Exchanging Saturated Fatty Acids for (n-6) Polyunsaturated Fatty Acids in a Mixed Meal May Decrease Postprandial Lipemia and Markers of Inflammation and Endothelial Activity in Overweight Men1–3

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Abstract

Postprandial lipemia, low-grade systemic inflammation, and endothelial activity are related to metabolic disorders. It is well known that dietary fatty acid composition modulates postprandial lipemia, but information on the other metabolic risk markers is limited. We therefore studied the acute effects of a meal rich in SFA compared with those of a meal rich in (n-6) PUFA on postprandial responses in overweight men who are at an increased risk to develop the metabolic syndrome and its comorbidities. In a crossover design, the effects of 50 g butter (rich in SFA) on lipemia and markers for inflammation and endothelial activity were compared with those of 50 g sunflower oil (rich in (n-6) PUFA) during an 8-h postprandial mixed meal tolerance test in 13 overweight men. Postprandial changes in serum TG were comparable between the meals (P = 0.38), except for a reduction in the incremental area under the curve (P = 0.046) in the late postprandial phase after (n-6) PUFA (125 ± 96 mmol·min⁻¹·L⁻¹) compared with SFA (148 ± 98 mmol·min⁻¹·L⁻¹). Compared with the SFA meal, the (n-6) PUFA meal decreased plasma IL-6 (P = 0.003), TNFα (P = 0.005), soluble TNF receptors I and II (sTNFr; P = 0.024 and P < 0.001, respectively), and soluble vascular cell adhesion molecule-1 (sVCAM-1; P = 0.030) concentrations. These results indicate that exchanging SFA from butterfat for (n-6) PUFA in a mixed meal may decrease postprandial lipemia and concentrations of IL-6, TNFα, sTNFr-I and -II, and sVCAM-1 in overweight men. J. Nutr. 141: 816–821, 2011.

Introduction

Postprandial lipemia, which is positively related to cardiovascular risk (1), is influenced by the composition of the diet. In this respect, linoleic acid from vegetable oils may be more beneficial than SFA (2). Results, however, are controversial; especially, conflicting results have been found for butterfat, because both decreased and increased TG responses have been reported compared with those of linoleic acid (3,4). In addition, results among studies are difficult to compare due to differences in experimental designs. In the latter studies (3,4), e.g., the test fats were added to an energy-free soup or tomato sauce or were consumed as a spread. In the soup and tomato sauce, the solid fat content of the meal was probably lowered due to its high temperature, which may aggravate postprandial lipemia (5). Also, genetic background may be important. Recently, it was shown that single nucleotide polymorphisms of the TCFL2 gene may affect the postprandial responses to (n-6) PUFA (6), indicating the complexity of postprandial metabolic responses.

Less is known about the effects of dietary composition on markers of low-grade systemic inflammation and endothelial activation, which are both involved in the process of atherosclerosis (7). Several in vitro studies have suggested that SFA are proinflammatory, whereas PUFA are antiinflammatory (8,9). On the other hand, in another in vitro study (10), incubation with (n-6) PUFA (linoleic or arachidonic acid) resulted in higher expression of the intercellular adhesion molecule (ICAM)-14 and vascular cell adhesion molecule (VCAM)-1 than did incubations with SFA (palmitic acid) and (n-3) PUFA (DHA). In vivo, it was recently shown that acute ingestion of carbohydrate meals with or without 0.6 g fat/kg body weight resulted in higher late-postprandial IL-6 concentrations in obese than in

1 Supported by the Dutch Dairy Association, Zoetermeer, The Netherlands.
2 Author disclosures: C. J. Masson and R. P. Mensink, no conflicts of interest.
3 Supplemental Tables 1–5 and Supplemental Figure 1 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
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lean women, but cream (SFA), olive oil (MUFA+ SFA), and canola oil (MUFA+ n-6 PUFA) meals did not differ (11). However, other relevant markers of inflammation and endothelial activation were not measured. Another study showed no differences in inflammatory markers in normal weight men after altering the ratio of SFA/unsaturated fatty acids of a high-fat [3.1 MJ; 70.8% of energy (En%) from fat] test meal (12). In addition, the acute effects of different types of fat on markers of postprandial inflammation and endothelial activation have not been studied, to our knowledge, in overweight men, who are at increased risk for developing metabolic syndrome (13).

We therefore decided to examine in overweight men changes in concentrations of plasma markers related to inflammation and endothelial activation after the consumption of a mixed meal providing 50 g of fat from butter or margarine, which are rich in SFA and (n-6) PUFA (linoleic acid), respectively.

Materials and Methods

Participants. Volunteers were recruited through announcements in local newspapers or among participants who had participated in earlier studies at the department. Female participants were excluded to avoid any possible variations in postprandial responses due to hormonal effects. Participants were invited for 2 screening visits if they met the following inclusion criteria: aged between 18 and 70 y, BMI between 25 and 30 kg/m², stable body weight (weight gain or loss < 5% in the previous 3 mo), no intention to change physical activity pattern during the study, no use of lip-lowering medication or a prescribed diet, and no participation in another biochemical trial for the past 30 d. Fifteen participants were included (Supplemental Table 1). They had mean fasting serum concentrations < 8.0 mmol/L for total cholesterol and < 1.7 mmol/L for TG; no indications for treatment for hyperlipidemia according to the Dutch Cholesterol Consensus (14); no inflammatory disease; no clinical condition known to affect lipid metabolism; no drug or alcohol abuse; and no history of coronary artery disease, heart failure (class III or IV), cardiomyopathy, or kidney, liver, or pancreatic disease or malignancy < 5 y ago. All participants gave written informed consent before entering the study. The study protocol was approved by the Ethics Committee of Maastricht University.

Study design. A randomized, double-blind, crossover design was used in which all participants received 2 different interventions. On the day before each test, participants were asked to not engage in any strenuous physical exercise or consume alcohol. Participants were also asked to refrain from high-fat foods, including fried foods and sausages. After a 12-h overnight fast (from 2000 h), participants visited the department by public transport or car. After resting for 20 min in a supine position, an iv. cannula was inserted into a vein of the nondominant arm. At T = 0 min (T0), a blood sample was collected. Participants were then requested to consume within 10 min 1 of the 2 test meals: a meal rich in butterfat or a meal rich in sunflower oil. Both meals provided 50 g fat. Subsequent blood samples were collected at T = 15 min after meal consumption (T15) and at T = 30 min (T30), T = 45 min (T45), T = 60 min (T60), T = 90 min (T90), T = 120 min (T120), and T = 180 min (T180). After T180, participants received a low-fat lunch. Additional blood samples were drawn at T = 240 min (T240), T = 300 min (T300), T = 360 min (T360), T = 420 min (T420), and T = 480 min (T480). After sampling, the cannula was rinsed with 1 mL 1% heparin solution and stored at −80°C until analysis.

Serum TG with correction for free glycerol was determined hourly (GPO Trinder; Sigma-Aldrich) and serum apo B-48 concentrations were measured 0, 120, 240, 360, and 480 min after meal consumption (Shibayagi). Plasma inflammatory markers (IL-6, IL-8, and TNFα) were measured at the same time points in plasma with a commercially available Multi Spot ELISA kit (Meso Scale Discovery). TNF receptors 1 and 2 were measured at the same time points in plasma with a commercially available Multi Spot ELISA kit (Meso Scale Discovery). Serum monocyte chemoattractant protein-1 (MCP-1) was measured 0, 60, 120, 240, and 480 min after test meal consumption with a single spot ELISA kit (Meso Scale Discovery). Cellular adhesion molecules soluble ICAM (sICAM) and sVCAM were measured in plasma at T0, 120, 240, 360, and 480 with a Multi Spot ELISA kit (Meso Scale Discovery).

Statistical analysis. All results are presented as mean ± SD. Differences in baseline concentrations between test days were tested using a paired t test. Changes in concentrations were analyzed by ANOVA with diet and time as fixed factors and with diet × time as interaction term. If the term was not significant, it was omitted from the model. If factor time was significant, post hoc tests with Bonferroni correction were carried out to compare concentrations to baseline concentrations. For both meals, the maximum change from baseline (peak change) and the time period between meal consumption and peak change (time to peak) were calculated. Also, the incremental AUC [iAUC; the area above baseline (T0) concentrations] or the decremental AUC (dAUC; the area below baseline concentrations) was calculated using the trapezoidal rule (15). Because serum TG concentrations peaked ≈4 h after meal consumption, we divided the postprandial period into an early (0–240 min) and a late postprandial phase (240–480 min). Comparable analyses were performed for apoB-48, glucose, and insulin concentrations. The differences in peak changes, time to peak, and iAUC or dAUC between the meals were compared using paired samples t tests. Differences were considered significant at P < 0.05. Statistical analyses were performed using SPSS 16.0 software.

Results

After screening, 15 participants started the study. One subject dropped out due to other commitments and 14 participants...
completed both postprandial tests. One of these participants was excluded from the statistical analyses, because he was not fasting on 1 of the 2 test days, as indicated by a large difference between fasting TG concentrations on these 2 d (5.08 vs. 1.34 mmol/L).

**Postprandial lipemia.** Fasting TG concentrations were comparable between the test days (P = 0.60). After meal consumption, postprandial TG concentrations rose until 3–4 h and returned to baseline after 8 h (Fig. 1). Changes in TG concentrations did not differ between the meals (P = 0.38 for diet effect). The iAUC of serum TG over the 8-h postprandial period and in the early phase (T0–T240) were comparable after consumption of the meals, but the iAUC in the late postprandial phase (T240–T480) was higher (P = 0.046) after the butter meal (148 ± 98 mmol·min·L⁻¹) than after the margarine meal (125 ± 96 mmol·min·L⁻¹). There was a delay in time to peak of 55 min after consuming the butter meal (P = 0.008), whereas the maximum changes from baseline were comparable (Supplemental Tables 3 and 4). Serum concentrations of apoB-48 increased over time (Fig. 1). The changes in apoB-48 concentrations after the meals did not differ (P = 0.95) and the iAUC, the iAUC for the early and late postprandial phases (data not shown), maximum changes, and the time to peak did not differ (Supplemental Tables 3–5).

**Postprandial glycaemia.** Glucose (P = 0.58) and insulin (P = 0.24) concentrations at baseline did not differ between the test days. Following each meal, there was a rapid increase in both glucose and insulin, which was significant for the factor time (P < 0.001) (Supplemental Fig. 1). Plasma glucose concentrations increased during the first 60 min (P < 0.05 at all time points) and serum insulin during the first 2 h (P < 0.001 at all time points) after both interventions. The glucose (P = 0.83) and insulin (P = 0.65) responses did not differ between the meals.

The postprandial responses, also between the early (T0–120) and late (T120–480) postprandial phases, between the test meals were comparable for all other variables (Supplemental Tables 3–5).

**Postprandial markers of inflammation and endothelial activation.** Baseline concentrations of all the markers related to inflammation and endothelial activation were comparable between the test days, except for soluble TNF receptor-II (sTNFRII), which was lower (P = 0.049) at the butter test day than at the margarine test day.

Changes in plasma IL-6 concentrations were not significant for time (P = 0.14) but increased more after butter than after margarine (P = 0.003) (Fig. 2). The dAUC tended to be higher after margarine than butter (P = 0.09) (data not shown), whereas other variables were comparable (Supplemental Tables 4 and 5).

Changes in IL-8 concentrations after the meals showed a time effect (P = 0.011) (Fig. 2). After adjustment for multiple comparisons, however, none of the time points differed from baseline. Moreover, there was no significant meal effect (P = 0.12). Other variables were also comparable between the meals (Supplemental Tables 4 and 5).

For TNFα, postprandial concentrations were lower after margarine than after butter (P = 0.005 for diet effect) (Fig. 2) and there was a trend for a higher maximum decrease in plasma TNFα concentrations after the margarine than after the butter meal (P = 0.10) (Supplemental Table 4).

sTNFRI concentrations decreased (P < 0.001) and sTNFRII tended to decrease after meal consumption (P = 0.06 for time effect) (Fig. 2). Plasma sTNFRI concentrations (P = 0.024) and sTNFRII concentrations (P < 0.001) were lower after margarine consumption than after butter consumption. The maximum decrease in sTNFRI was higher after margarine than after butter consumption (P = 0.049). For sTNFRII, there was a trend for a larger dAUC (P = 0.06; data not shown) and a higher maximum decrement (P = 0.06) after margarine consumption (Supplemental Tables 4 and 5).

Postprandial MCP-1 concentrations decreased after meal consumption over time (P < 0.001) and were lower than at baseline at all time points (P < 0.001) (Fig. 3), whereas the other variables did not differ between the meals (Supplemental Tables 4 and 5).

Postprandial sICAM-1 and soluble VCAM (sVCAM)-1 concentrations did not change over time (P = 0.63 and P = 0.62, respectively) (Fig. 3). There was a diet effect for sVCAM-1 (P = 0.030), with higher concentrations after butter than after margarine. For sICAM-1, concentrations tended to be lower after consumption of the margarine meal than after the butter meal (P = 0.07). Other variables were comparable (Supplemental Tables 4 and 5).

**Discussion**

**Postprandial lipemic response.** Serum TG concentrations peaked after ~3 h after consumption of the margarine meal but 4 h after consumption of the butter meal. It should, however, be realized that postprandial concentrations are the result of 2 main coinciding processes, e.g. absorption and clearance. It has been suggested that absorption is delayed if the melting point of a fat is high, resulting in a higher solid fat content at body temperature (5,16). The solid fat content of butter fat is close to 0% at body temperature (17). We do not know whether this affected absorption. For both meals, differences in absorption and clearance were comparable during the early postprandial phase,
resulting in similar TG responses. However, it is still possible that (n-6) PUFA (linoleic acid) are absorbed faster and that the clearance rate is higher, because increased postprandial LPL activity was found after consumption of linoleic acid compared with stearic and palmitic acids (18), which may explain the increased postprandial lipemia in the late postprandial phase after SFA. Additionally, it has been suggested that large chylomicrons are cleared from the circulation faster than are smaller chylomicrons when equal fat loads are given (19). In rats, safflower oil consumption resulted in larger chylomicrons compared with coconut oil, rich in SFA (20). In our study, however, the equal apoB-48 responses after the meals indicated that the number of intestinally derived lipoproteins was equal, but whether particle size differed is not known. Dworatzek et al. (21) reported similar responses for total serum TG when butter was compared with safflower oil. Chylomicron TG responses, however, decreased after the butter meal. The authors speculated that this may have been due to the fact that a part of the TG after the butter meal was packed into smaller, denser lipoproteins, which were lost during isolation. Alternatively, it was mentioned that the lower TG concentrations may have resulted from the absorption of medium-chain fatty acids into the portal circulation, which does not result in the formation of chylomicrons. However, Nielsen et al. (22) found no differences in postprandial lipemia when either palm oil or butter was added to a low-fat meal, which suggests that medium-chain fatty acids are not an important determinant for total serum TG responses during the postprandial phase.

Postprandial markers of inflammation and endothelial activation. After the consumption of the butter muffins, IL-6 concentrations increased in our study, whereas IL-6 concentrations decreased after consumption of the margarine muffins. In
studies with lean and overweight children (23), men with metabolic syndrome (24), abdominally obese men (25), CHD patients (26), and healthy participants (27), an increase in IL-6 concentrations was observed after a high-fat meal, whereas a carbohydrate-rich meal did not influence IL-6 concentrations (28). In contrast, no differences in IL-6 responses were found in obese men when the (n-6):(n-3) fatty acid ratio was decreased from 20:1 to 2:1 (24) or in lean and obese women when SFA was exchanged for MUFA or (n-6) PUFA (11). Reasons for inconsistencies may be found in differences between study populations, i.e. men vs. women. Additionally, we compared mixed meals with a low (3% of total fatty acids) or high (33% of total fatty acids) linoleic acid content, whereas in the study by Manning et al. (11), differences in linoleic acid content between the cream meal and canola oil meal were less pronounced (4 vs. 19%).

IL-8 did not significantly change from baseline. In contrast, van Oostrom et al. (27) found increased concentrations of IL-8 (50% increase) 1 h after ingestion of a glucose bolus, but no significant effects after intake of a fat bolus. The latter effect, however, was much more pronounced (130% increase) and the lack of significance was therefore related to high inter-subject variations. The discrepancy between our study and the findings by van Oostrom et al. (27) might be explained by the timing of sampling. In the latter study, IL-8 concentrations were measured only at 0, 1, and 2 h after the glucose bolus and after the fat bolus. We measured IL-8 concentrations at 2-h intervals for 8 h after the intake of a mixed meal. Therefore, it is possible that we missed an early increment in IL-8 concentrations. Our findings in overweight men, however, are in agreement with the aforementioned study of Manning et al. (11), who found no early peak in IL-8 concentrations or time- or meal-dependent variations in IL-8 concentrations after 1, 4, and 6 h. These findings suggest that the type of fatty acid consumed does not acutely affect IL-8 concentrations during an 8-h postprandial period.

In our study, we found a potentially favorable reduction in TNFα concentrations after consumption of the muffins rich in linoleic acid, whereas TNFα concentrations remained stable after consuming the butter muffins and slightly increased 6–8 h postprandially after both meals. In studies with abdominally obese men (25) and with both men and women covering a broad range of adiposity (29), TNFα concentrations were significantly reduced. Unfortunately, the compositions of the test meals used in these 2 studies (25,29) were not reported, which makes a comparison with our study difficult. On the other hand, Nappo et al. (28) found a time-dependent increase in TNFα concentrations in both healthy participants and type 2 diabetic patients after consumption of a test meal rich in SFA and MUFA.

With respect to variations in TNFα concentrations, the responses in sTNF-I and -II should also be considered, although their exact roles are under debate (30–34). In our study, the response in plasma sTNF-II after consumption of both meals followed that of plasma TNFα. sTNF-I concentrations, however, were significantly reduced after consumption of both types of muffins. To the best of our knowledge, no other intervention studies have compared postprandial responses in sTNF-I and -II between meals with a different fatty acid composition.

Postprandial serum MCP-1 concentrations decreased significantly after both meals, irrespective of the type of fat consumed. In contrast, endothelial MCP-1 mRNA expression was previously shown to increase after incubation with fasting or postprandially derived TG-rich lipoproteins from hypertriglyceridemic participants (35) or chylomicron remnants (36). In vivo, high intake of mainly saturated fat increased MCP-1 concentrations in hypertriglyceridemic patients with metabolic syndrome (37). In contrast, in HIV-infected participants (38), postprandial MCP-1 concentrations were not significantly affected after an oral fat load. The reason for these inconsistencies is unknown.

In our study, we did not find time-dependent differences in sICAM-1 and sVCAM-1. However, there was a significant meal-dependent reduction in sVCAM-1 concentrations and a trend for lower sICAM-1 concentrations after the margarine muffins. In contrast, most other studies found postprandial increases in sICAM-1 and sVCAM-1 after fat loads mainly providing SFA in healthy participants, type 2 diabetic patients, and hyperlipidemic participants (28,39,40). In contrast, Rubin et al. (41) did not find postprandial variations in sICAM-1 and sVCAM-1 after a mixed meal with mainly SFA and MUFA. The authors, however, noted that this could have been the result of the high retinol content of the test meal, because increases in sICAM-1 and sVCAM-1 were found when retinol was excluded from the test meal. On the other hand, no increases or rather decreased sICAM-1 and sVCAM-1 concentrations were found in premature coronary heart disease patients and healthy controls (26) or mildly obese men (42) after a high-fat meal with MUFA or (n-6) PUFA. Thus, large differences exist between the studies for which we do not have an explanation.

In summary, our data indicate that exchanging SFA from butterfat for (n-6) PUFA in a mixed meal may decrease postprandial lipemia and affects several postprandial markers of inflammation and endothelial activation in overweight men. Postprandial glucose and insulin concentrations were not affected differently. The impact of these findings on long-term health remains to be elucidated.

Acknowledgments

We thank M. Hulsbosch and Y. Verhulst for their excellent technical assistance. C.J.M. and R.P.M. designed research; C.J.M. conducted research; C.J.M. and R.P.M. analyzed data; C.J.M. and R.P.M. wrote the paper; and R.P.M. had primary responsibility for final content. Both authors read and approved the final manuscript.

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