Atherosclerosis 204 (2009) e70–e76

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Olive oil and walnut breakfasts reduce the postprandial inflammatory response in mononuclear cells compared with a butter breakfast in healthy men

Yolanda Jiménez-Gómez[a], José López-Miranda[a,*], Luis M. Blanco-Colio[b], Carmen Marín[a], Pablo Pérez-Martínez[a], Juan Ruano[a], Juan A. Paniagua[a], Fernando Rodríguez[c], Jesús Egido[b], Francisco Pérez-Jiménez[a]

[a] *The Lipids and Arteriosclerosis Unit, Reina Sofía University Hospital, University of Córdoba, CIBER de Fisiopatologia de la Obesidad y Nutricion (CIBEROBN), Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain*
[b] *The Vascular Research Laboratory, Jiménez Díaz Foundation, Autonomous University of Madrid, Spain*
[c] *The Clinical Analyses Service, Reina Sofía University Hospital, Spain*

ARTICLE INFO

Article history:
Received 17 August 2007
Received in revised form 4 August 2008
Accepted 9 September 2008
Available online 17 September 2008

Keywords:
Breakfast
Mononuclear cells
Proinflammatory cytokines
Fatty acids
Postprandial lipemia

ABSTRACT

Background: Inflammation is crucial in all stages of atherosclerosis, and few studies have investigated the effect of dietary fat on markers of inflammation related to this disease during the postprandial period.
Objective: To evaluate the chronic effects of dietary fat on the postprandial expression of proinflammatory genes in peripheral blood mononuclear cells (PBMCs) in healthy subjects.
Design: 20 healthy men followed three different diets for 4 weeks each, according to a randomized crossover design: Western diet: 15% protein, 47% carbohydrates (CHO), 38% fat (22% saturated fatty acid (SFA)); Mediterranean diet: 15% protein, 47% CHO, 38% fat (24% monounsaturated fatty acid (MUFA)); CHO-rich and n-3 diet: 15% protein, 55% CHO, <30% fat (8% polyunsaturated fatty acid (PUFA)). After 12-h fast, volunteers were given a breakfast with a fat composition similar to that consumed in each of the diets—butter breakfast: 35% SFA; olive oil breakfast: 36% MUFA; walnut breakfast: 16% PUFA, 4% α-linolenic acid (LNA).
Results: The butter breakfast induced a higher increase in tumor necrosis factor (TNF)-α messenger RNA (mRNA) expression than the olive oil or walnut breakfasts ($P = 0.014$) in PBMCs. Moreover, we found a higher postprandial response in the mRNA of interleukin (IL)-6 with the intake of butter and olive oil breakfasts than with the walnut breakfast ($P = 0.025$) in these cells. However, the effects of the three fatty breakfasts on the plasma concentrations of these proinflammatory parameters showed no significant differences (P = N.S.).
Conclusion: Consumption of a butter-enriched meal elicits greater postprandial expression of proinflammatory cytokine mRNA in PBMCs, compared to the olive oil and walnut breakfasts.

1. Introduction

Atherosclerosis is the major cause of death in western societies [1] and there is evidence that inflammation plays a central role in all phases of the atherosclerotic process [2]. A first step in this condition is the adhesion of circulating monocytes to the endothelium and its migration to the intima layer [3]. A crucial chemokine responsible for the recruitment of monocytes to inflammatory lesions in the vasculature is monocyte chemoattractant protein-1 (MCP-1) [4,5]. This chemokine is highly expressed in the macrophage-rich area of the atherosclerotic lesions in human [6] and its expression and secretion from vascular cells have been proved to account for the increased monocyte chemotatic activity [7]. Once monocytes have reached the subendothelial space, the modified LDL and various molecules produced by the T lymphocytes, endothelial cells and smooth muscle cells stimulate the transformation of the monocytes into macrophages, which are important mediators of inflammation. In the final stage, the plaque is ruptured in the shoulder [8], area more vulnerable, which is enriched in T lymphocytes and macrophages.

The diet, and particularly its fat content, can modulate the cardiovascular risk factors and the mechanisms related to the formation and development of the atheroma plaques [9,10]. However, the influence of the diet on atherosclerosis goes beyond its known effects on the classic cardiovascular risk factors [9]. Fatty acids and other components of the diet modulate the expression of several

* Corresponding author. Tel.: +34 957 218250; fax: +34 957 218250.
E-mail address: md1lomij@uco.es (J. López-Miranda).

0021-9150/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2008.09.011

genes involved in the inflammatory and immune response, such as proinflammatory cytokines, adhesion molecules, chemokines and inflammatory enzymes [11–13].

Changes in postprandial metabolism take place every time we eat a meal and alterations in this state may play an important role in the development of cardiovascular and associated diseases [14–16]. During postprandial lipemia, there is an increase in circulating triacylglycerol-rich lipoproteins (TRL), which may be deposited into the arterial wall and accumulated in atheromatous plaques [16], formation of highly oxidisable small, dense LDL and a reduction in the concentration of HDL [17]. Furthermore, it has been found that during this phase, when triacylglycerols (TG) and glucose rise, the neutrophil count increases with the subsequent production of proinflammatory cytokines and oxidative stress, with these changes possibly contributing to endothelial dysfunction [18,19]. Moreover, van Oostrom et al. [20] provided evidence that postprandial triglyceridemia is related to the proinflammatory state due to the high expression of the activation markers in neutrophils and monocytes. Our group has also shown that butter and walnuts, but not olive oil, elicit postprandial activation of nuclear factor-κB (NF-κB) in PBMCs in healthy men [21]. Since human beings spend much of the day in the postprandial state it is important to understand the inflammatory changes that take place during this period in terms of the type of fat consumed. Our aim was therefore to evaluate the chronic effect of the type of fat on the postprandial expression of proinflammatory genes in PBMCs from healthy men. Because apolipoprotein (apo)E is an important mediator of the clearance of circulating TRL by receptor [22] and the apoE E2/E3/E4 polymorphism is implicated in a variable lipid postprandial response [23], we realized the study in subjects with the apoE3/E3 genotype, the most common allele in the population.

2. Methods

2.1. Study subjects

Twenty male medical students all gave informed consent and underwent a comprehensive medical history, physical examination and clinical chemistry analysis before enrolment. None of the subjects showed signs of any chronic disease or obesity, and none practiced unusually high levels of physical activity. The volunteers had normal biochemical parameters. They were selected on the basis of having the apoE3/E3 genotype, in order to avoid the allele effects of this gene locus on postprandial lipemia [23]. None was taking medications or vitamins known to affect plasma lipids. The study protocol was approved by the Human Investigation Review Committee of the Reina Sofía University Hospital, according to Institutional and Good Clinical Practice guidelines.

2.2. Study design

Each volunteer in the trial was subjected to three diet intervention periods of 4 weeks of duration, in a randomized crossover design. The composition of three diets is shown in Table 1. The PUFA enrichment of the high-CHO diet was achieved via the use of natural food components rich in α-LNA of vegetable origin (based on walnuts (*Juglands regia* L.)). The cholesterol content of the diets was <300 mg/day, and it was kept at a constant level throughout the three dietary intervention periods.

The composition of the experimental diets was calculated using the United States Department of Agriculture [24] food tables and Spanish food composition tables for local foodstuffs [25]. All meals were prepared in the hospital kitchen and were supervised by a dietitian. Lunch and dinner were eaten in the hospital dining room, whereas breakfast and an afternoon snack were eaten in the medical school cafeteria. Fourteen menus were prepared with regular solid foods and rotated during the experimental period. Duplicate samples from each menu were collected, homogenized and stored at −70 °C. The study took place during January and March to minimize seasonal effects and academic stress. Subjects were encouraged to maintain their regular physical activity and lifestyle and were asked to record in a diary any event that could affect the outcome of the study, such as stress, changes in smoking habits and alcohol consumption or intake of foods not included in the experimental design.

At the end of the dietary intervention period and after a 12-h fast, at time 0, the subjects were given a fatty breakfast with a fat composition similar to that consumed in each of the diets, consisting of 50–66% of the subject's daily normal intake of calories and composed of 1 g fat, 7 mg cholesterol and 40 equiv. retinal/kg body weight, with the following caloric distribution: 60% fat, 15% protein, and 25% CHO. The composition of three breakfasts is shown in Table 1. The butter breakfast was based on the consumption of butter, wholemeal bread, hard-boiled egg and whole milk. The olive oil breakfast was administered in the form of a typical Mediterranean food with extra virgin olive oil, bread and tomato, accompanied by skimmed milk and hard-boiled egg. The walnut breakfast consisted of walnuts (*Junglans regia* L.), wholemeal bread, jam and skimmed milk.

The amount of each ingredient was calculated as a function of individual body weight so that all subjects consumed the same type of food at different amounts.

2.3. DNA amplification and genotyping of apoE

DNA was extracted from 10 mL blood containing EDTA. A region of 266-bp of the apoE gene was amplified by PCR with 250 ng of genomic DNA and 0.2 mmol of each oligonucleotide primer (E1, 5′-GAACAACTGACCCCGGTGGCGGAG-3′, and E2, 5′-TCGCGGGCCCCGGCCTGGTACACTGCCA-3′) and 10% dimethyl sulfoxide in 50 μL. DNA was denatured at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 63 °C for 1.5 min, and extension at 72 °C for 2 min. The 20 μL of the PCR product were digested with 10 units of restriction enzyme CfoI (BRL, MD, U.S.A.) in a total volume of 35 μL. Digested DNA was separated

Table 1
Diet and breakfast composition (% of energy intake)

	Western diet rich in SFA	Mediterranean diet enriched in virgin olive oil	High-CHO enriched in vegetal n-3 fatty acids	Butter breakfast	Olive oil breakfast	Walnut breakfast
Protein	15	15	15	15	15	15
CHO	47	47	55	25	25	25
Fat	38	38	<30	60	60	60
SFA	22	<10	<10	35	20	20
MUFA	12	24	12	22	36	24
PUFA	4	4	8	4	4	16
α-LNA	0.4	0.4	2	0.7	0.7	4

CHO: carbohydrates; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; α-LNA: α-linolenic acid.

by electrophoresis on an 8% non-denaturing polyacrylamide gel at 150 V for 2 h. Bands were visualized by silver staining.

2.4. Lipid analysis

Venous blood samples were collected in tubes containing 1 mg/mL EDTA in fasting, at time 0, and every 3 h until the 9th hour after the ingestion of the breakfasts. Plasma was obtained by low-speed centrifugation (1500 × *g*) for 15 min at 4 °C within 1 h of venipuncture. In order to reduce interassay variation, plasma samples were stored at −80 °C and analyzed at the end of the study. Lipid parameters were assessed with a DDPPII Hitachi modular analyzer (Roche, Basel, Switzerland), using specific reagents (Boehringer-Mannheim, Mannheim, Germany). Total plasma cholesterol (C) and TG concentrations and lipoprotein fractions were measured by colorimetric enzymatic techniques [26,27]. HDL–C levels were measured using colorimetric assay after precipitating the lipoproteins containing apoB with polyethylene glycol [28]. LDL–C concentrations were calculated by using the Friedewald formula based on the C, TG, and HDL–C values [29].

2.5. Plasma fatty acid composition

Plasma lipids were first methylated and an aliquot of fatty acid methyl esters was analyzed by gas chromatograph (Hewlett-Packard 5890; series II) equipped with a flame ionization detector and a SP-2380 (Sulpeco, Bellefonte, PA, U.S.A.) fused silica capillary column (60 m in length and with an internal diameter of 0.25 mm) coated with cyanopropylpolysiloxane (0.20 μm film thickness). The oven temperature program was isothermal at 160 °C for 8 min before rising to 220 °C at a rate of 2 °C/min. The temperature was kept at 220 °C for 12 min. Hydrogen was used as carrier gas at a column-head pressure of 20 psi. The injector and detector temperatures were 210 and 250 °C, respectively. Sample injections were performed in the split mode.

2.6. Adhesion molecules immunoassay

Plasma concentration of MCP-1, IL-6 and TNF-α were determined in duplicate with commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Inc.) according to the manufactures guidelines.

2.7. Isolation of PBMCs

Blood samples were diluted 1:1 in PBS, and cells were separated in 5 mL Ficoll gradient (lymphocyte isolation solution, Rafer, Zaragoza, Spain) by centrifugation at 2000 × *g* for 30 min at 4 °C. PBMCs were collected, washed twice with cold PBS, and resuspended in TRIZOL (Tri® Reagent, Sigma, St. Louis, MO). Approximately 95% of the cells were mononuclear cells (flow cytometer, data not shown).

2.8. Total RNA isolation and real-time RT-PCR

Total cellular RNA from PBMCs was extracted using the trizol method according to the recommendations of the manufacturer (Tri® Reagent, Sigma, St. Louis, MO). Next, since PCR can detect even a single molecule of DNA, RNA samples were digested with DNase I (AMP-D1, Sigma) before RT-PCR. The expression levels of the TNF-α, IL-6 and MCP-1 genes, and of 18S ribosomal RNA (rRNA) as a housekeeping gene, were measured by real-time RT-PCR using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, U.S.A). RT-PCR was performed in two steps as follows: 2 μg of total RNA underwent random primed reverse transcription for 10 min at 25 °C and 2 h at 37 °C using the High-Capacity cDNA kit (Applied Biosystems) and RNase inhibitor (rRNasin 40 U/μL, Promega, Madison, U.S.A.) to synthesize the cDNA. Real-time PCR was realized with 2 μL of cDNA and 18 μL of reaction mixture (10 μL of 2× TaqMan Universal PCR Master Mix (Applied Biosystems), 1 μL of 20× Assays-on-Demand™ Gene Expression Assay Mix (Applied Biosystems) and 7 μL RNase-free water). After an initial hold of 2 min at 50 °C and 10 min at 95 °C, the samples were cycled 40 times at 95 °C for 15 s and 60 °C for 60 s. For all quantitative cDNA analysis, the ΔC_t technique was utilised [30]. The expression of each target gene was normalized to housekeeping gene transcript. All measurements were performed in duplicate. Controls consisting of double distilled H_2O were negative in all runs.

2.9. Statistical analysis

Statistical analysis used SPSS statistical software, version 11.0 (SPSS Inc., Chicago, IL). ANOVA for repeated measures was used to analyze the differences in plasma lipid and lipoprotein concentrations and plasma fatty acid composition. ANCOVA for repeated measures, using the basal values of each mRNA and plasma concentration as covariate, was utilised to study the differences in the expression and production of proinflammatory cytokines under study. In these analysis, we studied the statistical effects of the time alone or the change in the variable after ingesting fatty food over the entire lipemic period (represented as *P*1) and the effect of the breakfast (represented as *P*2), independently of the time in the postprandial study. We also studied the effect of the interaction

Table 2
Plasma fatty acid composition (percentage relative to the total fatty acids) according to the type of fat consumed during the postprandial[a]

Time and breakfast	Fatty acid (% relative to the total fatty acids)				
	14:0	16:0	18:1	18:2	18:3
0 h					
Butter	0.53 ± 0.18a	21.95 ± 1.40c	18.24 ± 2.13b	23.30 ± 2.15b	0.34 ± 0.07
Olive oil	0.40 ± 0.12b	21.16 ± 1.54	20.12 ± 2.70a	22.00 ± 2.90b	0.35 ± 0.08
Walnuts	0.38 ± 0.16b	20.9 ± 1.04b	17.8 ± 2.42b	25.9 ± 1.64a	0.41 ± 0.15
3 h					
Butter	1.40 ± 0.43a	23.10 ± 0.71a	18.35 ± 2.30b	23.10 ± 1.10b	0.38 ± 0.12b
Olive oil	0.30 ± 0.15b	20.05 ± 0.84b	27.43 ± 3.98a	21.90 ± 2.28b	0.38 ± 0.04b
Walnuts	0.40 ± 0.15b	20.10 ± 1.38b	17.50 ± 1.21b	30.15 ± 1.60a	1.88 ± 0.63a
Global analysis *P* values					
Time effect	0.001	0.076	0.022	0.129	0.001
Breakfast effect	0.001	0.009	0.001	0.001	0.001
Breakfast × Time effect	0.001	0.001	0.001	0.002	0.001

[a] $n = 20$. Means in a column with different letters are significantly different, $P < 0.05$ (ANOVA for repeated measures). Values are means ± S.D. (all such values).

Table 3
Plasma lipid and lipoprotein concentrations according to the type of fat consumed during the postprandial phase[a]

Time and breakfast	Lipids and lipoproteins (mmol/L)			
	Total C	TG	HDL–C	LDL–C
0 h				
Butter	3.85 ± 0.12a	0.78 ± 0.07	1.17 ± 0.05	2.17 ± 0.08a
Olive oil	3.63 ± 0.12b	0.78 ± 0.07	1.18 ± 0.04c	1.91 ± 0.07b
Walnuts	3.57 ± 0.14b	0.72 ± 0.06	1.12 ± 0.05b	1.96 ± 0.10b
3 h				
Butter	3.62 ± 0.12	1.42 ± 0.12	1.05 ± 0.04	1.76 ± 0.09
Olive oil	3.59 ± 0.12	1.59 ± 0.18	1.04 ± 0.04	1.65 ± 0.09
Walnuts	3.49 ± 0.12	1.34 ± 0.12	1.04 ± 0.04	1.57 ± 0.13
6 h				
Butter	3.58 ± 0.14	0.88 ± 0.09a	1.06 ± 0.04	1.90 ± 0.09
Olive oil	3.53 ± 0.14	0.64 ± 0.04b	1.07 ± 0.04	1.99 ± 0.10
Walnuts	3.46 ± 0.13	0.64 ± 0.04b	1.07 ± 0.04	1.92 ± 0.08
9 h				
Butter	3.72 ± 0.14	0.59 ± 0.05	1.14 ± 0.04	2.15 ± 0.10d
Olive oil	3.58 ± 0.13	0.53 ± 0.03c	1.12 ± 0.04	2.07 ± 0.10
Walnuts	3.55 ± 0.15	0.62 ± 0.04b	1.10 ± 0.04	1.95 ± 0.09b
Global analysis *P* values				
Time effect	0.001	0.001	0.001	0.001
Breakfast effect	0.614	0.862	0.940	0.503
Breakfast × Time effect	0.036	0.006	0.025	0.020

[a] $n = 20$. Means in a column with different letters are significantly different, $P < 0.05$ (ANOVA for repeated measures). Values are means ± S.E.M. (all such values).

of both factors – breakfast and time – which is indicative of the magnitude of the postprandial response in each meal (represented as *P*3). When statistically significant effects were found, an ANOVA was used to identify group differences in each time. A study of the relation among parameters was carried out using Pearson's linear correlation coefficient. A probability of less than 0.05 was considered significant. All data presented in the text and tables are expressed as mean ± S.E.M.

3. Experimental results

3.1. Plasma fatty acid composition

The data suggest that the type of diet consumed during the dietary intervention period and the intake of the fatty breakfasts at the end of each period has a direct influence on plasma fatty acid composition (Table 2). At the end of the dietary intervention period, we observed that the Western diet raised the proportion of palmitic and myristic fatty acids ($P < 0.05$). On the other hand, the Mediterranean diet produced an increase in oleic acid ($P < 0.05$), while the high-CHO diet enriched in n-3 fatty acids augmented linoleic ($P < 0.05$) and linolenic acids. In the postprandial period, we found an increase ($P < 0.05$) of palmitic and myristic fatty acids and of oleic acid with the butter and olive oil breakfasts, respectively. Moreover, we showed that the walnut breakfast increased concentrations of linolenic and linoleic fatty acids ($P < 0.05$).

3.2. Diet intake and postprandial lipemia

At the end of the dietary intervention period and after a 12-h fast (Table 3), we observed an increase in total C and LDL–C with the Western diet, compared with the Mediterranean diet and the high-CHO diet rich in α-LNA ($P < 0.05$), as well as, a reduction in HDL–C concentrations with the high-CHO diet rich in α-linolenic acid respect to the Mediterranean diet ($P = 0.015$). Throughout the whole period of postprandial lipemia, we demonstrated an increase in total TG and a reduction in C, HDL–C and LDL–C concentrations ($P < 0.001$). Furthermore, we found a higher increase in total postprandial TG with the butter breakfast than with the walnut- or olive oil-enriched breakfasts at 6 h ($P < 0.05$). By 9 h, there was a lower concentration of TG with the olive oil breakfast than with the walnut breakfast ($P < 0.05$), as well as an increase in LDL–C concentrations with the butter breakfast compared to the walnut breakfast ($P = 0.013$) (Table 3).

3.3. Diet intake and proinflammatory cytokines

In order to determine whether the intake of the three fat-load breakfasts could regulate the expression of different inflammation parameters in PBMCs during the postprandial phase, we studied the mRNA levels for TNF-α, IL-6 and MCP-1 in these cells (Fig. 1). We observed that the ingestion of the butter breakfast induced a higher postprandial expression of mRNA TNF-α than the olive oil or walnut-enriched breakfasts ($P = 0.014$). At 3 h after the intake of the olive oil and walnuts breakfasts, we found a lower postprandial expression of mRNA TNF-α than after the butter-rich breakfast ($P < 0.05$). When we studied the expression of mRNA IL-6, we showed a higher postprandial response in the mRNA of this cytokine with the intake of the butter and olive oil breakfasts than with the walnut breakfast ($P = 0.025$). We observed an increase in the mRNA IL-6 after the butter- and olive oil-enriched breakfasts compared to the walnut breakfast after 3 h ($P < 0.05$). Finally, we did not find any significant differences in mRNA MCP-1 expression following the intake of the three types of fatty breakfasts throughout the postprandial phase.

In addition, we also analyzed plasma levels of TNF-α, IL-6 and MCP-1 during the postprandial phase (Fig. 2). Comparisons of the effect of the time, the fatty breakfast, and the interaction of both factors (time and breakfast) on the concentrations of these parameters revealed no significant differences (P = N.S.).

3.4. Correlations between proinflammatory and lipid parameters/fatty acid composition

We studied the relationship between the mRNA levels for TNF-α and IL-6, and lipid and lipoprotein concentrations, as well as between these proinflammatory cytokines and the plasma fatty acid composition during the first 3 h after the intake of a fatty

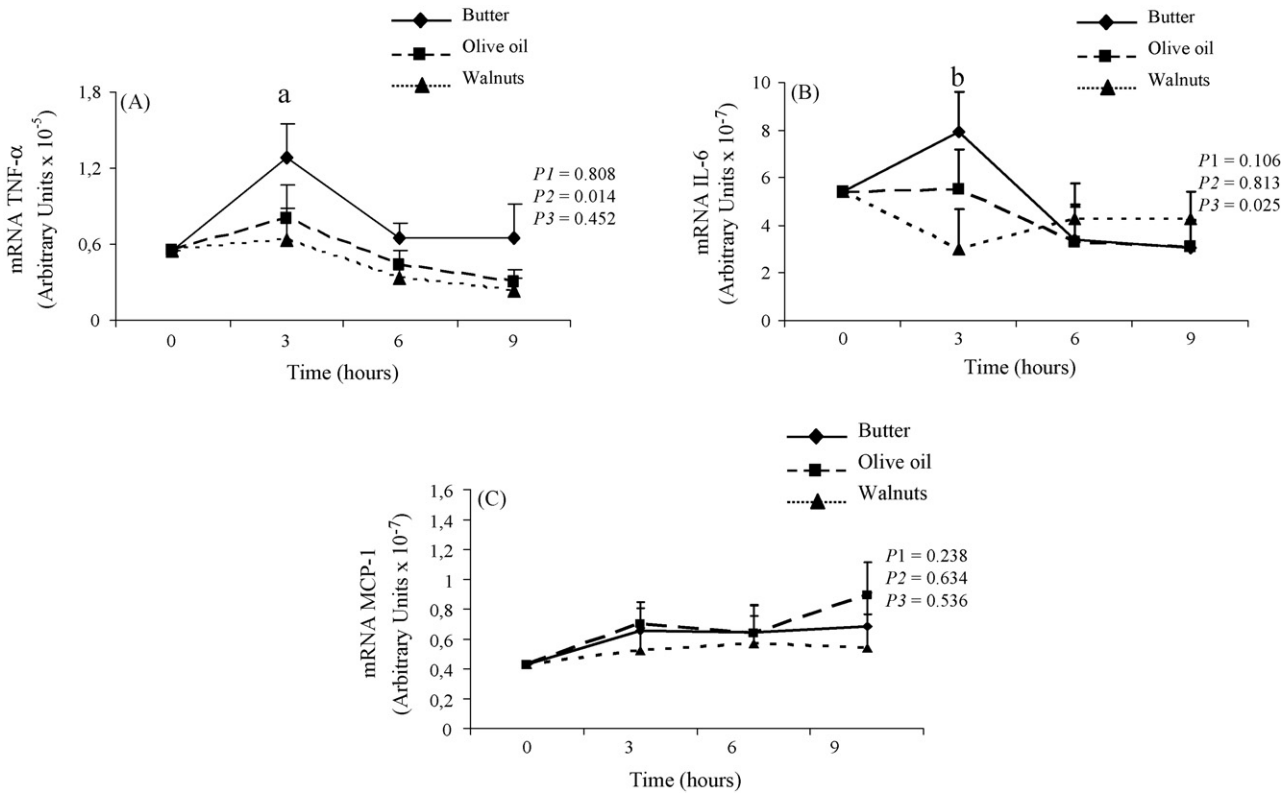

Fig. 1. Mean (±S.E.M.) mRNA TNF-α (A), IL-6 (B) and MCP-1 (C) expression in PBMCs, $n = 20$. Results are expressed in arbitrary units. ANCOVA for repeated measures: $P1$: time effect; $P2$: breakfast effect; $P3$: breakfast by time interaction. $^{a}P < 0.05$ butter vs. olive oil and walnuts breakfasts and $^{b}P < 0.05$ walnut vs. butter and olive oil breakfasts at that specific point in time.

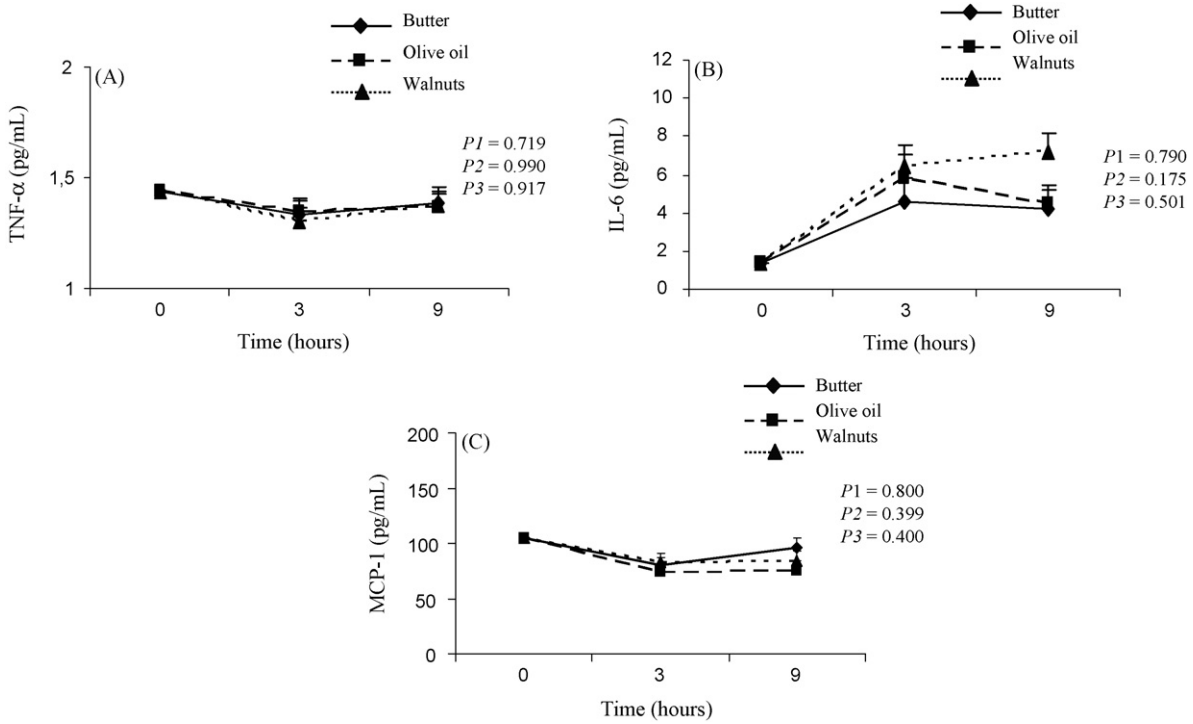

Fig. 2. Mean (±S.E.M.) plasma TNF-α (A), IL-6 (B) and MCP-1 (C) levels, $n = 20$. Results are expressed in arbitrary units. ANCOVA for repeated measures: $P1$: time effect; $P2$: breakfast effect; $P3$: breakfast by time interaction.

breakfast without considering the type of fat consumed. We did not find correlation among the different studied parameters (data no shown).

4. Discussion

Ours results show that a butter-enriched breakfast increases postprandial expression of mRNA TNF-α in PBMCs from healthy men with apoE3/E3 genotype compared with a breakfast rich in olive oil or walnuts. Moreover, we observed a higher postprandial response of mRNA IL-6 in these cells with the butter and olive oil breakfasts compared to the breakfast rich in walnuts.

Few studies have investigated changes in inflammatory markers related to atherosclerosis during the postprandial state [31,32], which is the normal metabolic condition of the human beings throughout the day, and none of them considered the effect of the type of fat on such response. Our group has already demonstrated that the consumption of an olive oil-enriched breakfast does not activate NF-κB in PBMCs as do butter- and walnut-enriched breakfasts [21]. Nevertheless, the chronic effect of the type of dietary fat on the postprandial inflammatory response is not known. MCP-1 regulates the transmigration of monocytes and other mononuclear cells on inflammatory sites [33]. Moreover, MCP-1 also recruits monocytes into atherosclerotic lesions and into the infarct zone after myocardial infarction [34]. However, when we analyzed this chemokine, we did not observe significant differences in the expression and plasma levels of MCP-1 throughout the postprandial phase. This result may be explained, at least in part, by the characteristics of our sample population, which consisted of completely healthy young people. On the other hand, TNF-α and IL-6 can mediate the systemic effects of inflammation, including fever, loss of appetite, mobilization of protein and fat, and acute phase protein synthesis. The production of sufficient amounts of these cytokines is clearly beneficial in response to infection, but inappropriate quantities or overproduction may be harmful. In order to alleviate inflammation, therefore, it is important to inhibit the production of proinflammatory cytokines. Nutrition strategies may be desirable to manipulate the secretion of these cytokines and, in this regard, our present study showed a higher expression of proinflammatory cytokines in PBMCs following a butter-enriched breakfast than after those rich in olive oil or walnuts. However, we did not observe significant postprandial differences in plasma levels of these proinflammatory cytokines. The fact that we only found differences in the expression of TNF-α and IL-6 at mRNA levels in PBMCs following the intake of the three breakfasts may be due to that the synthesis and secretion processes of these proteins do not happen simultaneously, and to the short half-life of both cytokines [35,36].

Biologically, TNF-α acts as a trigger that activates a cascade of cytokine production. A number of regulatory agents, including glucorticoids, acute-phase proteins, eicosanoids and soluble receptors [37], limit TNF-α production. Lipids have been shown to be potent modulators of inflammation since a large number of the modulatory compounds cited previously are derived from the hydrolysis of membrane phospholipids. Because of this effect of lipids in the inflammation and, to the fact that apoE genotype is know to have significant effects on postprandial lipemia [23], we study only individual homozygous for the most common allele, apoE3/E3. When we investigated whether the effect observed in the proinflammatory cytokines was due to the change in the lipid profile [38] or in the plasma fatty acid composition, we did not observe a correlation between these parameters and mRNA for TNF-α and IL-6. Therefore, it is possible that other factors take part in the process. We speculated that, as indicated in our previous study [21], an increase in activation of NF-κB may be one of the main mechanisms of the inflammatory properties of the butter breakfast, since this nuclear factor plays a central role in regulating the cytokine network. It has also been observed that the modulatory effect of fatty acids on the synthesis of proinflammatory cytokines may be due to a peroxisome proliferative activated receptor γ (PPARγ)-dependent mechanism. Desreumaux et al. [39] showed that activation of PPAR-γ in the colon inhibits mucosal production of IL-1β and TNF-α by downregulation of the NF-κB and mitogen-activated protein kinase signal pathways. Furthermore, we observed a higher mRNA IL-6 expression in PBMCs following the butter and olive oil breakfasts than with the walnut breakfast. A mechanism possibly capable of explaining this result is that n-3 polyunsaturated fatty acids induce changes in both cycloxygenase and lipoxygenase products, such as a reduction in the production of prostaglandin E_2 and leukotriene B_4 [40]. Since both metabolites enhance the release of IL-6 in vitro [41], a reduction in these eicosanoids could explain the reduction in mRNA IL-6 that we observed in this study with the walnut-rich breakfast.

To summarize, this study has shown that breakfasts rich in olive oil or walnuts had an anti-inflammatory effect, which may provide an additional beneficial mechanism of both types of fat in the primary and secondary prevention of cardiovascular disease. The final objective in the prevention and treatment of coronary atherosclerosis is to reduce the risk of new heart attacks and mortality due to cardiovascular failure. Identifying a suitable diet is thus fundamental for avoiding the development of this disease and associated cardiovascular events.

Acknowledgments

Supported in part by research grants from the MCYT (AGL 2004/07907, AGL2006-01979/ALI to JL-M and SAF2003-05770 to FP-J), the Spanish Ministry of Health (FIS PI041619 to CM, FIS 01/449 to JL-M, and CB06/03/0047 (CIBER Fisiopatologia de la Obesidad y Nutricion is an initiative of ISCIII) to FP-J), Consejería de Salud, Junta de Andalucía (03/75, 04/238 to JL-M, 03/73, 04/191 to FP-J, 05/396 to CM), the Diputación Provincial de Córdoba (to FP-J), the CAM (08.4/0021.1/2003 to JE) and the Spanish Cardiovascular Network (to JE). We extend our thanks to Canoliva (Antonio Cano e Hijos SA, Luque, Córdoba), who generously donated the olive oil for the dietary experiments, and also to the volunteers.

References

[1] Braunwald E. Shattuck lecture—cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. N Engl J Med 1997;337:1360–9.
[2] Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
[3] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801–8.
[4] Gu L, Okada Y, Clinton SK, et al. Absence of CC chemokine receptor-2 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. Mol Cell 1998;2:275–81.
[5] De Lemos JA, Morrow DA, Sabatine MS, et al. Association between plasma chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 2003;107:690–5.
[6] Yla-Herttuala S, Lipton BA, Rosenfeld ME, et al. Expression of monocyte chemottractant protein-1 in macrophage-rich areas of human and rabbit atherosclerosis lesions. Proc Natl Acad Sci USA 1991;88:5252–6.
[7] Okada M, Matsumori A, Ono K, et al. Cyclic stretch upregulates production of interleukin-8 and monocyte chemotactic and activating factor/monocyte chemoattractant protein-1 in human endothelial cells. Atheroscler Thromb Vasc Biol 1998;18:894–901.
[8] Fuster V. Elucidation of the role of plaque instability and rupture in acute coronary events. Am J Cardiol 1995;76:24C–33C.
[9] Perez-Jimenez F, Lopez-Miranda J, Mata P. Protective effect of dietary monounsaturated fat on arteriosclerosis: beyond cholesterol. Atherosclerosis 2002;163:385–98.
[10] Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb 1992;12:911–9.

[11] Paschos GK, Rallidis LS, Liakos GK, et al. Background diet influences the anti-inflammatory effect of alpha-linolenic acid in dyslipidaemic subjects. Br J Nutr 2004;92:649–55.
[12] Baer DJ, Judd JT, Clevidence BA, Tracey RP. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomised crossover study. Am J Clin Nutr 2004;79:969–73.
[13] Grimble RF. The modulation of immune function by dietary fat. Br J Int Care 1994;4:159–67.
[14] Patsch W, Esterbauer H, Foger B, Patsch JR. Postprandial lipemia and coronary risk. Curr Atheroscler Rep 2000;2:232–42.
[15] Parks EF. Recent findings in the study of postprandial lipemia. Curr Atheroscler Rep 2001;3:462–70.
[16] Zilversmit DB. Atherogenesis: a postprandial phenomenon. Circulation 1979;60:473–85.
[17] Patsch JR, Prasad S, Gotto Jr AM, Bengtsson-Olivecrona G. Postprandial lipemia. A key for the conversion of high density lipoprotein2 into high density lipoprotein3 by hepatic lipase. J Clin Invest 1984;74:2017–23.
[18] van Oostrom AJ, Sijmonsma TP, Rabelink TJ, Van Asbeck BS, Cabezas MC. Postprandial leukocyte increase in healthy subjects. Metabolism 2003;52: 199–202.
[19] van Oostrom AJ, Sijmonsma TP, Verseyden C, et al. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. J Lipid Res 2003;44:576–83.
[20] van Oostrom AJ, Rabelink TJ, Verseyden C, et al. Activation of leukocytes by postprandial lipemia in healthy volunteers. Atherosclerosis 2004;177: 175–82.
[21] Bellido C, López-Miranda J, Blanco-Colio LM, et al. Butter and walnuts, but not olive oil, elicit postprandial NF-κB activation in peripheral mononuclear blood cells from healthy male volunteers. Am J Clin Nutr 2004;80:1487–91.
[22] Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988;240:622–30.
[23] Boerwinkle E, Brown S, Sharrett AR, Heiss G, Patsch W. Apolipoprotein E polymorphism influences postprandial retinyl palmitate but not triglyceride concentrations. Am J Hum Genet 1994;54:341–60.
[24] Human Nutrition Information Service, Agriculture Handbook No. 8. Department of Agriculture Composition of Foods. Washington, DC: US Government Printing Office, 1987.
[25] Varela G. Tablas de composición de alimentos (Spanish) (Food composition tables). Madrid: Instituto de Nutrición. CSIC; 1980.
[26] Bucolo G, David H. Quantitative determination of serum triglycerides by use of enzymes. Clin Chem 1973;19:476–82.
[27] Allain CC, Poon LS, Chang CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470–5.
[28] Brigg CJ, Anderson D, Johnson P, Deegan T. Evaluation of the polyethylene glycol precipitation method for the estimation of high-density lipoprotein cholesterol. Ann Clin Biochem 1981;18:177–81.
[29] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of a preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[30] Cohen CD, Franch K, Schlondorff D, Kretzler M. Cuantitative gene expression analysis in renal biopsies: a novel protocol for a high-throughput multicenter application. Kidney Int 2002;61:133–40.
[31] Nappo F, Esposito K, Cioffi M, et al. Postprandial endothelial activation in healthy subjects and type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol 2002;39:1145–50.
[32] Jellema A, Plat J, Mensink RP. Weigh reduction, but not a moderate intake of fish oil, lowers concentrations of inflammatory markers and PAI-1 antigen in obese men during the fasting and postprandial state. Eur J Clin Invest 2004;34: 766–73.
[33] Krsihnaswamy G, Kelley J, Yerra L, Smith JK, Chi DS. Human endothelium as a source of multifunctional cytokines: molecular regulation and possible role in human disease. J Interferon Cytokine Res 1999;19:91–104.
[34] Kumar AG, Ballantyne CM, Michael LH, et al. Induction of monocyte chemoattractant protein-1 in the small veins of the ischemic and reperfused canine myocardium. Circulation 1997;95:693–700.
[35] Futterman LG, Lemberg L. High-sensitivity C-reactive protein is the most effective prognostic measurement of acute coronary events. Am J Crit Care 2002;11:482–6.
[36] Kishimoto T. Interleukin-6; form basic science to medicine-40 years in immunology. Annu Rev Immunol 2005;23:1–20.
[37] Grimble RF. Interaction between nutrients, proinflammatory cytokines and inflammation. Clin Sci 1996;91:121–30.
[38] Khan BV, Parthasarathy SS. Modified low-density lipoprotein and its constituents augment cytokine-activated vascular cell adhesion-1 gene expression in human vascular endothelial cells. J Clin Invest 1995;95:1262–70.
[39] Desreumaux P, Dubuquoy L, Nutten S, et al. Attenuation of colon inflammation through activators of the retinoid × receptor (RXR)/peroxisome proliferators-activated receptor gamma (PPARgamma) heterodimer. A basis for new therapeutic strategies. J Exp Med 2001;193:827–38.
[40] Chavali SR, Zhong WW, Forse RA. Dietary α-linolenic acid increases TNF, and decreases IL-6, IL-10 in response to LPS: effects of sesamin on the Δ-5 desaturation of ω6 and ω3 fatty acids in mice. Prostaglandins Leukot Essent Fatty Acids 1998;58:185–91.
[41] Leal-Berumen I, O'Byrne P, Gupta A, Richards CD, Marshal JS. Prostanoid enhancement of interleukin-6 production by rat peritoneal mast cells. J Immunol 1995;154:4759–67.