Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents\textsuperscript{1–3}

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ABSTRACT

Background: Together with adiposity, plasma fatty acid (FA) composition can modulate the development of the metabolic syndrome (MS).

Objective: Our aim was to investigate the relations of FA composition in plasma phospholipids and cholesterol esters (CEs) with weight status, MS, and inflammation in adolescents.

Design: Plasma FA composition was measured by gas-liquid chromatography in 120 (60 normal-weight and 60 overweight) 12-y-old adolescents. We also measured the presence of MS, insulin resistance with the homeostasis model assessment, and interleukin 6 and C-reactive protein concentrations in the adolescents.

Results: MS was present in 25% of the overweight adolescents but in none of the normal-weight adolescents. Compared with overweight adolescents, normal-weight adolescents had lower saturated FAs (SFAs) in both phospholipids (P<0.001) and CEs (P<0.01) and higher docosahexaenoic acid in phospholipids (P<0.001). In overweight subjects, FA composition was associated with MS features independent of body fat. The odds ratios of MS for a 0.1 increase in the ratio of polyunsaturated FAs (PUFA) to SFAs (PUFA:SFA) were 0.91 in phospholipids (P=0.03) and 0.90 in CEs (P=0.06). In phospholipids, PUFA:SFA and linoleic acid were associated positively with HDL cholesterol (P<0.01 for both). PUFA:SFA in phospholipids and CEs were associated inversely with interleukin 6 (P<0.05 for both). Eicosapentaenoic acid in phospholipids (P=0.06) and CEs (P<0.05) and linolenic acid in CEs (P<0.05) were inversely related to C-reactive protein. These relations remained significant after adjustment for the waist-to-hip ratio. No significant relation between FA composition and the homeostasis model assessment was observed.


KEY WORDS Adolescents, plasma fatty acid composition, low-grade inflammation, metabolic syndrome, obesity

INTRODUCTION

Much evidence exists that the metabolic syndrome (MS), which affects adults but also affects a worrisome proportion of adolescents (1, 2), is closely connected to cardiovascular disease risk (3). MS is defined by the clustering of several metabolic abnormalities in the same person, mainly overweight status and more specifically abdominal adiposity, insulin resistance, dyslipidemia, and high blood pressure. Besides these factors, low-grade inflammation is increasingly viewed as a significant component of MS (4, 5). Different cytokines and chemical messengers, which induce their effects individually or in interaction with each other, constitute the main regulators of this inflammatory process. Among them, interleukin (IL) 6, a proinflammatory cytokine produced by different cells including adipose tissue, is overexpressed in adults with MS (6) and in obese adolescents (4, 7). These disorders are important because elevated IL-6 and C-reactive protein (CRP) concentrations are associated with a higher risk of developing type 2 diabetes and cardiovascular disease (8).

Although the association between adiposity and MS is well recognized, other factors, such as diet, are thought to be significant contributors. Epidemiologic and experimental studies conducted on adults have shown that dietary fat and plasma fatty acid (FA) composition, which is at least partly influenced by the diet ingested over the preceding several weeks (9, 10), are related to insulin sensitivity and several features of MS, such as blood pressure, plasma lipid profile (11, 12), and inflammation (13). Studies of diet or plasma FA composition conducted in children, mostly in obese children (14–16), focused mainly on lipid variables; none has examined low-grade inflammation. The study of children or young adolescents may furnish new insight into the early mechanisms that underlie the development of MS, because they are not confounded by the consequences of advanced metabolic disorders or atheromatous disease. These young persons are also free of several lifestyle confounders, such as smoking and alcohol consumption.

In the present study, we investigate the cross-sectional relations between the FA composition in both plasma phospholipids and cholesterol esters (CEs) and different features of MS as well as 2 markers of low-grade inflammation (IL-6 and CRP) in 60

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Received April 18, 2005.

Accepted for publication July 8, 2005.

normal-weight and 60 overweight adolescents who participated in the Intervention Centered on Adolescents’ Physical Activity and Sedentary Behavior Study (ICAPS). Our hypothesis was that plasma FA composition is associated with obesity and, more specifically, with MS. We also examined whether the contribution of plasma FA composition to components of MS is independent of adiposity and fat localization and whether it might modulate the relation between obesity and metabolic or inflammatory profiles.

SUBJECTS AND METHODS

Population

ICAPS is an ongoing randomized intervention study that began in 2002 and was aimed at encouraging physical activity in adolescents to reduce weight gain and cardiovascular disease risk (17). Briefly, 1048 adolescents aged 12 y from 8 public schools of the Department of the Bas-Rhin (eastern France) were eligible to participate. Of the eligible subjects, 91% participated and ≈66% agreed to provide a blood sample. The present study included a randomized subsample of 60 normal-weight (30 adolescent females and 30 adolescent males) and 60 overweight (30 adolescent females and 30 adolescent males) subjects. ICAPS was reviewed and approved by the local Ethics Committee and the schools’ governing bodies. Written consent to participate was obtained from all adolescents and their parents.

Anthropometric and medical measurements

An initial examination was conducted by trained professionals according to standardized methods (17). Height, weight, and waist and hip circumferences were measured, and the waist-to-hip ratio (WHR) was calculated. The percentage of body fat (BF) was evaluated by bioelectrical impedance (Tanita TBF-310, Tanita Corporation, Tokyo, Japan). Body mass index was calculated as weight (in kg)/height² (in m), and overweight was defined according to international references (18). Blood pressure and heart rate were measured in adolescents while they were seated and after a 5-min rest with a fully automatic blood pressure monitor (Omron M4-I; Omron Healthcare Inc, Bannockburn, IL) and an adapted cuff size. Self-assessed Tanner stages and plasma follicle-stimulating hormone concentrations were used to measure the sexual maturity of the adolescents. Physical activity was assessed with the Modifiable Activity Questionnaire for Adolescents (19). The practice of structured physical activity outside of school (yes or no) was taken into account.

Laboratory analysis and determination of the metabolic syndrome

The subjects were asked to fast for ≥10 h before blood sampling, which was performed with minimal venous stasis in the morning before the medical examination. All samples were processed within 3 h of sampling, and serum and plasma samples were divided into aliquots for immediate analysis or for long-term storage at −80 °C until assayed for IL-6 and CRP concentrations.

Plasma glucose concentrations were measured with the use of a glucose oxidase method with an intraassay CV <1.8%. Plasma HDL-cholesterol concentrations were analyzed with a cholesteryl esterase method (CV: 3–6%) in the supernatant fraction after precipitation of non-HDL with a magnesium-dextran precipitating reagent. Plasma triacylglycerol concentrations were measured with the use of a standard glycerol-blanked enzymatic triacylglycerol method (CV: 1.6%). Plasma insulin and follicle-stimulating hormone concentrations were measured by direct chemiluminescence immunoassays performed with an Advia-Centaur automatic analyzer (Bayer Diagnostics, Tarrytown, NY) that had a mean intraassay CV of 4.5% (for insulin) and 2.5% (for follicle-stimulating hormone). CRP was measured by an immunoassay with the Synchron-LX system (Beckman Coulter, Galway, Ireland) with inter- and intra-assay CVs of 7.5% and 5.0%, respectively. IL-6 was measured by an ultrasensitive enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) with a sensitivity of 0.039 pg/mL and with inter- and intra-assay CVs of 4.5% and 2.8%, respectively. Insulin resistance was estimated by the homeostasis model assessment (HOMA), which was calculated as insulin (in µU/mL) × glucose (in mmol/L)/22.5.

Because no standard definition exists for MS in children or adolescents, the subjects were classified as having MS if they met ≥3 criteria of the National Cholesterol Education Program Adult Treatment Panel III definition for MS (20) with adapted cutoffs, as was recently proposed (21). The cutoffs that were chosen to define the presence of the MS features were the following: >90th percentile for triacylglycerol concentrations, <5th percentile for HDL-cholesterol concentrations, >90th percentile for blood pressure measurements, >6.1 mmol/L for glucose concentrations, and >95th percentile for waist measurements. The percentiles for triacylglycerols, HDL cholesterol, and blood pressure were based on US age and sex reference curves (22, 23), and the percentiles for waist measurements were based on British reference curves (24).

Fatty acid composition of phospholipids

For FA analyses, lipids were extracted with a chloroform-methanol mix (1:1, by vol). The extract was washed with a saline solution to remove proteins. The chloroform phase was concentrated under a stream of nitrogen and redissolved in chloroform-methanol. The lipid classes were separated by thin-layer chromatography on silica gel plates (Merck KgaA, Darmstadt, Germany) with petroleum ether-ethyl ether-acetic acid (87:13:2) as the developing solvent. The plates were sprayed with bromophenol blue, and individual bands of phospholipids and CEs were scraped off into separate tubes. The phospholipid fraction was saponified and transesterified with sodium methylate, and the CE fraction was methylated with sulfuric acid and dehydrated methanol. The methyl esters of each fraction were removed with hexane and analyzed by gas-liquid chromatography on a Fison GC-8000 gas chromatograph (Thermo Separation Products, Les Ulis, France) equipped with a CP-SIL fused silica capillary column (25 m, 0.25 mm internal diameter) coated with 100% cyanopropyl siloxane 88 phase 0.2 μm (Chrompack, Les Ulis, France) with helium as the carrier gas and a split ratio of 1:20.

The identity of each FA peak was ascertained by comparison of the peak’s retention time with the retention times of synthetic standards with known FA composition. The relative amount of each FA (% of total FA) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids.
Fatty acid composition of plasma lipids, components of the metabolic syndrome, and low-grade inflammation

Weight status had a modifying effect on the relation between FA composition and the different features of MS. Whereas no significant relation was observed between plasma FA composition and any of the components of MS in normal-weight subjects, significant relations were observed in overweight subjects. The relation between plasma FA lipid composition and the components of MS in overweight adolescents with adjustment for sex, sexual maturity, physical activity, and BF are presented in Table 3. PUFA:SFA was associated positively with HDL-cholesterol concentrations in phospholipids and inversely with IL-6 in both the phospholipid (Figure 1) and the CE fractions. In phospholipids, but not in CEs, 18:2 n-6 PUFA was positively associated with HDL-cholesterol concentrations and tended to be inversely associated with triacylglycerol concentrations (P = 0.07). CRP concentrations were inversely related to linolenic acid (18:3n-3) in CEs and to eicosapentaenoic acid (20:5n-3) in CEs (P < 0.05) and marginally in phospholipids (P = 0.06). In contrast, insulin resistance, as measured by HOMA, was not significantly associated with FA composition. All the significant relations observed in overweight subjects remained significant after additional adjustment for WHR.
DISCUSSION

Our study confirms that the FA composition of plasma lipids, particularly SFA proportion, is associated with the weight status of otherwise healthy adolescents. It additionally indicates that the PUFA:SFA and the high long-chain PUFA concentrations are inversely related to MS and inflammation in overweight, but not in normal-weight adolescents, independent of body fat and fat distribution. More specifically, 18:2n–6 PUFA was positively associated with plasma HDL-cholesterol concentrations, whereas the PUFA:SFA and long-chain n–3 PUFA concentrations were inversely associated with IL-6 and CRP concentrations, 2 markers of low-grade inflammation. In contrast, no significant association was observed between FA composition and insulin resistance. The lower risk factor variances in

<table>
<thead>
<tr>
<th>Fatty acid type</th>
<th>HOMA&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Triacylglycerols&lt;sup&gt;2&lt;/sup&gt;</th>
<th>HDL cholesterol</th>
<th>Systolic blood pressure</th>
<th>IL-6</th>
<th>CRP&lt;sup&gt;2&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Phospholipids</td>
<td></td>
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<tr>
<td>Total SFA</td>
<td>−0.01 ± 0.03</td>
<td>−0.01 ± 0.02</td>
<td>−0.02 ± 0.01</td>
<td>0.36 ± 0.72</td>
<td>0.07 ± 0.03</td>
<td>0.01 ± 0.06</td>
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<tr>
<td>PUFA:SFA</td>
<td>0.16 ± 0.66</td>
<td>−0.03 ± 0.55</td>
<td>0.82 ± 0.33&lt;sup&gt;3&lt;/sup&gt;</td>
<td>−11.96 ± 17.33</td>
<td>1.63 ± 0.71</td>
<td>0.28 ± 1.42</td>
</tr>
<tr>
<td>18:2n–6</td>
<td>0.01 ± 0.02</td>
<td>−0.04 ± 0.02</td>
<td>0.04 ± 0.01&lt;sup&gt;4&lt;/sup&gt;</td>
<td>−0.20 ± 0.62</td>
<td>−0.03 ± 0.03</td>
<td>0.07 ± 0.05</td>
</tr>
<tr>
<td>18:3n–3</td>
<td>0.43 ± 0.49</td>
<td>−0.24 ± 0.42</td>
<td>0.24 ± 0.25</td>
<td>−20.91 ± 12.63</td>
<td>0.19 ± 0.56</td>
<td>−0.54 ± 1.06</td>
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<tr>
<td>20:5n–3</td>
<td>−0.32 ± 0.49</td>
<td>−0.03 ± 0.42</td>
<td>−0.08 ± 0.26</td>
<td>6.06 ± 13.08</td>
<td>−0.06 ± 0.57</td>
<td>−1.95 ± 1.04</td>
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<tr>
<td>22:6n–3</td>
<td>−0.08 ± 0.17</td>
<td>0.16 ± 0.14</td>
<td>0.02 ± 0.09</td>
<td>0.56 ± 4.48</td>
<td>−0.14 ± 0.19</td>
<td>−0.23 ± 0.37</td>
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<tr>
<td>Cholesterol esters</td>
<td></td>
<td></td>
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<tr>
<td>Total SFA</td>
<td>0.03 ± 0.05</td>
<td>−0.01 ± 0.04</td>
<td>−0.04 ± 0.03</td>
<td>1.00 ± 1.34</td>
<td>0.15 ± 0.05</td>
<td>−0.01 ± 0.11</td>
</tr>
<tr>
<td>PUFA:SFA</td>
<td>−0.04 ± 0.11</td>
<td>−0.03 ± 0.09</td>
<td>0.09 ± 0.05</td>
<td>−2.69 ± 2.90</td>
<td>−0.27 ± 0.12</td>
<td>0.04 ± 0.24</td>
</tr>
<tr>
<td>18:2n–6</td>
<td>0.001 ± 0.01</td>
<td>−0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>−0.39 ± 0.38</td>
<td>−0.01 ± 0.02</td>
<td>0.01 ± 0.03</td>
</tr>
<tr>
<td>18:3n–3</td>
<td>0.42 ± 0.52</td>
<td>−0.18 ± 0.45</td>
<td>0.17 ± 0.27</td>
<td>−7.74 ± 14.14</td>
<td>0.37 ± 0.58</td>
<td>−2.24 ± 1.10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:5n–3</td>
<td>−0.37 ± 0.75</td>
<td>−0.37 ± 0.63</td>
<td>−0.002 ± 0.390</td>
<td>3.20 ± 20.19</td>
<td>0.48 ± 0.83</td>
<td>−3.32 ± 1.57&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>22:6n–3</td>
<td>0.65 ± 1.16</td>
<td>1.27 ± 0.96</td>
<td>−0.38 ± 0.60</td>
<td>35.10 ± 30.96</td>
<td>−2.16 ± 1.26</td>
<td>−0.63 ± 2.53</td>
</tr>
</tbody>
</table>

<sup>1</sup> All values are β estimates ± SEs. HOMA, homeostasis model assessment; IL, interleukin; CRP, C-reactive protein; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

<sup>2</sup> Tested with the log-transformed values.

<sup>3</sup> General linear regression models were used with adjustment for sex, sexual maturity, physical activity, and body fat. P < 0.05. P < 0.01.
normal-weight than in overweight persons may partly explain why no significant relations were seen in the former. Our results may also indicate that overweight represents a necessary prerequisite that catalyzes the deleterious relations of FA composition, MS, and inflammation.

A positive association was reported between the dietary fat contribution to energy intake and the level of obesity. In accordance with previous studies in adults (25–27) and adolescents (14), higher SFA and lower n−3 PUFA concentrations were found in overweight subjects than in normal-weight adolescents in the present study. Although the increase in SFAs concerned mainly stearic acids (18:0) in our overweight adolescents with no MS, higher proportions of palmitic acids (16:0) were associated with higher palmitoleic acid (16:1n−7) concentrations (which reflect mostly endogenous desaturation of 16:0 FAs) in obese adults (26, 27) and in our adolescents with MS. Similarly, both n−3 PUFA and 18:2n−6 FA concentrations were low in obese adults (27) and in our overweight adolescents with MS, whereas n−3 PUFA concentrations, but not 18:2n−6 FA concentrations, were low in the overweight adolescents without MS. The finding of lower 22:6n−3 and total n−3 PUFA concentrations in our overweight subjects agree with the reduction in BF that was observed in healthy adults after substitution of visible fat with fish oil (28). Whether higher 16:0 and 16:1 FAs and lower 18:2n−6 PUFA concentrations are specifically associated with the risk of MS or only reflect a lower PUFA:SFA in overweight subjects with MS than in subjects without MS remains to be determined.

Significant relations have been reported between the type of dietary fat or plasma FA composition and several classic and nonclassic cardiovascular disease risk factors, which include components of inflammation in adults (11–13). In children, the FA profile of plasma lipids was related to the presence of MS in one study (14, 15). On the other hand, abdominal obesity has been shown to be a central component of MS and of the associated low-grade inflammation (29). Our results suggest that this excess in adipose tissue may be necessary to observe a synergistic influence of FA composition on MS and inflammation. We hypothesize an indirect amplifying effect via adipocyte products; conversely, because plasma FA composition is influenced not only by diet but also by de novo synthesis and transformation of exogenous FAs (12), one cannot exclude that the composition of plasma FAs is partly influenced by excess fat. Also, genetic determinants may interact with FA metabolism and contribute to both FA composition and some MS features (30, 31). However, in contrast with most of the studies conducted in adults, we ruled out that the relations observed here were the consequences of severe insulin resistance, evolutive atheromatous lesions, or chronic diseases. Because of the young age of our subjects, we can exclude the confounding effect of smoking or alcohol consumption. The relations were also independent of physical activity levels.

Our data showed no significant association between FA composition and insulin or HOMA. This contrasts with several studies in adults that related serum phospholipid SFAs to incident diabetes and to increased fasting and meal-induced insulinemia in healthy, obese, or diabetic adults (12, 32, 33). Insulin resistance has been attributed to changes in membrane composition and fluidity. A similar association with insulinemia has been found in obese children but was especially apparent postprandially (34). The relatively low concentrations and variability of insulin in our moderately obese subjects may explain why we did not see the same relations.

Different epidemiologic data have shown a weak negative relation between the proportions of dietary and plasma n−3 PUFA concentrations and plasma triacylglycerol concentrations, with a positive association with HDL-cholesterol concentrations (11, 12). Moreover, supplementation with long-chain n−3 PUFAs has been shown to be associated with a decrease in triacylglycerol concentrations in hypertriglyceridemic persons and in persons with type 2 diabetes. Differences in the dietary fat content (12) and the absence of established dyslipidemia or insulin-resistance in our adolescents may partly explain why we did not find such a relation. The relations between n−6 PUFA concentrations and plasma lipids are less consistent among studies, possibly because of the FAs studied. Note that the beneficial association found here between plasma lipid concentrations and 18:2n−6 PUFA concentrations was found only for the phospholipid fraction.

Supplementation with 18:3n−3 PUFAs has been shown to decrease CRP concentrations (35, 36) and also IL-6 concentrations in dyslipidemic patients (35). Moreover, supplementation with 20:5n−3 and 22:6n−3 PUFAs (37) resulted in a modulation of the phospholipid FA composition and reduced the production of tumor necrosis factor α and IL-6 by mononuclear cells, even at doses <1g/d. Conversely, palmitate (16:0) was shown to induce the expression of IL-6 in human myotubules (38). The
influence of n-6 PUFAs on inflammation is less consistent. Although arachidonic acid (20:4n-6 PUFAs) is a potent proinflammatory factor (30), 18:2n-6 PUFAs has been shown to inhibit the palmitate-induced IL-6 production (38). The present findings support a stimulation of IL-6 secretion by SFAs and an inhibition of low-grade inflammation by 18:2n-6 and n-3 PUFAs as early as adolescence. Because this relation was observed in overweight subjects only, we can speculate that this inhibition concerns, at least partly, the production and secretion of IL-6 by adipose tissue. It was shown that 10–35% of basal circulating human IL-6 derives from this tissue (39).

In conclusion, our findings suggest that plasma FA composition contributes to the heterogeneity that exists in the relations between BF and features of MS. A high ratio of PUFAs to SFAs, and more specifically a high intake of long-chain n-3 PUFAs, may protect obese persons against the development of metabolic complications as early as adolescence.

We thank the medical staffs for their participation, F. Goupilleau and Equipe d’Accueil 3072 for biochemical measurements of adipokines, and F. Ghazlane for her technical assistance.

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