Atherosclerosis 225 (2012) 166–172

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

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

Effects of omega-3 fatty acids on postprandial triglycerides and monocyte activation

Stephan H. Schirmer*, Christian M. Werner, Stephan B.G. Binder, Maria E. Faas, Florian Custodis, Michael Böhm, Ulrich Laufs

Klinik für Innere Medizin III (Kardiologie, Angiologie und Internistische Intensivmedizin), Universitätsklinikum des Saarlandes, 66421 Homburg/Saar, Germany

ARTICLE INFO

Article history:
Received 21 March 2012
Received in revised form
4 September 2012
Accepted 4 September 2012
Available online 13 September 2012

Keywords:
Triglycerides
Postprandial
Polyunsaturated fatty acids
Monocytes
Atherosclerosis

ABSTRACT

Objective: Epidemiologic studies suggest that elevated postprandial triglycerides (ppTG) are associated with future cardiovascular events. Monocyte activation plays an important role in vascular diseases. Omega-3 fatty acids (n3-FA) lower fasting TG levels. The effects of n3-FA on ppTG and the role of ppTG for monocyte activation are insufficiently understood.

Methods and results: 23 healthy volunteers and 30 non-diabetic patients with documented coronary artery disease were subjected to an oral TG tolerance test (OTTT) consisting of 80 g cream fat or to water as control (H_2O). Patients were treated with 4 g n3-FA/day or placebo for 3 weeks in a randomized, placebo-controlled, double-blind, crossover study. Relative postprandial TG increase reached its maximum 4 h after fat intake (185.1 ± 10.9% of baseline). n3-FA reduced fasting TG from 137.1 ± 12.9 to 112.2 ± 8.6 mg/dl ($p < 0.05$), and maximum ppTG concentrations from 243.6 ± 24.6 to 205.8 ± 17.1 mg/dl ($p < 0.05$), while relative TG increase (192.8 ± 12.7%) was comparable to placebo. Relative monocytopenia and neutrophilia were detected following fat intake, which was unaffected by n3-FA and also detectable in the H_2O group. Serum chemotactic cytokine (MCP1 and fractalkine) concentrations and monocyte migration were not affected by fat intake or n3-FA. Monocyte activation markers CD11b and CD14, monocyte subpopulations $CD16^{+}CD14^{high}$ and $CD16^{+}CD14^{low}$, sICAM serum levels and markers of oxidative stress remained unchanged by fat intake or n3-FA.

Conclusion: The postprandial TG increase does not stimulate monocytes beyond their circadian activation patterns. n3-FA reduce fasting TG and the postprandial TG increase. n3-FA may therefore allow to prospectively study whether selected patients benefit from TG-lowering independent of LDL- and HDL-cholesterol.

1. Introduction

Serum lipids and inflammatory cells contribute to the pathogenesis of atherosclerosis. While the causal role of LDL-cholesterol in the development of atherosclerotic plaques is well established, the role of triglycerides (TG) in the pathophysiology and epidemiology of vascular disease is less clear [1]. There is an association between fasting serum TG and the incidence of cardiovascular events, however, the extent of this association is heterogeneous between studies and lost in multivariable analyses [2]. The prognostic benefit of selective TG-lowering remains a matter of debate and has never been tested in a prospective study [3]. Mechanistic studies point towards a direct effect of triglyceride-rich lipoproteins and chylomicron remnants on the vascular wall [1]. Hydrolysis of TG-rich lipoproteins (TRL) yields small cholesterol-enriched remnant lipoprotein particles that have been shown to exert atherogenic effects and the lipolysis of TG-rich lipoproteins along the vessel wall produces potentially toxic oxidized fatty acids [1]. Many years ago, atherosclerosis was suggested to be a postprandial disease [4]. In fact, recent epidemiologic studies (Women's Health Study [5], Copenhagen City Heart Study [6]) suggest that postprandial (ppTG) rather than fasting TG are associated with cardiovascular events in primary prevention.

Circulating inflammatory cells, particularly monocyte activation, play an important role in lipid-mediated vascular diseases [7]. Mononuclear cells become attracted to sites of plaque formation via specific chemokines and bind to endothelial adhesion molecules.

* Corresponding author. Klinik für Innere Medizin III, Kardiologie, Angiologie und Internistische Intensivmedizin, Universitätsklinikum des Saarlandes, Kirrberger Straße, 66424 Homburg/Saar, Germany. Tel.: +49 6841 1623000; fax: +49 6841 1623434.
E-mail address: Stephan.schirmer@uks.eu (S.H. Schirmer).

0021-9150/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.atherosclerosis.2012.09.002

An inflammatory environment contributes to the progression and destabilization of the atherosclerotic plaque and monocyte functional state may influence the severity of atherosclerotic vascular disease [8]. Uncontrolled studies of healthy volunteers report an activation and enhanced adhesion of monocytes following hypertriglyceridemia [9–11].

Omega-3 fatty acids (n3-FA) at a higher dose (3–4 g daily) are known to lower fasting TG levels by about 30% [12], while their effects on non-fasting, postprandial TG (ppTG) metabolism have not been systematically characterized in non-hyperlipidemic patients with coronary artery disease (CAD). N3-FA have been described to have anti-inflammatory effects in blood monocytes [13]. Therefore, this study aimed to investigate ppTG following treatment with n3-FA and to dissect the effects of ppTG on monocyte activation as a possible mechanism of TG-stimulated atherosclerosis using a standardized test protocol in controls and CAD patients.

2. Methods

2.1. Subjects

This study was approved by the institutional medical ethics committee (Nr. 170/07), and informed consent was obtained from all participants. First, 23 healthy volunteers were enrolled. Subjects exhibited no signs of cardiovascular, inflammatory or malignant disease, had no lipid disorders and were not on continuous drug treatment. In addition, 30 patients with angiographically documented stable CAD were studied. No CAD patient had diabetes as confirmed by oral glucose tolerance test and HbA1c measurements.

2.2. Triglyceride tolerance test

Healthy controls and patients were subjected to an oral TG tolerance test of 250 ml cream (OTTT) or to water as control (H_2O) in a crossover design. Ingested cream consisted of 80 g cream fat (31.9–32.5 g per 100 ml), 5.75 g protein, 7.75 g carbohydrates and 0.1 g sodium. All study participants fasted overnight and drank liquid cream or water at 8 o'clock in the morning. Blood was drawn after a 5 min rest in supine position before (0 h) and 3, and 5 h following fat load (controls) and at 0, 3, 4 and 5 h (patients) for measurements of serum lipids and assessment of inflammatory parameters.

The following parameters of triglyceride metabolism were assessed: fasting TG concentration, absolute TG maximum, absolute increase of TG (in mg/dl), relative TG increase (maximal TG/fasting TGx100). Also, the TG curve was integrated to calculate the 5 h area under the curve (AUC). Furthermore, total and HDL-cholesterol were measured.

2.3. Inflammatory and monocyte parameters

C-reactive protein and serum cortisol were assessed on a Cobas 8000 (Roche, Grenzach-Wyhlen, Germany), differential blood counts were assessed on a XE-5000 (Sysmex, Norderstedt, Germany). From 200 μl of blood drawn at each time point, flow cytometric analysis of monocyte activation and composition was performed. Blood was processed within 20 min after blood drawing to avoid ex-vivo activation. Whole blood was stained using monoclonal antibodies against CCR2 (RnD Systems, Wiesbaden, Germany), CD14, CD11b, CD16, CX3CR1 (all Serotec, Düsseldorf, Germany) at 4° for 45 min. Following red blood cell lysis and washing, samples were subjected to fluorescence activated cell sorting (FACS) analysis in a FACSCalibur (BD Biosciences, Heidelberg, Germany). Plasma concentrations of the chemotactic cytokines MCP1 and fractalkine and soluble intercellular adhesion molecule-1 (sICAM1) were determined using ELISA kits (RnD Systems). Plasma oxidative stress was estimated by measuring levels of 8-isoprostane (Cayman Chemicals, Tallinn, Estonia), plasma lipid peroxidation was assessed in a TBARS (thiobarbituric acid reactive substances) assay (RnD Systems) measuring malondialdehyde (MDA). Migration of THP1 monocytes towards 10% serum of study participants (diluted in RPMI cell culture medium) was tested using a fluorometric cell migration assay (Innocyte, Calbiochem, Darmstadt, Germany). To assess function of the test, migration towards 20% foetal calf serum (FCS) was used as a positive control, 0% serum was used as negative control. Migrated cells were quantified using a fluorescence reader (Infinite 200 pro, Tecan, Männedorf, Switzerland). Peripheral blood monocytes were isolated using Ficoll density gradient centrifugation and immunomagnetic negative isolation as previously described [14]. Following RNA-isolation and reverse transcription, mRNA expression of inflammatory cytokines IL1b, IL6, TNFalpha and MCP1 were measured using real-time RT-PCR.

2.4. Randomized crossover study design

30 non-diabetic patients with stable CAD were treated with 4 g n3-FA/day or placebo for 3 weeks in a randomized, placebo-controlled, double-blind, crossover study. Randomization was performed by the pharmacy of the Universitätsklinikum des Saarlandes. N3-FA (Zodin®) was provided as red–brown soft capsules of 1 g by the manufacturer (Trommsdorff GmbH, Alsdorf, Germany). Placebo capsules containing olive oil were made with identical look by Pronova BioPharma, Lysakar, Norway. Placebo was tested to contain no eicosapentaenoic or docosahexaenoic acid.

Patients in each group (n3-FA or placebo) were treated for 3 weeks. A three week washout phase was allowed before then switching between n3-FA and placebo. The treatment drug contained 92% omega-3 fatty acids (n3-FA), of which were 470 mg eicosapentaenoic acids (EPA) and 371 mg docosahexaenoic acids (DHA) and was administered as 2 × 2 tablets of 1 g each. Adherence to study medication was assessed by pill counting. Before (day 0) and after the 21 day treatment period (d21) with either n3-FA or placebo, patients underwent OTTT including assessment of lipid and inflammatory parameters as described above.

2.5. Statistical analysis

Continuous variables were tested for normal distribution by the Kolmogorov–Smirnov test. Parameters were analysed in the relation to baseline following OTTT using repeated measures ANOVA. In the patient study, differences between treatment groups were analysed. Changes in triglyceride concentrations or inflammatory parameters over time were compared between treatment groups by general linear model for repeated measurements using Wilks Lambda for overall and Dunett test for post-hoc comparisons. A p-value <0.05 was considered significant. Statistical analyses were performed with SPSS version 18.0.

3. Results

3.1. Healthy controls – effects of OTTT on monocyte activation

Healthy controls were 26.4 ± 1.1 years old, 11 of them (48%) were male, mean body mass index was 23.0 ± 0.6 kg/m^2. All healthy volunteers completed the OTTT. Total cholesterol levels were 174.6 ± 8.4 mg/dl before OTTT, and remained unchanged in the 5 h after fat load. Similarly, mean HDL- and LDL- (calculated from Friedewald formula) cholesterol concentrations were

unaffected by OTTT. Baseline HDL-cholesterol was 58.9 ± 6.6 mg/dl, LDL-cholesterol 99.3 ± 8.6 mg/dl. Serum triglyceride concentration was 89.7 ± 8.1 mg/dl before OTTT and rose to a maximum of 155.5 ± 13.3 mg/dl after 3 h ($p < 0.001$ compared to baseline, 73% increase), before concentrations fell again but remained elevated at 5 h (130.7 ± 12.3 mg/dl, $p < 0.01$ compared to baseline). No change in serum triglycerides was observed in the group that received water. Fig. 1 depicts the effects of OTTT on lipid levels in healthy individuals.

To evaluate potential inflammatory effects of postprandial lipids, we first evaluated leukocyte composition. Differential blood count revealed neutrophilia, lymphocytopenia and monocytopenia 3 h after fat load. Neutrophil count rose from 3011 ± 176 to 4116 ± 252/μl ($p < 0.001$) 3 h after OTTT, but also from 3231 ± 261 to 4172 ± 538/μl in the control (H_2O) group ($p < 0.001$). There was no correlation between TG and neutrophils (data not shown). Absolute lymphocyte counts decreased from 2611 ± 187/μl before to 1989 ± 73/μl ($p < 0.001$) 3 h after OTTT, and from 2547 ± 231 to 2109 ± 118/μl in the control group ($p < 0.05$). Monocyte numbers decreased from 537 ± 33 to 487 ± 34/μl ($p < 0.01$) 3 h after OTTT, and from 557 ± 35 to 453 ± 38 in the control group ($p < 0.001$) (Fig. 2).

Because of changes in leukocyte composition and their particular role in atherogenesis we next examined the effects of postprandial triglycerides on monocyte activation and subsets. We could not observe relevant changes in the expression of the surrogate activation markers CD11b or CD14 on monocyte surface following OTTT. In the control group, expression of CD14 fell from a mean fluorescence intensity (MFI) of 486 ± 57 to 414 ± 57 ($p < 0.01$). Neither the monocyte subpopulation $CD16^+CD14^{low}$ (regenerative subtype) nor $CD16^+CD14^{high}$ (inflammatory subtype) was affected in the 5 h observation period following OTTT. To investigate if diurnal regulating mechanisms could be the cause for monocyte changes in the control group, we investigated serum cholesterol concentrations, which fell during morning hours as expected and were not affected by ppTG (Fig. 2).

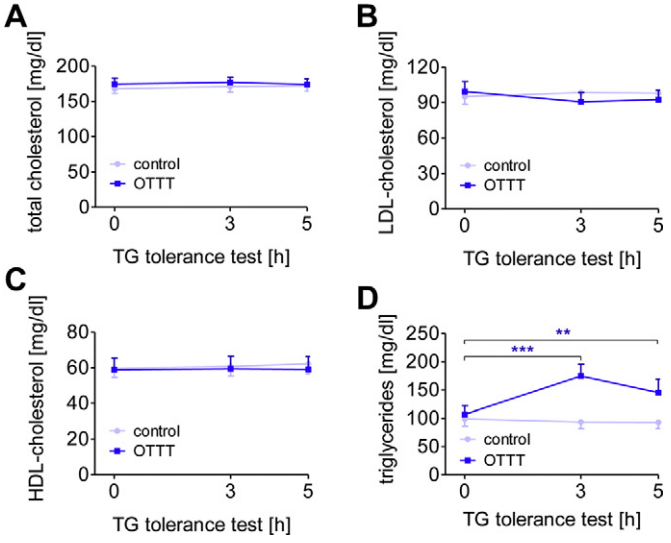

Fig. 1. *Triglyceride tolerance test in healthy controls.* Total (A), LDL- (B) and HDL-cholesterol (C) were unchanged following oral triglyceride tolerance test in healthy control subjects. Triglycerides increased to their maximum at 3 h following oral triglyceride tolerance test (dark blue bars), which was absent in the control group (light blue bars) (D). $^{**}p < 0.01$, $^{***}p < 0.001$ versus baseline. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

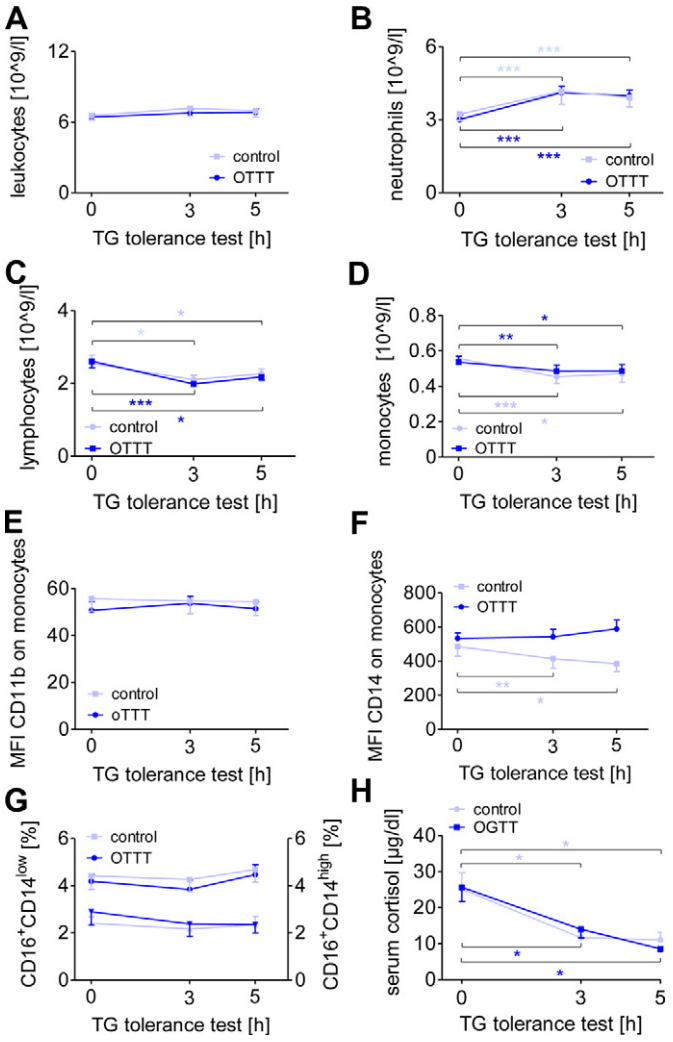

Fig. 2. *Leukocyte counts, monocyte activation markers and subpopulations and serum cortisol concentrations in healthy individuals.* Neutrophils (B) increased, while lymphocytes (C) and monocytes (D) decreased both after oral triglyceride tolerance test and after ingestion of water in healthy controls, while total leukocytes (A) increased only mildly. Oral triglyceride tolerance test did not influence monocyte surface expression of activation marker CD11b (E). Surface expression of CD14 decreased in the course of time (controls). This decrease was abrogated following OTTT (F). Monocyte 16^+CD14^{low} (upper lines)and $CD16^+CD14^{high}$ (lower lines) subpopulations were unaffected by fat loading (G). Serum cortisol decreased during the test hours (8:00–13:00) independent of fat ingestion (H). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus baseline in OTTT (dark blue) or control (light blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

For monocytes, the cytokines chemoattractant protein-1 (MCP1) and fractalkine (CX3CL1) are strong chemoattractant proteins. MCP1 concentration in serum of the control group (H_2O) fell from 254 ± 28 to 189 ± 21 pg/ml after 3 h ($p < 0.001$) and from 251 ± 24 to 198 ± 33 pg/ml ($p < 0.05$) following OTTT. Fractalkine serum concentrations remained unchanged. The MCP1-receptor CCR2 decreased on monocytes from control patients (from a MFI of 25.8 ± 2.7 to 19.4 ± 2.3 after 3 h ($p < 0.001$)), which was not detected following OTTT. Similarly, the fractalkine-receptor CX3CR1 decreased in the control group over time (from a MFI of 116 ± 7 to 84 ± 5 after 5 h ($p < 0.001$)), and decreased to a smaller extent in the OTTT group. At the functional level, reflecting MCP1 expression, THP1 monocyte migration towards participants' serum was not significantly affected by OTTT compared to the control group (Fig. 3).

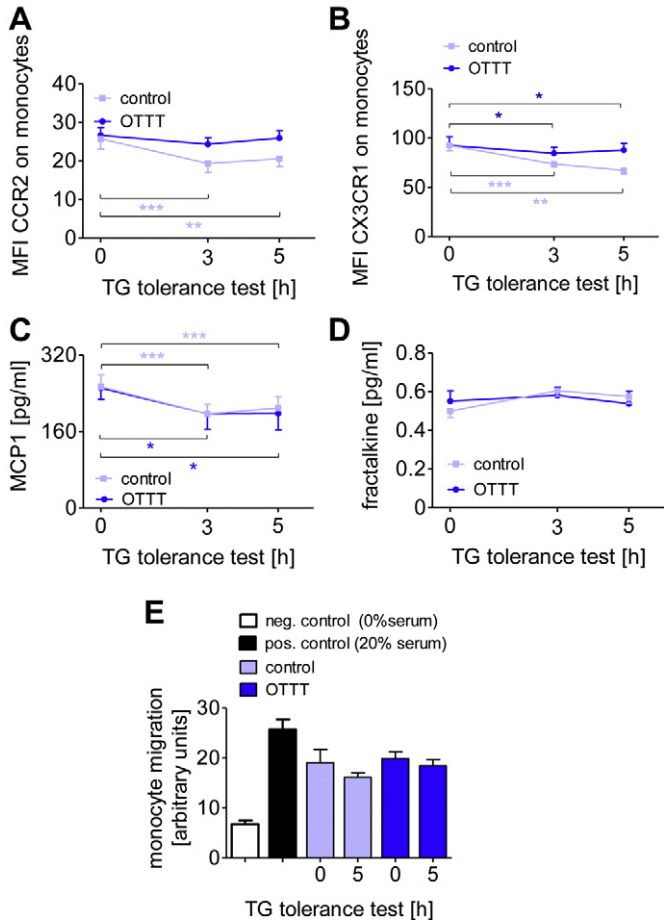

Fig. 3. *Chemokines and monocyte migration following triglyceride tolerance test*. Monocyte surface expression of the MCP1-receptor CCR2 (A) and the fractalkine-receptor CX3CR1 (B) decreased during the morning hours of the test when subjects ingested water. Oral triglyceride tolerance test abolished this decrease. Monocyte chemoattractant protein-1 (MCP1) was reduced following fat loading but also after water ingestion (C). Fractalkine (D) was unchanged following fat loading. THP1 monocyte migration (compared to 0% or 20% foetal calf serum (negative and positive control, respectively)) towards participants' serum was not significantly affected following fat loading (E). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus baseline in OTTT (dark blue) or control (light blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Because surface marker measurements using flow cytometry are only surrogate markers of cellular activation and to evaluate if monocyte activation status would potentially not be directly influenced by ppTG, but whether TG might render circulating cells more or less susceptible to other inflammatory stimuli, isolated peripheral blood monocytes were treated with LPS following fat load. Following LPS-stimulation, mRNA expression of inflammatory cytokines IL1b, IL6, TNFalpha and MCP1 was not significantly changed (data not shown).

3.2. Patient study – effects of n3-FA on postprandial triglycerides and monocyte activation

One of the 30 patients undergoing treatment with n3-FA withdrew consent because of inability to complete the OTTT (nausea and vomiting). The remaining 29 patients were 66.7 ± 7.5 years old, 22 of them (76%) were male, and mean body mass index was 27.4 ± 3.4. None of the patients had diabetes but 13 (45%) had impaired glucose tolerance in an OGTT. The majority of patients was treated according to guidelines for secondary prevention: all patients received aspirin, and 25 (90%) took a statin. Baseline LDL-cholesterol was 95.8 ± 21.4 mg/dl and serum triglycerides 128.8 ± 48.2 mg/dl. For further baseline characteristics see Supplemental Table 1.

OTTT and n3-FA did not affect total, LDL- or HDL-cholesterol. Serum glucose and insulin concentrations changed slightly during OTTT, but were not affected in a biologically relevant magnitude when compared to a glucose tolerance test started at 3 h into the OTTT protocol (Supplemental Fig. 1). TG rose from 137.1 ± 12.9 mg/dl at baseline to their maximum at 4 h of 244.8 ± 24.6 md/dl ($p < 0.001$, 78% increase). Three week treatment with 4 g n3-FA/day reduced fasting TG by 24.9 mg/dl (18%)–112.2 ± 8.6 mg/dl ($p < 0.05$ compared to placebo). Postprandial TG 3, 4 and 5 h following OTTT were reduced by n3-FA ($p < 0.05$). Maximum TG were reduced by 38.9 mg/dl (16%)–205.86 mg/dl ($p < 0.05$). Calculation of the 5 h area under the curve showed a reduction following n3-FA (873.3 ± 59.4 versus 1033.0 ± 86.7 mg/dl following placebo, $p < 0.05$). Relative TG increase (maximum 192.8 ± 12.7%) following n3-FA was comparable to placebo (183.2 ± 9.3%, p = ns) (Fig. 4).

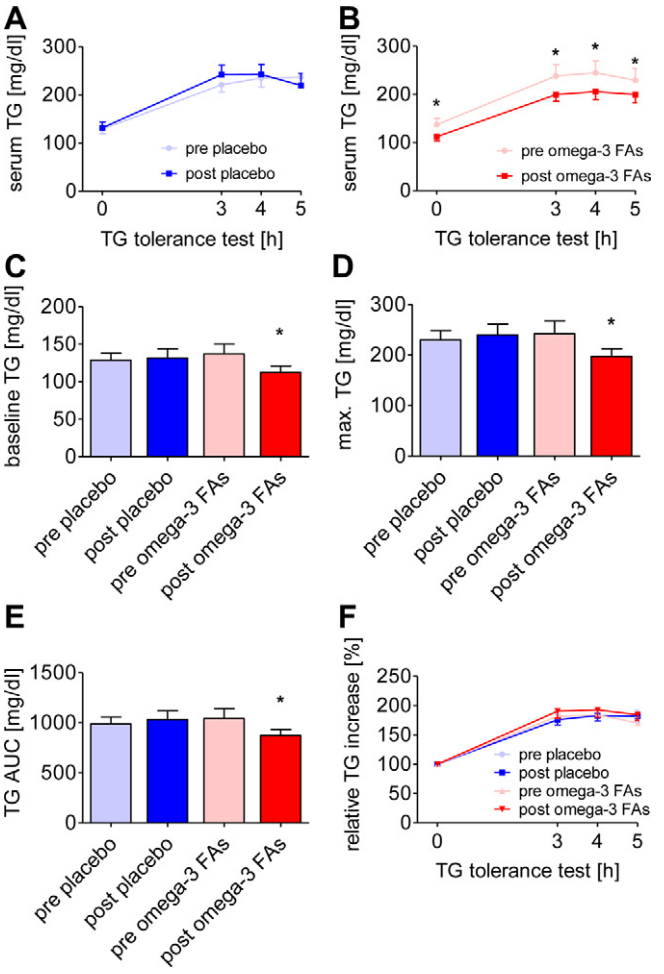

Fig. 4. *Effects of n3-FA on fasting and postprandial triglycerides*. In the patient study, significant differences between treatment groups are marked in the graphs. While no change was noted following placebo treatment (A), treatment with omega-3 fatty acids reduced both fasting and postprandial triglyceride levels (B). The bar graphs separately show reduction of fasting triglycerides by omega-3 fatty acids (C) and maximum triglycerides after oral triglyceride tolerance test (D). The 5 h area under the curve was reduced following treatment (E). Plotting the relative triglyceride increases demonstrated that curves were parallel shifted without significant change by omega-3 fatty acids (F). $^{*}p < 0.05$, post-treatment versus pre-treatment.

Because of its predictive value in cardiovascular disease [15], neutrophil/lymphocyte count and neutrophil/monocyte counts were calculated. Neutrophil/lymphocyte ratio increased 3 h following OTTT from 1.86 ± 0.15 to 2.56 ± 0.24, $p < 0.001$ compared to baseline and fell again thereafter. Treatment with n3-FA did not affect this increase (increase from 1.85 ± 0.14 to 2.40 ± 0.23, $p < 0.01$). Similarly, neutrophil/monocyte ratio increased to its maximum 3 h following OTTT from 6.27 ± 0.40 to 8.15 ± 0.66, $p < 0.001$ compared to baseline, which remained unaffected by n3-FA (increase from 6.77 ± 0.44 to 8.64 ± 0.72, $p < 0.01$). As in healthy individuals, OTTT in patients with CAD did not change monocyte $CD16^{+}CD14^{low}$ and $CD16A^{+}CD14^{high}$ subpopulations. These monocyte populations were not affected by treatment with n3-FA (Fig. 5).

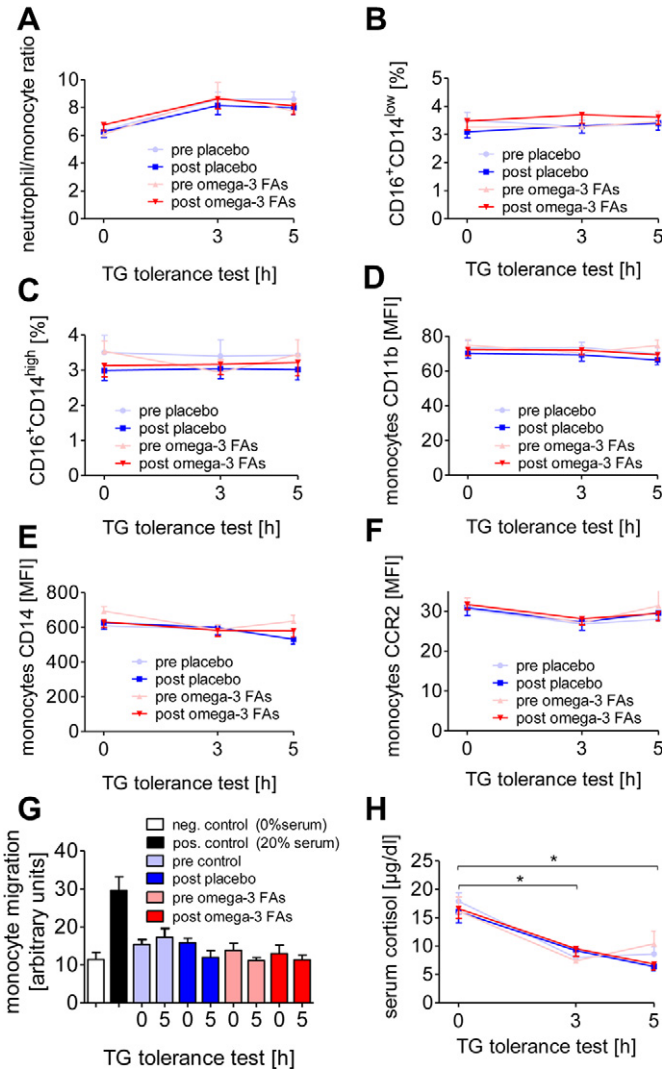

Fig. 5. *Leukocyte subpopulations, monocyte migration and activation in coronary artery disease patients.* Calculations of inflammatory ratios in peripheral blood showed an increase in neutrophil/monocyte ratio (A) following oral triglyceride tolerance test in patients with coronary artery disease. This increase was not attenuated by treatment with omega-3 fatty acids. Both $16^{+}CD14^{low}$ (B) and $CD16^{+}CD14^{high}$ (C) monocyte subpopulations were not affected by the triglyceride tolerance test or treatment with omega-3 fatty acids. Surface marker expression of the activation marker CD11b (D) and of CD14 on monocytes (E) was unchanged by either triglycerides or omega-3 fatty acids treatment. Also, the MCP1-receptor CCR2 on monocytes was not changed (F). Migration of THP1 monocytes towards patients' serum (compared to 0% or 20% foetal calf serum (negative and positive control, respectively)) was not significantly affected by fat loading or omega-3 fatty acids (G). Decrease in serum cortisol during the test hours (8:00–13:00) was unaffected by omega-3 fatty acids (H).

Flow cytometric analyses of monocyte surface markers CD11b, CD14 and the MCP1-receptor CCR2 as surrogate activation markers demonstrated these molecules to remain unchanged in the course of OTTT and unaffected by treatment with n3-FA. THP1 monocyte migration towards patients' serum was not changed by n3-FA. To assess if circadian rhythms as a possible cause for monocyte and inflammatory changes during the day were affected by n3-FA, serum cortisol concentrations were measured. Data show a decrease during morning hours that was not affected by n3-FA (Fig. 5).

Because circulating cell composition and activation status seemed untouched by either fat load or treatment with n3-FA we then examined plasma levels of soluble intercellular adhesion molecule-1 (sICAM1) as a marker of endothelial cell activation. As parameters of oxidative stress, 8-isoprostane [16] and MDA (assessing plasma lipid peroxidation) were measured. Neither plasma concentration of sICAM1 nor 8-isoprostane or MDA levels were significantly different 5 h following OTTT. Both parameters were unaffected by treatment with n3-FA. Also, C-reactive protein was found comparable between placebo and n3-FA before and after treatment (Supplemental Fig. 2).

4. Discussion

In this study, a standardized oral triglyceride tolerance test (OTTT) increased serum TG by 73% in healthy controls and 78% in non-hyperlipidemic patients with coronary artery disease, without affecting cholesterol concentrations. Leukocyte subpopulations and monocyte activation were only mildly affected by the fat intake. Treating stable, non-diabetic, non-hyperlipidemic CAD patients with omega-3 fatty acids in a randomized, double-blind, crossover trial decreased not only fasting but also postprandial TG by 18 and 16%, respectively. Compared to placebo, three weeks treatment with n3-FA did not alter fasting and postprandial leukocyte composition and parameters of monocyte activation and migration.

On the background of the epidemiologic data on the predictive value of non-fasting triglycerides in cardiovascular disease [5,6] we hypothesized that postprandial TG may influence monocyte activation and thereby contribute to the pathophysiology of atherosclerotic vascular disease. Postprandial TG may accelerate atherogenesis by entering the vessel wall as TG-rich remnant particles promoting the generation of macrophage-derived foam cells [17]. In addition, lipolysis of TG-rich lipoproteins generates inflammatory lipids that may contribute to monocyte activation [18]. Monocyte activation represents an important component of vascular inflammation promoting atherogenesis [7]. In contrast to the initial hypothesis of the study, our analyses do not provide evidence for a stimulatory effect of triglycerides on monocyte activation and migration parameters. The observed parallel postprandial changes after water ingestion likely reflect the circadian regulation of inflammatory status, which may relate to changing levels of cortisol during the day [19]. As with monocyte parameters, cortisol levels were unaffected by n3-FA. Alternatively, several hours fasting in the morning period may be a stressor of similar strength as postprandial hypertriglyceridemia in otherwise normolipidemic individuals. If this is a finding of chance or functional significance remains to be elucidated in further studies on the circadian regulation of monocyte function. MCP1 and monocyte migration were reduced following OTTT, indicating even a possible buffering effect of postprandial triglycerides on inflammatory cytokines. Other authors found more pronounced effects on monocyte surface marker expression following fat ingestion [11]. When studying the influence of TG on inflammatory cells, it is important to use a glucose-free test, because glucose is known to elicit increases in CD11b monocyte surface marker expression [20].

Decrease in glucose and rise in insulin concentrations over time were very mild and within the physiological range of these parameters, making a biologically relevant effect unlikely. Other studies of fat-induced monocyte activation in healthy volunteers used similar amounts of ingested oral fat loading. Some, however, lack a control group and are therefore difficult to interpret [9]. To prevent analytical errors, we have eliminated unwanted cellular activation by processing blood samples directly after blood drawing and performing all laboratory steps at 4 °C.

Low dose (1 g/day) n3-FA have been analysed in large studies on secondary preventive effects on cardiovascular events [21–23]. This treatment form has been suggested to exert "pleiotropic effects" including stabilization of cellular membrane potentials, however, the detailed molecular mechanisms are incompletely understood [24]. While low dose therapy does not affect lipid levels, high-dose (3–4 g/day) treatment with n3-FA selectively lowers TG [25]. The effect of the high-dose n3-FA used in clinical practise on postprandial TG kinetics has not been previously studied in detail. More than 20 years ago, a reduction in ppTG has been described in subjects on a fish oil diet containing n3-FA [26]. Our test protocol and the randomized, crossover study now shows that treatment of non-diabetic CAD patients with 4 g/d n3-FA reduces ppTG to a similar extent as fasting TG.

In line with the paucity of effects of ppTG on inflammatory parameters in healthy young controls, no quantitatively impressive changes were observed in non-diabetic patients with stable CAD either. The increase in neutrophils parallelled by a reduction in lymphocytes and monocytes in peripheral blood are most likely due to diurnal regulation similar to the healthy individuals studied, and possibly triggered by physiological changes in serum cortisol. Treatment with n3-FA did not change leukocyte composition, activation status or migration of peripheral blood monocytes. Particularly, surface expression of adhesion molecules, which are thought to mechanistically contribute to a pro-atherogenic role of TG [27], were unchanged. Activated cells adhere to the endothelium, become part of the marginal pool and can thus be missed in the analysis of circulating cells and cytokines. The counterpart of circulating cells in atherosclerotic vascular disease, the vessel wall, is more difficult to investigate in humans. As a surrogate parameter, we studied levels of sICAM1, which are known to reflect endothelial activation and to predict future cardiovascular events [28], and 8-isoprostane and MDA as markers of oxidative stress. The effects of n3-FA on lipid peroxidation were earlier investigated elsewhere, but results remained inconclusive or reported an increase in oxidative stress by n3-FA [29,30]. In our study, vascular parameters were not altered by n3-FA, suggesting that n3-FA treatment did not change monocyte or endothelial activation. The three weeks treatment period might be too short for an effect on the vessel wall. For circulating monocytes, we assumed cells to have renewed completely due to turnover in peripheral blood within this time frame.

Importantly, 90% of the study patients were on statin treatment and showed LDL-cholesterol serum concentrations <100 mg/dl. It is therefore possible that statin treatment had already reduced the response of circulating inflammatory cells to TG [31]. However, from the clinical perspective this is the relevant patient population to study. Furthermore, we observed similar monocyte activation patterns in both patients on statin and healthy controls without drug treatment. Subanalyses within our patient population showed similar patterns of ppTG and inflammatory parameters in statin users and non-users at a qualitative level, although the latter failed to meet statistical significant because of low numbers.

Perhaps the most important limitation of the study is the time course of OTTT. All parameters assessed were measured no earlier than 3 and no later than 5 h following fat loading. Changes occurring before or, more importantly, after this time period, might have been missed. Earlier studies have already shown that n3-FA lower ppTG. However, they less rigorously applied controls or less standardized postprandial test protocols or only investigated healthy controls or patients not on statin therapy or hyperlipidemic subjects [32–34]. We extended the standardized analyses of the effects n3-FA in non-diabetic, non-hyperlipidemic statin-treated CAD patients to measures of postprandial monocyte activation and oxidative stress. As in every study reporting negative findings we cannot exclude that the parameters used (such as monocyte expression of the Mac1-subunit CD11b expression) are suboptimal and our experiments thus miss inflammatory activation via other routes. However, by looking at subpopulations, surface markers as well as secreted cytokines and their receptors, although each can be considered as surrogate for cellular activation only, we tried to overcome this limitation.

In conclusion, our study shows that high-dose n3-FA selectively reduce both fasting and postprandial TG in patients with cardiovascular disease on statin treatment. Monocyte activation and migration do not appear to be relevant mechanisms behind possible TG-mediated effects on vascular pathophysiology. n3-FA provide an ideal tool to prospectively study whether selected patients benefit from TG-lowering independent of LDL- and HDL-cholesterol.

Acknowledgements

This study was supported by a research grant of the Solvay foundation to SHS. The Universität des Saarlandes received a research grant from Trommsdorff.

We would like to thank Julia Marhofer and Ellen Becker for expert technical assistance.

Supplemental Table 1

Characteristics	Patients ($n = 29$), mean ± SD
Age – years	66.7 ± 7.5
Male sex. – no. (%)	22 (75.9)
Body mass index (BMI)	27.4 ± 3.4
Waist circumference – cm	100.6 ± 10.5
Hip circumference – cm	105.5 ± 6.4
Waist/hip ratio	1.0 ± 0.1
Body surface area (BSA) – m^2	1.94 ± 0.17
Blood pressure – mmHg	128.9/68.4 ± 17.3/8.5
Heart rate – per min.	66.7 ± 8.6
Hypertension – no. (%)	29 (100)
Hypercholesterolaemia – no. (%)	28 (96.6)
Family history of CAD – no. (%)	12 (41.4)
Current smoker – no. (%)	4 (13.8)
Ex-smoker – no. (%)	14 (48.3)
Diabetes – no. (%)	0 (0)
Impaired glucose tolerance – no. (%)	13 (44.8)
Beta-blockers – no. (%)	28 (96.6)
Statins – no. (%)	25 (89.7)
Aspirin – no. (%)	29 (100)
ACE-inhibitors/ARBs – no. (%)	23 (79.3)
Diuretics – no. (%)	14 (48.3)
C-reactive protein – mg/l	2.0 ± 0.4
Glucose – mg/dl	107.2 ± 11.6
Total cholesterol – mg/dl	167.1 ± 4.7
LDL-cholesterol – mg/dl	95.8 ± 21.4
HDL-cholesterol – md/dl	51.8 ± 13.3
Non-HDL-cholesterol – mg/dl	111.3 ± 5.7
Triglycerides – mg/dl	128.8 ± 48.2

Baseline characteristics of study patients. Parameters are depicted as mean ± SEM.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2012.09.002.

References

[1] Goldberg IJ, Eckel RH, McPherson R. Triglycerides and heart disease. Arterioscler Thromb Vasc Biol 2011;31:1716–25.
[2] Miller M, Stone NJ, Ballantyne C, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation 2011;123:2292–333.
[3] Chapman MJ, Ginsberg HN, Amarenco P, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. Eur Heart J 2011; 32:1345–61.
[4] Zilversmit DB. Atherogenesis: a postprandial phenomenon. Circulation 1979; 60:473–85.
[5] Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. J Am Med Assoc 2007;298:309–16.
[6] Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. J Am Med Assoc 2007;298:299–308.
[7] Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 2006;6:508.
[8] Schirmer SH, Fledderus JO, van der Laan AM, et al. Suppression of inflammatory signaling in monocytes from patients with coronary artery disease. J Mol Cell Cardiol 2009;46:177–85.
[9] Alipour A, van Oostrom AJHHM, Izraeljan A, et al. Leukocyte activation by triglyceride-rich lipoproteins. Arterioscler Thromb Vasc Biol 2008;28:792–7.
[10] Gower RM, Wu H, Foster GA, et al. CD11c/CD18 expression is upregulated on blood monocytes during hypertriglyceridemia and enhances adhesion to vascular cell adhesion molecule-1. Arterioscler Thromb Vasc Biol 2011;31: 160–6.
[11] van Oostrom AJ, Rabelink TJ, Verseyden C, et al. Activation of leukocytes by postprandial lipemia in healthy volunteers. Atherosclerosis 2004;177:175–82.
[12] Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. Atherosclerosis 2006;189:19–30.
[13] Lee JY, Plakidas A, Lee WH, et al. Differential modulation of toll-like receptors by fatty acids: preferential inhibition by n-3 polyunsaturated fatty acids. J Lipid Res 2003;44:479–86.
[14] Schirmer SH, Fledderus JO, Bot PT, et al. Interferon-beta signaling is enhanced in patients with insufficient coronary collateral artery development and inhibits arteriogenesis in mice. Circ Res 2008;102:1286–94.
[15] Azab B, Zaher M, Weiserbs KF, et al. Usefulness of neutrophil to lymphocyte ratio in predicting short- and long-term mortality after non-ST-elevation myocardial infarction. Am J Cardiol 2010;106:470–6.
[16] Morrow JD, Frei B, Longmire AW, et al. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med 1995;332:1198–203.
[17] Zilversmit DB. A proposal linking atherogenesis to the interaction of endothelial lipoprotein lipase with triglyceride-rich lipoproteins. Circ Res 1973;33: 633–8.
[18] Chung BH, Segrest JP, Smith K, Griffin FM, Brouillette CG. Lipolytic surface remnants of triglyceride-rich lipoproteins are cytotoxic to macrophages but not in the presence of high density lipoprotein. A possible mechanism of atherogenesis? J Clin Invest 1989;83:1363–74.
[19] Petrovsky N, McNair P, Harrison LC. Diurnal rhythms of pro-inflammatory cytokines: regulation by plasma cortisol and therapeutic implications. Cytokine 1998;10:307–12.
[20] Sampson MJ, Davies IR, Brown JC, Ivory K, Hughes DA. Monocyte and neutrophil adhesion molecule expression during acute hyperglycemia and after antioxidant treatment in type 2 diabetes and control patients. Arterioscler Thromb Vasc Biol 2002;22:1187–93.
[21] Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 1999;354:447–55.
[22] Tavazzi L, Maggioni AP, Marchioli R, et al. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. Lancet 2008;372:1223–30.
[23] Rauch B, Schiele R, Schneider S, et al. OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. Circulation 2010;122:2152–9.
[24] De Caterina R. n-3 fatty acids in cardiovascular disease. N Engl J Med 2011; 364:2439–50.
[25] Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. J Lipid Res 2003;44:455–63.
[26] Harris WS, Connor WE, Alam N, Illingworth DR. Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. J Lipid Res 1988;29: 1451–60.
[27] Zhang WY, Schwartz E, Wang Y, Attrep J, Li Z, Reaven P. Elevated concentrations of nonesterified fatty acids increase monocyte expression of CD11b and adhesion to endothelial cells. Arterioscler Thromb Vasc Biol 2006;26:514–9.
[28] Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet 1998;351:88–92.
[29] Carrepeiro MM, Rogero MM, Bertolami MC, Botelho PB, Castro N, Castro IA. Effect of n-3 fatty acids and statins on oxidative stress in statin-treated hypercholestorelemic and normocholesterolemic women. Atherosclerosis 2011;217:171–8.
[30] Rumley AG, Woodward M, Rumley A, Rumley J, Lowe GDO. Plasma lipid peroxides: relationships to cardiovascular risk factors and prevalent cardiovascular disease. QJM 2004;97:809–16.
[31] Tuomisto TT, Lumivuori H, Kansanen E, et al. Simvastatin has an anti-inflammatory effect on macrophages via upregulation of an atheroprotective transcription factor, Kruppel-like factor 2. Cardiovasc Res 2008;78: 175–84.
[32] Griffin MD, Sanders TA, Davies IG, et al. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45–70 y: the OPTILIP study. Am J Clin Nutr 2006;84:1290–8.
[33] Tinker LF, Parks EJ, Behr SR, Schneeman BO, Davis PA. (n-3) fatty acid supplementation in moderately hypertriglyceridemic adults changes postprandial lipid and apolipoprotein B responses to a standardized test meal. J Nutr 1999;129:1126–34.
[34] Westphal S, Orth M, Ambrosch A, Osmundsen K, Luley C. Postprandial chylomicrons and VLDLs in severe hypertriacylglycerolemia are lowered more effectively than are chylomicron remnants after treatment with n-3 fatty acids. Am J Clin Nutr 2000;71:914–20.