Butter and walnuts, but not olive oil, elicit postprandial activation of nuclear transcription factor κB in peripheral blood mononuclear cells from healthy men1–3

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ABSTRACT
Background: Nuclear transcription factor κB (NF-κB) plays an important role in atherosclerosis by modulating gene expression. Postprandial lipemia has been correlated with an increase in NF-κB activation in vascular cells and it is associated with an increase in postprandial triacylglycerol-rich lipoproteins, which are involved in the development of atherosclerotic plaque.

Objective: The objective of this study was to determine the effect of the intakes of 3 different foods with different fat compositions on the postprandial activation of monocyte NF-κB.

Design: Eight healthy men followed a 4-wk baseline diet and then consumed 3 fat-load meals consisting of 1 g fat/kg body wt (65% fat) according to a randomized crossover design. Each meal had a different fatty acid composition, and the consumption of each meal was separated by 1 wk. The compositions of the 3 test meals were as follows: olive oil meal (22% saturated fatty acids (SFAs), 38% monounsaturated fatty acids (MUFAs), 4% polyunsaturated fatty acids (PUFAs), and 0.7% α-linolenic acid), butter meal (38% SFAs, 22% MUFAs, 4% PUFAs, and 0.7% α-linolenic acid), and walnut meal (20% SFAs, 24% MUFAs, 16% PUFAs, and 4% α-linolenic acid).

Results: Ingestion of the olive oil meal did not elicit NF-κB activation compared with ingestion of either the butter meal at 3 h (P < 0.05) or the walnut meal at 9 h (P < 0.05). There was no significant difference in the postprandial triacylglycerol response between the 3 meals.


KEY WORDS Diet, blood cells, nuclear transcription factor, NF-κB, butter, walnuts, olive oil, postprandial lipemia

INTRODUCTION
Epidemiologic evidence indicates that the Mediterranean diet, in which olive oil is the principal source of fat, reduces the risk of coronary heart disease (CHD) (1). Data from controlled clinical studies have shown that monounsaturated fatty acid (MUFA) intake favorably affects many risk factors related to the development of CHD. Compared with saturated fatty acids (SFAs), MUFAs lower plasma total and LDL-cholesterol concentrations, increase HDL-cholesterol concentrations, and decrease total plasma triacylglycerol concentrations (2, 3). Previous studies have shown that the lipid composition of plasma and tissues is closely related to dietary fat intake (4, 5).

In vitro studies have recently suggested that olive oil and red wine antioxidant polyphenols, at nutritional concentrations, transcriptionally inhibit endothelial adhesion molecule expression. Furthermore, the contribution of nuclear factor κB (NF-κB) in this process has also been shown (6). NF-κB is a redox-sensitive transcription factor composed of members of the Rel family; it is found in the cytosol as a trimer consisting of p50, p65, and IkB. Several stimuli activate NF-κB through the phosphorylation of the IkB subunit and subsequent release of IkB from the trimer, which results in the translocation of the p50/p65 heterodimers from the cytoplasm into the nucleus, where they bind to target genes and stimulate transcription (7). This process activates genes involved in immune and inflammatory responses, such as interleukins, growth factors, and cytokines (8). NF-κB is rapidly activated in response to a variety of inflammatory and other stimuli, such as tumor necrosis factor α, oxidized LDL, oxidant stress, interleukin 1, hyperglycemia, and platelet activation (9). A previous study (10) showed that the consumption of a fat-enriched breakfast increased NF-κB activation in a time-dependent manner in peripheral blood mononuclear cells (PBMCs) in humans. In addition, in this study it was observed that the simultaneous consumption of red wine avoided the activation of NF-κB.

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Postprandial lipemia is associated with the induction of inflammatory genes, and increases in postprandial triacylglycerol-rich lipoproteins (TRLs) are involved in the development of atherosclerotic plaque (11). Our aim is to determine whether the consumption of 3 foods with different fat contents has a selective influence on the postprandial activation of NF-κB in the PBMCs of healthy volunteers.

SUBJECTS AND METHODS

Subjects

Eight male medical students were included in this study, all of whom gave informed consent and underwent a comprehensive medical history, physical examination, and clinical chemistry analysis before enrollment. Subjects showed no signs of any chronic disease or obesity, and none had unusually high levels of physical activity. None of the subjects were taking medications or vitamins known to affect plasma lipids. The study protocol was approved by the Human Investigation Review Committee of the Hospital Universitario Reina Sofia.

Study design

Each subject, after 4 wk of consuming a typically Western-style stabilization basal diet rich in saturated fat (fat 38% with: 16% SFAs, 16% MUFAs, and 6% PUFA; 15% proteins and 47% carbohydrates), underwent 3 postprandial lipemia studies in which they consumed meals with the same fat content (1 g fat/kg body wt, 7 mg cholesterol/kg, and 40 retinol equivalents/kg body wt) but with different fatty acid compositions following a random administration order. After fasting for 12 h, at time 0, the subjects were provided a fat meal consisting of 50–66% of the subjects’ body wt, 7 mg cholesterol/kg, and 40 retinol equivalents/kg body wt, but with different fatty acid compositions following a random administration order. After fasting for 12 h, at time 0, the subjects were provided a fat meal consisting of 50–66% of the subjects’ body wt, 7 mg cholesterol/kg, and 40 retinol equivalents/kg body wt, but with different fatty acid compositions following a random administration order.

Lipid analysis and isolation of large triacylglycerol-rich lipoproteins

Plasma was obtained by low-speed centrifugation at 1500 × g for 15 min at 4 °C. To reduce interassay variation, plasma was stored at −80 °C and analyzed at the end of the study. Cholesterol and triacylglycerols in plasma and lipoprotein fractions were measured with the use of enzymatic procedures (12, 13). The large TRLs (Sf > 400) were isolated from 4 mL plasma overlayed with 0.15 mol NaCl/L and 1 mmol EDTA/L (pH 7.4, density < 1.006 kg/L) with a single ultracentrifugal spin (20 000 rpm, 30 min, 4 °C) in a type 50 rotor (Beckman Instruments, Fullerton, CA). Large TRLs, contained in the top layer, were removed by aspiration after cutting the tubes. Large TRL fractions were stored at −70 °C.

Fatty acid composition of large triacylglycerol-rich lipoproteins

The analysis of fatty acids was performed by gas–liquid chromatography. Lipids were transmethylated as previously described (14). The resulting fatty acid methyl esters were analyzed by gas–liquid chromatography with a model 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector and a capillary silica column (Supelcowax 10; Supelco, Bellefonte, PA) 60 m in length and with an internal diameter of 0.25 mm; hydrogen was used as the carrier gas.

Isolation of PBMCs

The blood samples were diluted 1:1 in phosphate-buffered saline, and cells were separated in 5 mL Ficoll gradient (lymphocyte isolation solution; Rafer, Zaragoza, Spain) by centrifugation at 2000 × g for 30 min. PBMCs were collected, washed twice with cold phosphate-buffered saline, and resuspended in buffer A (see Protein Extraction); ≈95% of the cells were mononuclear cells (flow cytometry; data not shown).

Protein extraction and electrophoretic mobility shift assays

Proteins of PBMCs were extracted as described by Hernández-Presa et al (15). Gel shift assays were performed with a commercial kit according to the instructions of the manufacturer (Promega, Madison, WI). Briefly, NF-κB consensus oligonucleotide (5'-AGTTGAGGGGACTTTCCCAGGC-3') was end-labeled with 32P by incubation with 10 U T4 polynucleotide kinase (Promega) in a reaction containing 10 μCi [γ-32P]ATP (3000 Ci/mmol) (Amersham Biosciences, Buckinghamshire, United Kingdom), 70 mmol Tris-HCl/L, 10 mmol MgCl2/L, and 5 mmol DTT/L. The gel was dried and exposed to an X-ray film. The autoradiograph was subjected to densitometry with an Image Quant densitometric scanner (Molecular Dynamics; Amersham Biosciences). Data values were calculated in relation to the basal value in each subject and situation.

Intracellular adhesion molecule 1 immunoassay

Plasma concentrations of soluble intracellular adhesion molecule 1 (sICAM-1) were determined in duplicate with commercially available enzyme-linked immunosorbent assay kits (R&D systems); 15-μL plasma samples were assayed in parallel to known standard concentrations. Each assay was calibrated with an sICAM-1 standard curve. The minimum detectable level of sICAM-1 was 0.35 ng/mL. Intraassay and interassay CVs were 5.4% and 6.2%, respectively.

Statistical analysis

Statistical analyses were carried out with SPSS statistical software (version 8.0; SPSS Inc, Chicago). Analysis of variance (ANOVA) for repeated measures was used to analyze the differences in plasma lipid concentrations and NF-κB activation. When statistically significant effects were found, Tukey’s post hoc comparison test was used to identify group differences. Analysis of covariance (ANCOVA) was used to analyze the sICAM-1 data, with the difference between time 0 and time 9 as
RESULTS

Postprandial lipemia

The lipid profile showed an increase in total triacylglycerols and large TRLs at 3 h \((P < 0.001)\), but there were no significant differences between the postprandial responses after the intake of the 3 meals (Figure 1). No significant differences in the other lipid variables studied (total LDL and HDL cholesterol; data not shown) between the 3 meals were shown.

Fatty acid composition of postprandial large triacylglycerol-rich lipoproteins

No significant differences in the fatty acid composition of large TRLs were found at baseline. Palmitic acid (16:0) acid during the butter meal was significantly higher \((P < 0.001)\) than during the olive oil and walnut meals. Linoleic (18:2) and linolenic (18:3) acids were highest during the walnut meal \((P < 0.001; \text{Table 1})\).

Diet intake and NF-κB activation

To examine the effects of meal intake on NF-κB activation, blood samples were taken before and 3, 6, and 9 h after the meals. As shown in Figure 2, olive oil meal intake did not elicit NF-κB activation, compared with either the ingestion of the butter meal at 3 h \((P < 0.05)\) or with the walnut meal at 9 h \((P < 0.05)\).

Plasma soluble intracellular adhesion molecule 1 concentrations

Plasma sICAM-1 concentrations at 0 and 9 h are shown in Figure 3. An increase was found after the ingestion of the butter-enriched meal.

DISCUSSION

Our results showed that the acute intake of an olive oil meal did not elicit postprandial activation of NF-κB compared with the butter and walnut meals in PBMCs. Also, plasma sICAM-1 concentrations increased after the ingestion of a butter-enriched meal.

TABLE 1

<table>
<thead>
<tr>
<th>Time and meal</th>
<th>Fatty acid</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h (% of total fatty acids)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td></td>
<td>16.55 ± 1.7</td>
<td>9.61 ± 0.8</td>
<td>23.10 ± 10.4</td>
<td>8.00 ± 2.6</td>
<td>0.61 ± 0.1</td>
</tr>
<tr>
<td>Olive oil</td>
<td></td>
<td>15.72 ± 2.8</td>
<td>8.78 ± 2.5</td>
<td>25.91 ± 18.3</td>
<td>9.19 ± 4.9</td>
<td>0.58 ± 0.1</td>
</tr>
<tr>
<td>Walnuts</td>
<td></td>
<td>17.54 ± 3.1</td>
<td>9.68 ± 2.1</td>
<td>19.69 ± 5.5</td>
<td>8.20 ± 2.2</td>
<td>0.55 ± 0.1</td>
</tr>
<tr>
<td>3 h (% of total fatty acids)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td></td>
<td>23.82 ± 2.3a</td>
<td>9.57 ± 1.3</td>
<td>23.85 ± 4.3</td>
<td>8.47 ± 1.4b</td>
<td>0.58 ± 0.0b</td>
</tr>
<tr>
<td>Olive oil</td>
<td></td>
<td>14.55 ± 1.6b</td>
<td>6.91 ± 0.9</td>
<td>37.17 ± 7.5</td>
<td>9.96 ± 1.8b</td>
<td>0.79 ± 0.0b</td>
</tr>
<tr>
<td>Walnuts</td>
<td></td>
<td>16.04 ± 2.3b</td>
<td>8.16 ± 1.8</td>
<td>19.55 ± 3.6</td>
<td>23.51 ± 2.9b</td>
<td>3.91 ± 0.7a</td>
</tr>
<tr>
<td>Global analysis P values</td>
<td>0.002</td>
<td>0.062</td>
<td>0.013</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

\(^1 \text{n} = 8. \text{Means in a column with different superscript letters are significantly different, } P < 0.05 \text{ (ANOVA followed by post hoc Tukey’s test).} \)

\(^2 \bar{x} \pm \text{SD (all such values).} \)
NF-κB may play an important role in atherosclerosis, and activated NF-κB has been identified in human atherosclerotic plaques. Brand et al. (16) detected its presence in smooth muscle cells, macrophages, and endothelial cells of atherosclerotic lesions, but it was absent or scarce in healthy vessels. A recent study showed that the consumption of a fat-enriched breakfast increased NF-κB activation in a time-dependent manner in PBMCs in humans, and the simultaneous consumption of red wine prevented this effect despite the postprandial increment in plasma chylomicrons and total triacylglycerol concentrations, which suggests that the antioxidant compounds of the red wine could modulate this process (6). Similar findings were observed by Mekki et al. (17), but they did not find any differences between monounsaturated and polyunsaturated fats, probably because the source of polyunsaturated fat was sunflower, not walnuts as in the current study.

NF-κB is a redox-sensitive transcription factor (7); therefore, agents that diminish oxidant stress may stabilize the NF-κB system, which would attenuate atherosclerotic lesion formation. Hennig et al. (18) exposed porcine endothelial cells to 18-carbon fatty acids. Both linoleic and stearic fatty acids activated endothelial cells more markedly than did either oleic or linolenic fatty acids. Also, compared with control cultures, treatment with stearic and linoleic acids decreased glutathione concentrations, which suggested an increase in cellular oxidative stress. This increase in oxidative stress with the subsequent activation of NF-κB could be one of the mechanisms of the inflammatory properties of 18:0 and 18:2. In our postprandial study, we observed that the intake of an olive oil meal diet did not induce the postprandial activation of NF-κB, which appeared during the butter and walnut meal intakes. These findings suggest that olive oil–enriched meals could prevent the activation of the NF-κB system, and this effect could be associated with either the intake of MUFAs or the protective influence of the antioxidant components contained in virgin olive oil. In conclusion, this study suggests that the cardioprotective effect of MUFAs could be motivated in part by their protective effect on NF-κB activation.

FIGURE 2. Nuclear transcription factor κB (NF-κB) activation in peripheral blood mononuclear cells obtained at 0, 3, 6, and 9 h of fat intake from the 3 meals studied (butter, olive oil, and walnuts). A: Electrophoretic mobility shift assay of nuclear proteins from peripheral blood mononuclear cells. B: Mean (±SEM) NF-κB activity in peripheral blood mononuclear cells determined by densitometer quantification. n = 8. Two-factor repeated-measures ANOVA: diet effect, P = 0.081; time effect, P = 0.050; diet × time interaction, P = 0.013. *Significantly different from olive oil, P < 0.05 (Tukey’s test).

FIGURE 3. Mean (±SEM) plasma concentrations of soluble intracellular adhesion molecule 1 (sICAM-1) at 0 and 9 h of fat intake from 3 meals (butter, olive oil, and walnuts). n = 8. Analysis of covariance for the difference between time 0 and time 9 with time 0 as covariate: F for corrected model = 0.009. *Significantly different from butter at 9 h, P < 0.05 (Tukey’s test).

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CB, JL-M, JE, and FP-J were responsible for the conception and design of the study. PP-M, FF, CM, PG, and CB were responsible for the provision of study materials or subjects. CB, LMB-C, JLM-V, JL-M, FJM, and JE were responsible for the collection and assembly of data. CB, PP-M, JL-M, LMB-C, JLM-V, and FP-J were responsible for the analysis and interpretation of the data. JL-M, PG, CM, FF, and FP-J provided statistical expertise. CB, PP-M, JL-M, and FP-J were responsible for drafting the manuscript. JE, LMB-C, JLM-V, JL-M, and FP-J were responsible for the critical review of the manuscript for important intellectual content. JE, JL-M, and FP-J obtained funding. None of the authors had any conflict of interest.
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