and negatively related to 18:2n-6. Similar results were observed for E-selectin, i.e.; positive associations with 16:0, 16:1n-7, 18:3n-6, 20:3n-6, and an inverse association with 18:2n-6. The correlations with E-selectin, a marker of endothelial function, were dependent of obesity and lifestyle factors since the associations were abolished after adjusting for BMI, smoking, alcohol intake, physical activity and lipid-lowering therapy. Thus, E-selectin concentrations may be driven by
obesity and lifestyle rather than the dietary fat composition. The abolished correlation between FA and E-selectin but not between FA and CRP may also be explained by a wider distribution of E-selectin compared to CRP in our population. Further, none of the other inflammatory or endothelial function markers were related to FA composition, perhaps indicating that they are weaker markers of systemic inflammation.

Interestingly, serum 16:1n-7 and SCD-1 index were significantly related to CRP independently of obesity and lifestyle factors, findings that accord with our recent results in a population of elderly men [8]. It should be noted that 16:1n-7 only constitutes a small part of the FAs in the Swedish diet and may rather reflect endogenous conversion from 16:0 by SCD-1. Indeed, both 16:1 and SCD-1 index change in response to saturated fat intake [11], results supported by animal data suggesting that saturated FAs induce SCD-1 activity and hence increase endogenous FA synthesis of monounsaturated FAs [12]. Moreover, in an US population, monounsaturated FAs in CE reflected the dietary intake of saturated fat, e.g. meats and dairy fat, rather than monounsaturated FAs derived from vegetable oils [5]. Thus, as discussed previously [8], the positive correlations between 16:1n-7, SCD-1, and CRP probably reflect high saturated fat intake, or possibly that high SCD-1 activity may promote inflammation. SCD-1 index could however also be influenced by dietary carbohydrates, in addition to saturated fat [13]. It is also possible that inflammation could be promoted by a metabolic disordered state associated with elevated SCD-1 activity.

A possible role of saturated FAs in inflammation has been demonstrated in vitro. When stimulating human cells with palmitic acid, the gene expression and protein production of IL-6 increased [14,15]. IL-6 is a central regulator of CRP. In contrast, linoleic acid prevented the palmitic acid-induced upregulation of IL-6 and thus seemed to act anti-inflammatory [14]. The exact mechanisms for these effects are unknown but proposed involved molecules are nuclear factor (NF)-κB [14] and protein kinase C [15]. It is possible that the pathways may differ depending on cell type. It should be noted that CRP and E-selectin may have a greater sensitivity in detection and lower variability than the other measured markers. This would be in favor of CRP and E-selectin with regard to finding significant correlations with FAs. Only CRP was significantly related to FA composition after adjustments, perhaps indicating CRP to be a more robust marker compared to the other inflammation molecules. Moreover, plasma concentrations of several chemokines are low and near the detection limits of enzyme immunoassays, why their use as biomarkers may be limited [16]. Also, different location of production or activity may play a role. The markers were measured in serum and different results may be observed if these markers are measured directly in the target tissues, e.g. endothelium or adipose tissue.

A serum FA pattern with high monounsaturated fat (probably as a reflection of high saturated fat intake) and low linoleic acid has previously been linked to elevated CRP among Swedish men [8], the metabolic syndrome [17], diabetes [18], and CVD [19,20]. Only a few other studies have examined the relationship between serum FAs and inflammatory markers. In a Spanish study, CRP was inversely associated with 18:2n-6 and n-3 FAs whereas IL-6 correlated positively with 14:0 and 16:0 [21]. An inverse relation between CRP and n-3 FAs was also seen in a French overweight population [22]. The Italian InCHIANTI study showed that lower proportions of 20:4n-6, 20:5n-3, and 22:6n-3 were associated with higher IL-6 concentrations and that 18:3n-3 was inversely related to CRP [23]. In line with some controlled

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>C-reactive protein</th>
<th>E-selectin</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td>14:0</td>
<td>r = -0.002, P-value = 0.98, PR-value = 0.58</td>
<td>r = 0.04, P-value = 0.49, PR-value = 0.77</td>
</tr>
<tr>
<td>16:0</td>
<td>0.03, 0.63, 0.51</td>
<td>0.20, 0.001, 0.54</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>0.22, 0.0004, 0.003</td>
<td>0.17, 0.005, 0.14</td>
</tr>
<tr>
<td>18:0</td>
<td>-0.03, 0.66, 0.02</td>
<td>-0.01, 0.85, 0.31</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>0.18, 0.004, 0.12</td>
<td>0.10, 0.09, 0.85</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>-0.19, 0.003, 0.03</td>
<td>-0.21, 0.0005, 0.29</td>
</tr>
<tr>
<td>18:3n-6a</td>
<td>0.13, 0.034, 0.03</td>
<td>0.16, 0.007, 0.15</td>
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<tr>
<td>18:3n-3</td>
<td>-0.004, 0.95, 0.71</td>
<td>-0.13, 0.038, 0.27</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>0.16, 0.008, 0.53</td>
<td>0.24, &lt;0.0001, 0.07</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>0.08, 0.18, 0.31</td>
<td>0.09, 0.14, 0.97</td>
</tr>
<tr>
<td>20:5n-3a</td>
<td>0.02, 0.76, 0.33</td>
<td>0.12, 0.06, 0.43</td>
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<tr>
<td>22:6n-3</td>
<td>-0.06, 0.31, 0.83</td>
<td>0.03, 0.59, 0.81</td>
</tr>
<tr>
<td>SCD-1</td>
<td>0.22, 0.0004, 0.001</td>
<td>0.14, 0.024, 0.16</td>
</tr>
</tbody>
</table>

r, Pearson’s correlation coefficient; SCD-1, stearoyl coenzyme A desaturase-1 index (16:1/16:0).

a Log-transformed variable.
b Adjusting for BMI, smoking, physical activity, alcohol intake and lipid-lowering drugs.

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trials [24], we did not observe any relation between n-3 FAs and inflammation.

There are limitations of this study. Since this is an observational cross-sectional study no causality can be drawn. Another drawback is that the desaturase activity is estimated by SCD-1 index (16:1/16:0). However, the SCD-1 index has been used as a marker of SCD activity in several other studies [7,8,13,17]. In humans, in response to rosiglitazone treatment, both SCD-1 mRNA expression in adipose tissue and serum SCD-1 index increased [25]. Inhibition of SCD-1 activity is reflected by decreased SCD-1 index in human hepatoma cells [26]. This index is also markedly reduced in mice lacking the SCD-1 gene [27]. Although the SCD-1 index is a useful marker, direct measurement of SCD-1 activity would have been optimal to investigate its involvement in subclinical inflammation. Strength of this study is the assessment of a large number of inflammatory markers, including adhesion molecules. Other studies have only measurements of a few markers of inflammation without any markers of endothelial function. The use of the serum FA composition is most likely an advantage before dietary registration since it is a more objective method avoiding reporting bias. Another advantage is the inclusion of both men and women, and thereby increasing generalisability. To confirm previous results [8], an elderly population was chosen. The applicability to other ages is unknown but a link between FA and inflammation has been indicated in younger subjects also [21,22]. Although the study population may seem small it appears to be representative for the whole cohort concerning several parameters (Table 1).

In this Swedish cohort of elderly subjects, SCD-1 index and palmitoleic acid correlated positively to CRP concentrations independently of obesity and lifestyle factors. The relations between CRP and other FAs as well as between E-selectin and FAs were also significant but attenuated after adjusting for covariates. Rather surprisingly, none of the other inflammatory or endothelial function markers were associated with FA composition. CRP may therefore be the preferred marker in future epidemiological studies investigating these relations. It cannot be excluded however that there may be significant associations between FA composition and these markers in larger populations also including younger subjects. In summary, our results suggest a potential role of saturated fat in systemic inflammation. It would therefore be of interest to investigate if inflammatory status may be affected by altering dietary fat quality. This study also indicates involvement of endogenous FA metabolism, i.e. SCD-1 activity, in low-grade inflammation. This needs further investigation in controlled trials with direct measures of desaturase activity.

Conflicts of interest

None.

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References


