Pro-atherogenic postprandial profile: Meal-induced changes of lipoprotein sub-fractions and inflammation markers in obese boys

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Abbreviations: MF, moderate fat; HF, high fat; Ox-LDL, oxidized LDL; IL-6, interleukine-6; IL-10, interleukine-10; TNF-alpha, tumor necrosis factor-alpha; CRP, C reactive protein; BMI, body mass index; AUC, area under the curve; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

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Abstract  Background and aims: Obesity is a pro-atherogenic condition and postprandial lipoprotein profile and circulating cytokines changes may contribute to promote the process. The aim of this study is to investigate postprandial metabolic response, lipoprotein oxidation and circulating cytokine levels, after the ingestion of two different meals with different fat/carbohydrate ratio.

Methods and results: Ten prepubertal obese boys consumed two meals with the same energy and protein content but with a different carbohydrate to fat ratio: 1) moderate fat (MF): 61% carbohydrate, 27% fat; 2) high fat (HF): 37% carbohydrate, 52% fat. The AUC of glucose and insulin were significantly \( p < 0.05 \) lower after the HF meal. HF meal was followed by a significant decrease in the cholesterol carried in the HDL fractions, while cholesterol in the small, dense LDL and in the VLDL particles increased, as compared to baseline \( p < 0.05 \) for all). No differences were found in the cholesterol distribution after the MF meal. Moreover, HDL-C concentration was lower \( p < 0.05 \) at 300 min after HF vs. MF meal. Oxidized LDL (ox-LDL) concentration increased after the HF meal but not after the MF meal [9.3(2.2) vs 1.8(2.2)% from baseline, \( P < 0.02 \)]. A positive association \( r > 0.3, P < 0.05 \) was observed between the densest LDL particles and the ox-LDL plasma levels. A reduction of IL-6 was found at 120 min after the MF \[ C_0 - 23.3(5.5) vs -8.4(3.8)% from baseline, P < 0.05 \] compared with the HF meal.
Conclusion: A simple change of ≈ 25% of energy load from fat to carbohydrate in a meal significantly improves postprandial pro-atherogenic factors in obese boys. © 2011 Elsevier B.V. All rights reserved.

Introduction

Postprandial lipoprotein profile affects the atherogenic process [1]. Postprandial lipemia is characterized by an increased and prolonged plasma concentration of triglyceride-rich very-low-density lipoprotein (VLDL) and their remnants, and qualitative changes in the low-density lipoprotein (LDL) particles. Inflammatory mechanisms and increased oxidative stress have been associated with the atherothrombotic risk linked to postprandial lipemia [2].

Fat intake and composition play a central role in modulating postprandial lipoprotein profile. In adults, a single meal high in saturated fat caused an immediate increase in triglycerides (TG), oxidative stress and inflammation, which promoted corresponding post meal worsening of endothelial dysfunction, vasoconstriction and systolic blood pressure [3].

Low grade inflammation is another contributing factor to atherosclerosis [4]. Adipose tissue of obese children secretes pro-inflammatory cytokines, especially in macrophage infiltrated areas [5]. The mechanisms to explain the development of the pro-inflammatory postprandial conditions are yet to be fully elucidated, although adipocytes, endothelial cells and leucocytes are all involved in the process [6].

As a significant increase in interleukine-6 (IL-6) four to eight hours after meal intake, greater in obese and insulin-resistant adults than in insulin-sensitive ones was reported [6,7]. No data are available in children.

The purpose of this study was to measure, in a group of obese children, postprandial metabolic response, lipoprotein oxidation and circulating cytokine changes after the ingestion of two different mixed meals with the same energy and protein content but with different fat/carbohydrate ratios: high fat/low carbohydrate and moderate fat/high carbohydrate, respectively.

Subjects and methods

Subjects

Ten prepubertal obese boys participated in the study (Table 1). Body composition was measured by total body dual-energy X-ray-absorptiometry. None of the boys had any overt condition other than obesity. Puberty development was clinically assessed on the basis of Tanner stages (Tanner Stage 1 for all of the subjects) [8]. Impaired glucose tolerance or type 2 diabetes were excluded by OGTT. Body mass index (BMI), using International Obesity Task Force BMI cut-offs as a reference, was used to define obesity [9]. Boys were at their maximal weight and none of them was dieting at the time of the study or was on any medication. Informed consent was obtained from the children and their parents. The protocol was approved by the Ethics Committee of the University Hospital of Verona.

Study design

The experiment was designed as a randomized, crossover study for repeated measures. We analyzed meal-induced changes in the profile of several metabolic parameters. Previously, we described thermogenic response, nutrient oxidation and gastrointestinal hormone changes induced by high fat (HF) versus moderate fat (MF) meals in obese boys [10]. In the present study, we analyzed the postprandial changes of lipoprotein sub-fractions and inflammation markers induced by the same meals in the same subjects.

Each boy arrived at the Department of Pediatrics at 8am on the day of the test. They had their last meal at 8pm the evening before. After baseline (preprandial) blood sample collection, the boys consumed the test meal (≤ 20 min). Blood samples were collected at 60, 90, 120, 150, 180, 240 and 300 min after meal ingestion.

One week after the first test meal the boys repeated the test with a different meal. The two meals were randomly assigned.

Dietary intakes

Two different meals were served and consumed on the two days of the experiment under medical supervision. They had the same energy and protein content but different carbohydrate to fat ratio: 1) a MF meal (61% carbohydrate, 27% fat); and 2) a HF meal (37% carbohydrate, 52% fat) (Table 2). The energy content was calculated as 25% of the estimated daily energy requirements for 10-year-old boys.

Table 1 Physical characteristics. Data are presented as median and interquartile range (IQR).

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>IQR</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>11.05</td>
<td>10.25–12.02</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>147.00</td>
<td>139.25–154.50</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.50</td>
<td>52.75–69.75</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.24</td>
<td>25.61–30.80</td>
</tr>
<tr>
<td>BMI z score</td>
<td>2.24</td>
<td>1.95–2.92</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>41.20</td>
<td>36.87–43.88</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>23.52</td>
<td>19.07–28.63</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>34.79</td>
<td>30.69–41.12</td>
</tr>
</tbody>
</table>
as reported [11]. The repeated measure design of the study did not require individualized meal loads. The energy and nutrient contents of the two meals were calculated using the National Institute of Nutrition tables for food composition [11]. The two meals had the same energy density (2.8 kcal/g) and similar fiber content.

**Biochemical analysis**

Plasma glucose, triglycerides (Wako Chemical GmbH) and cholesterol (CHOD-Pap, Roche/Hitachi) were evaluated by enzymatic methods. Plasma insulin was measured using a specific chemiluminescence method (Adaltis, Inc., Montreal, Canada). Serum collected at baseline, 120 min and 300 min after a meal was used to measure leptin (DBC, London, Canada), adiponectin (B-Bridge, Mountain View, CA), and C reactive protein (CRP) (Alpco, Salem, NH) concentrations by ELISA kit. IL-6, IL-10, TNF-alpha were quantified simultaneously by custom Searchlight chemiluminescent protein array kit by Aushon BioSystems (Billerica, MA).

**Density gradient ultracentrifugation (DGUC) for apo B-containing lipoproteins**

After creating a discontinuous salt density gradient in an ultracentrifuge tube (DGUC), the samples were centrifuged at 65,000 rpm for 90 min at 10 °C in a Sorvall TV-865B vertical rotor. Thirty-eight 0.45-ml fractions were then collected from the bottom of the centrifuge tube. Cholesterol and triglycerides were measured in each fraction. Each lipoprotein subclass elution range was defined as previously described [12].

**Oxidized LDL determination**

An enzymatic immunoassay, using the sandwich technique based on two monoclonal antibodies directed against different epitopes of the oxidized apolipoprotein B, was used [13].

**Statistical analysis**

All results are shown as mean and standard error of the mean (SEM) except when otherwise stated. The global responses of insulin and metabolites to meal ingestion were measured as net incremental area under the curve (AUC). A comparison of the mean values (and net incremental AUC) of the metabolic and inflammatory variables before and after the MF and HF meals was done by a Wilcoxon paired-sample test. Power analysis (two sided $\alpha = 0.05$) showed that the sample size ($n = 10$ subjects) was adequate ($1 - \beta > 0.80$) to detect (300 min after meal intake) a difference of ox-LDL of 7.1 U/L (estimated SD $\pm 7.1$). Association between lipoprotein subfractions, ox-LDL, cytokines and metabolite was calculated by Spearman correlation analysis. $P < 0.05$ indicated statistical significance. SPSS 16.0 software for windows (SPSS Inc., Chicago, IL, USA) was used.

**Results**

The physical characteristics of the subjects are shown in Table 1.

Blood glucose, TG and insulin increased after both meals. The AUC of glucose [107 (59) mmol · 300 min/L vs 202 (35) mmol · 300 min/L] and insulin [6014.7 (765.8) mU · 300 min/L vs 7922.5 (943.4) mU · 300 min/L] were significantly ($p < 0.05$) lower after the HF meal. The AUC of TG was significantly ($p < 0.05$) higher after the HF than after the MF meal [141.12 (30.26) mmol · 300 min/L vs 79.3 (23.8) mmol · 300 min/L].

Lipoprotein subclass cholesterol distribution across the density range was evaluated at baseline, 120 and 300 min after ingestion of the HF or MF meal. Earlier time points were not evaluated since the DGUC technique is not optimized for chylomicron detection and analysis. Differences

### Table 2: Energy and macronutrient composition of the test meals.

<table>
<thead>
<tr>
<th></th>
<th>Moderate fat meal</th>
<th>High fat meal</th>
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<tbody>
<tr>
<td></td>
<td>Proteins (12%)</td>
<td>Lipids (27%)</td>
</tr>
<tr>
<td>Pasta (100 g)</td>
<td>11</td>
<td>1.4</td>
</tr>
<tr>
<td>Olive oil (7 g)</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Grana Cheese (13 g)</td>
<td>4.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Ice Cream (40 g)</td>
<td>2</td>
<td>5.6</td>
</tr>
<tr>
<td>Nutrient (g)</td>
<td>17.4</td>
<td>17.7</td>
</tr>
<tr>
<td>Total Energy (kcal)</td>
<td>69.6</td>
<td>159.3</td>
</tr>
<tr>
<td>SFA: 7.8 g; PUFA 1.5 g; MUF 8.4 g; Cholesterol 31.27 mg.</td>
<td></td>
<td></td>
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<tr>
<td>SFA: C4-C10 ac. 2.5 g; Lauric ac. 0.18 g; Myristic ac. 0.71 g; Palmitic ac. 3.37 g; Stearic ac. 1.03 g; Arachidic ac. 0.03 g.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Behenic ac. 0.006 g.</td>
<td></td>
<td></td>
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<tr>
<td>PUFA: Linoleic ac. 1.34 g; α-Linolenic ac. 0.15 g.</td>
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<th>Moderate fat meal</th>
<th>High fat meal</th>
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<tr>
<td></td>
<td>Proteins (12%)</td>
<td>Lipids (51%)</td>
</tr>
<tr>
<td>Cheese-Burger (120 g)</td>
<td>15.8</td>
<td>13</td>
</tr>
<tr>
<td>French Fries (65 g)</td>
<td>2.4</td>
<td>9.7</td>
</tr>
<tr>
<td>Mayonnaise (14 g)</td>
<td>10.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Nutrient (g)</td>
<td>18.2</td>
<td>33.3</td>
</tr>
<tr>
<td>Total Energy (kcal)</td>
<td>72.8</td>
<td>299.7</td>
</tr>
<tr>
<td>SFA: 12.8 g; PUFA 8.3 g; MUF 12.1 g; Cholesterol 33.27 mg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA: C4-C10 ac. 0.48 g; Lauric ac. 0.14 g; Myristic ac. 1.03 g; Palmitic ac. 8.78 g; Stearic ac. 2.42 g; Arachidic ac. 0.04 g.</td>
<td></td>
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<tr>
<td>PUFA: Linoleic ac. 6.84 g; α-Linolenic ac. 1.31 g.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Where: SFA, saturated fatty acid, MUF, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.</td>
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</table>
between profiles were calculated by subtracting the post meal cholesterol value in each single fraction from the cholesterol value of the very same fraction at baseline. DGUC profiles were compared by calculating the mean and 95% confidence intervals of the difference between baseline and 300 min after HF meal for each fraction [14]. Baseline cholesterol profiles were similar before the HF and MF meals (Fig. 1, panels A and C). As compared to baseline, cholesterol distribution after 120 min was similar in the HDL-C and LDL-C density range after both MF and HF meals, while cholesterol was significantly and comparably higher in the VLDL-C particles (fractions # 36 and 37, *p* < 0.05 for both; data not shown). VLDL-C levels at 120 min time point were not significantly different after MF and HF meals. After 300 min (Fig. 1, panel B) post MF meal cholesterol distribution was similar to baseline, although we did observe a non significant trend toward decreased cholesterol in the HDL fractions and increased cholesterol in the VLDL fractions. On the contrary, 300 min after the HF meal, cholesterol was significantly reduced in the HDL fractions (fractions # 1–2), increased in the small, dense LDL particles (fractions # 10–12) and in the VLDL range (fractions # 36–37), versus baseline (Fig. 1, panels C and D). The increased cholesterol in fractions 11 and 12 (*p* = 0.046 and *p* = 0.038 respectively) and the reduced cholesterol in fractions 1 and 2 (HDL) remained significant (*p* = 0.041 and *p* = 0.036 respectively) after adjusting for changes in plasma TG. Moreover, at 300 min, cholesterol concentration in the HDL fractions (# 1–3) was significantly lower (*p* < 0.05) in the HF vs. the MF group (Fig. 2), with a trend, statistically significant only in fraction 9 (*p* = 0.041) and borderline not significant in fraction 10 (*p* = 0.058), higher cholesterol in the dense LDL sub-fractions (Fig. 2, panel B). Triglyceride distribution was similar at baseline while after HF meal there was a trend toward higher TG in the large VLDL particles (fraction# 36 and 37).

Ox-LDL concentrations were similar before the two meals and did not change significantly after the MF meal (Table 3). Following the HF meal, ox-LDL concentrations increased significantly vs. baseline [9.3(2.2) vs 1.8(2.2)%, *P* < 0.02]. Ox-LDL levels were significantly (*p* < 0.05) higher 300 min after the HF meal vs. the MF meal.

Both HF and MF meals induced a reduction of IL-6 below baseline levels at 120 min, followed by an increase at 300 min (Table 3). Reduction of circulating IL-6, expressed as a percent of baseline, was significantly higher after the MF than after the HF meal [−23.3(5.5) vs −8.4(3.8) % from baseline, *P* < 0.05]. In the interval 120–300 min after meal intake, no significant differences between the two meals were found. After both meals, IL-10, TNF-alpha, adiponectin and leptin did not change from baseline. No changes in CRP were observed after the HF or the MF meal.

**Correlation analysis**

Cholesterol levels in the LDL-C density range was associated with ox-LDL concentrations after the HF and MF meals. For this purpose, postprandial cholesterol in the corresponding fractions of the LDL-C density range (fractions 8–18) after both meals were pooled together. A significant positive

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**Figure 1** Cholesterol distribution profile before and after moderate fat (MF) and high fat (HF) meal. Panel A shows the cholesterol content of each lipoprotein subfraction at baseline (black circles) and 300 min after a MF meal (red triangles). Panel B shows the mean difference profile of cholesterol content of each lipoprotein subfraction after MF meal vs. baseline: values were obtained by subtracting cholesterol of each single fraction at baseline from the corresponding fraction after MF meal. DGUC profiles were compared by calculating the mean and 95% confidence intervals of the difference between baseline and 300 min after HF meal for each fraction. Panel C shows the cholesterol content of each lipoprotein subfraction at baseline (black circles) and 300 min after a HF meal (red triangles). Panel D shows the mean difference profile of cholesterol content of each lipoprotein subfraction after HF meal vs. baseline: values were obtained as explained for panel B and DGUC profiles were compared as reported in panel B. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
A moderate fat content (27% of total calories) is associated with a less atherogenic postprandial profile (a lower TG and ox-LDL concentration and greater reduction of IL-6 in the first postprandial phase) than a meal with the same energy and protein but with higher fat content (52%).

Our study is the first, to our knowledge, to show in a prepubertal population a transient increase in the cholesterol of small, dense LDL particles, specifically after a HF meal, associated with a significant increase in ox-LDL. Ox-LDL are key players at several stages of the atherothrombotic process, particularly at earlier stages of endothelial dysfunction [15], as is plausible to hypothesize in our young obese children. Small, dense LDLs are more susceptible to oxidation than native LDL-C particles [16], and the increased ox-LDL seen after HF meal is likely to be modulated by the observed trend toward an higher concentration of dense LDL particle after HF than MF meal. Our data support the association between plasma levels of dense LDL particles and ox-LDL. In an adult population, a non-fasting rise in plasma TG [17] and postprandial ox-LDL are strong predictors of the increased risk of severity and extent of coronary atherosclerosis, myocardial infarction, ischemic heart disease and death in men and women. Early stages of the atherothrombotic process, i.e. endothelial dysfunction, may be detected in obese children. Our study supports this finding by providing a plausible pathophysiological link between postprandial lipemia (increased TG, dense LDL-C, ox-LDL) which leads to a pro-atherogenic, pro-inflammatory state, and the initial steps of the atherothrombotic process on the arterial walls in young obese children.

PUFA intake with HF was higher than that with MF meal. Previous studies conducted in adults showed that PUFA intake was associated with a reduction of LDL-cholesterol [18]. However, our study demonstrated that a low fat intake, independently from the high PUFA content in the HF meal, was associated with a better postprandial lipid profile. Therefore, a high fat meal, also if PUFA content is high, seems less preferable than a low fat meal having the same energy and protein content.

The difference in fatty acid composition is unlikely a potential explanation of the different postprandial ox-LDL concentration after the two meals found in our study. In fact, in healthy adults, the comparison of three different emulsified lipid loads with different polyunsaturated/saturated (P/S) fatty acid ratio but identical lipid content revealed no difference in postprandial lipid response [19]. Moreover, in young healthy men, PUFA and SFA intakes had opposite effects on the expression of genes involved in liver X receptor signaling, that regulate the expression of multiple genes involved in the efflux, transport, and excretion of cholesterol. PUFA intake decreased whereas SFA intake increased the expression of genes in liver X receptor signaling and MUFA intake had an intermediate effect [20]. In our study, SFA load was pretty close in the two meals, whereas PUFA was higher in the HF meal than in the MF meal. Therefore, the liver X receptor expression should be decreased rather than increased after the HF meal. As a consequence, it is likely the quantity more than the composition of fat ingested that affects ox-LDL.

Interestingly, the high fat/low carbohydrate meal was followed by a higher increase of TG than the moderate fat/high carbohydrate meal. This finding disagrees with data association was observed between LDL-C in fractions 8, 9 and 10 and plasma levels of ox-LDL \( r = 0.29, r = 0.33, r = 0.29 \), respectively; \( P < 0.05 \). These fractions correspond to the densest LDL particles.

After the MF meal, IL-6 (percent change from baseline) was significantly correlated with the AUC of TG calculated in the 2 h after food intake \( r = -0.772, p < 0.01 \) but not with the AUC of glucose \( -0.36 \) or insulin \( -0.03 \). A correlation was also found between IL-6 (percent change between baseline and 120 min) and lipoprotein oxidation (percent of change from baseline) \( r = 0.66, p < 0.05 \).

**Discussion**

The results of this study, comparing two meals often part of the dietary habits of children, support the hypothesis that

![Figure 2](image-url)
reported in adults, showing lower TG postprandial levels after HF than MF diet [21]. This apparent discrepancy may be explained by insulin resistance. In fact, postprandial data reported in adults were obtained in obese or diabetic subjects who had a high level of insulin resistance, that affects substrate trafficking, promoting hypertriglyceridemia [22]. The children recruited in this study had a modest level of insulin resistance, as estimated by a HOMA-IR that averaged 1.88 (1.09) and TG levels into the normal values for children. Therefore, likely, high carbohydrate meal is accompanied by a greater reduction of IL-6, suggesting a link between postprandial fat metabolism and inflammation. Accordingly, after a high fat/low carbohydrate meal, which is followed by a significantly higher increase in TG than a MF meal, the reduction of IL-6 is almost undetectable. Therefore, postprandial triglycerides may be a pro-inflammatory co-factor also in obese prepubertal children, in agreement with analogous data obtained in adults [23].

Adipose tissue derived hormones such as adiponectin and leptin, could potentially be affected by meal composition. The results of this study showed that this is not the case: neither hormone significantly changed after both meals. Previous studies on adults failed to demonstrate a postprandial change in adiponectin concentrations [24], in agreement with our results; whereas other studies have reported that a high fat meal promoted an increase, a decrease or no change in plasma leptin concentrations [25]. The high variability of diurnal change of circulating leptin may contribute to explain the inconsistency of the results among studies.

A potential limitation of this study is ethnicity. In fact only Caucasian children have been recruited. We should be cautious to generalize these results to children of other ethnic groups. Another limit may be the lack of comparison with normal weight children, due to the Ethics Committee restrictions. However, the aim of the study was to compare different meals in obese children who may get the highest benefit from an healthy meal.
fact, prepubertal obese children are in their dynamic phase of weight gain, when most of the metabolic consequences of obesity are not evident. This is an advantage in comparison with studies performed in obese adults who have a far greater metabolic involvement.

In conclusion, as compared with an HF meal, a MF meal is followed by: 1) a slighter increase in triglyceride-rich lipoproteins; 2) a significantly lower reduction in HDL-C, and 3) a significantly lower concentration of ox-LDL particles, compatible with a trend toward lower concentration of small, dense LDL particles than a HF meal, in spite of modest changes in pro-inflammatory markers. This finding suggests that, for the same energy and protein intake, even a modest reduction of fat/carbohydrate ratio in the diet may be helpful to reduce atherogenic risk factors in obese children. Studies conducted in larger sample size are necessary to further investigate the effect of fat intake on atherogenic lipid profile in children.

Conflict of interest

There aren’t financial or other relationships that might lead to a conflict of interest for any of the authors.

Aknowledgements

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References