Individual saturated fatty acids and cardiovascular risk markers : studies in healthy women and men

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studies in healthy women and men

Elisabeth Helena Maria Temme

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Individual saturated fatty acids and cardiovascular risk markers

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Voor Chris en Riet

Abstract

Individual saturated fatty acids and cardiovascular risk markers: studies in healthy women and men

PhD thesis by Elisabeth HM Temme, Department of Human Biology, Maastricht University, Maastricht, The Netherlands, December 19, 1997.

A high intake of saturated fatty acid is associated with increased risk for cardiovascular diseases (CVD). Although it has been postulated that individual saturated fatty acids may have dissimilar effects on lipid and lipoprotein concentrations, those of some (MCFA and lauric acid) have hardly been studied, whereas results of others (myristic or palmitic acids) are conflicting. The effects of the individual saturated fatty acids on hemostatic factors have not been examined in detail, while mainly young and lean male subjects were studied. In two studies we, therefore, compared the effects of individual saturated fatty acids (MCFA, lauric acid, myristic acid and palmitic acid) with those of a monounsaturated fatty acid (oleic acid).

Eighteen women and fourteen men participated in the first study with a cross-over design, and consumed three 6 wk experimental diets enriched in lauric, palmitic or oleic acids. Thirty-seven women and twenty three men participated in the second study with a parallel design and consumed a 3 wk run-in diet enriched in oleic acid and 6 wk experimental diets enriched in either MCFA, or myristic acid or oleic acid. The calculated nutrient composition was the same in each diet except for 8.5 En% (study 1) and 10 En% (study 2) which was provided by lauric, palmitic or oleic acids (study 1) or MCFA, myristic or oleic acids (study 2).

Compared with a diet rich in oleic acid, diets rich in myristic acid raised LDL cholesterol concentrations most pronounced followed by lauric and then palmitic acid rich diets. Lauric and myristic acid enriched diets, in addition, increased HDL cholesterol concentrations whereas diets enriched in palmitic acid or MCFA had similar effects compared with oleic acid. Serum lipoprotein(a) concentrations were lower with the myristic acid diet compared with the oleic acid diets, but did not change with the MCFA, lauric or palmitic acid diets. The individual saturated fatty acids did not affect *in vitro* whole blood aggregation velocity compared with oleic acid. Lauric, myristic and palmitic acid significantly increased factor VIIam activity. This effect was much more pronounced in women than in men. The lauric acid diet tended to decrease antithrombin activity. The TFPI activity, which was not measured in the first study was decreased on the myristic acid diet. The palmitic acid diet increased PAI activity.

The increase of CVD-risk is most pronounced when elevated levels of LDL cholesterol go together with increased coagulant capacity. These studies, therefore, suggest that it will be advisable to decrease the intake of fats rich in lauric, myristic and palmitic acids in favor of a higher intake of unsaturated fatty acids for example from oils rich in monounsaturated fatty acids (oleic acids) such as olive oil, high oleic sunflower or rapeseed oil.

$$-f' = f'' = 0 \quad \text{if } y = 10^6 \cdot 2^{-1/2} \cdot 10^{-6} = 10^{-1/2}$$

$$y = 10^{-1/2} = 10^{-0.5} = 10^{-0.5}$$

$$\begin{aligned} \frac{1}{2} \cdot 10^{-0.5} &= 10^{-0.5} \\ \frac{1}{2} \cdot 10^{-0.5} &= 10^{-0.5} \end{aligned}$$

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1

General introduction

It is well established that diets rich in saturated fatty acids increase the risk for the development of cardiovascular diseases (CVD) (1, 2). Factors involved in blood processes of importance for the cardiovascular risk profile, such as factors involved in lipid metabolism are affected by changes in the saturated fatty acid content of diets (3-5). In addition, other processes that determine the thrombotic capacity of the blood (i.e. platelet aggregation, coagulation and fibrinolytic factors) are affected by dietary fatty acid composition (6, 7). Saturates with different chain length may, however, differ in their cholesterol-raising effects. For example, fats rich in stearic acid (C18:0) decrease serum total, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol concentrations compared with fats rich in lauric (C12:0) plus myristic acids (C14:0) (8). The effects of saturates on hemostatic factors might also depend on the particular effects of individual saturated fatty acids (8).

In this chapter the saturated fatty acids of different chain lengths are introduced. Then, epidemiological and clinical evidence for the role of lipid and hemostatic factors in the development of CVD is discussed. For the hemostatic factors the way of measurement will be focused on. Then, the present knowledge on the effects of (individual) saturates on lipid and hemostatic factors is discussed. Finally at the end of this chapter, the outline of the thesis will be given.

SATURATED FATTY ACIDS

Fats and oils always consist of a mixture of fatty acids, although one or two fatty acids are usually predominant. Table 1 shows the fatty acid composition of some edible fats rich in saturated fatty acids. An "average" Dutch person consumes 37 percent of energy from fat, or on average 92 grams. Saturated fatty acids contribute 14 percent of energy or about 35 grams a day (9). In the Western diet half of the saturated fatty acids consists of palmitic acid (C16:0) (10), thereby the major saturated fatty acid. Fats rich in palmitic acid are palm oil, dairy fat and meat fat. A smaller proportion of saturated fat intake is provided by stearic acid (C18:0), followed by myristic acid (C14:0), lauric acid (C12:0) and short and medium chain fatty acids (MCFA; $\leq$ C10:0). Stearic acid is a major fatty acid in cocoa fat, lauric and myristic acids are both found in coconut and palm kernel fat, and in dairy fat. Natural sources of medium and short chain fatty acids are coconut fat and dairy fat, but intakes via these sources will usually be very low. Higher doses of MCFA are ingested with structured lipids (caprenin), special (par)enteral or infant nutrition or certain sports drinks containing MCFA.

Table 1. Fatty acid composition of some edible fats and oils rich in saturated fatty acids.

	≤C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	Other
	per 100 gram of fatty acid								
Butter fat	9	3	17	25	13	27	3	1	2
Palm kernel fat	8	50	16	8	2	14	2		
Coconut fat	15	48	17	8	3	7	2		
Palm oil			1	45	5	39	9		1
Beef fat			3	26	22	38	2	1	8
Pork fat (lard)			2	25	12	44	10	1	6
Cocoa butter				26	35	35	3		1

EPIDEMIOLOGICAL EVIDENCE FOR EFFECTS OF LIPID AND HEMOSTATIC FACTORS ON CVD RISK MARKERS

CVD is a major cause of morbidity and mortality in the prosperous Western countries, in women as well as in men, and responsible for 30-50% of all deaths (11). The main clinical conditions of CVD are ischemic heart diseases (40%) and cerebrovascular diseases (24%) (12). Many factors, such as smoking, hypertension, age, gender, body mass index, genetic, hormonal, lipid and hemostatic factors may affect risk for CVD. Of these, diet amendable factors such as lipid and hemostatic factors, are discussed below.

Lipid factors

Well known risk markers for the development of CVD are high circulating levels of plasma total cholesterol, low density lipoprotein (LDL) or low levels of high density lipoprotein (HDL) cholesterol (13) (see table 2). Epidemiological studies (14, 15) suggest that for every increase of 1% (corresponding to approximately 0.05 mmol/L) in total cholesterol concentrations (14) or for every 0.03 mmol/L fall in HDL cholesterol concentrations (15), the risk for coronary heart diseases rises by 2-3%. Raised triacylglycerol concentrations may also imply a higher CVD risk, although controversies exist on the independence of this relationship (see (16), for review). In addition, ratios such as the total cholesterol to HDL cholesterol ratio (17, 18), or concentrations of the apolipoproteins associated with LDL or HDL -apolipoprotein B-100 (apoB) and apolipoprotein A-I (apoA-I), respectively- and their ratio (19) have been reported as potent predictors of CVD-risk.

Recently, associations between high lipoprotein(a) (Lp(a)) concentrations and CVD have been demonstrated in cross-sectional studies (20, 21). This relationship was not confounded by levels of LDL or HDL cholesterol (21). Whether Lp(a) is indeed an independent risk factor for CVD needs to be confirmed in further prospective studies. Because of the homology of the apolipoprotein(a) part of Lp(a) with plasminogen, Lp(a) may well cause its action via impaired fibrinolytic function (22, 23).

Most studies were carried out in men, but the few that were carried out in both men and women found that the associations between lipids and lipoproteins with CVD risk were essentially similar in both sexes (13, 15).

Hemostatic factors

A delicately balanced system of interactions keeps the blood circulating as a fluid through the blood vessels. In case of imbalance, for example when a vessel is damaged, the blood starts to clot and a stable thrombus will be formed. This, of course, is necessary to stop a wound from bleeding. However, if a stable thrombus is formed in a small coronary artery, the artery can become occluded, blood and oxygen supply is then hampered, and a heart attack can be the result.

The hemostatic system involves interacting processes for the formation of a stable thrombus -platelet activation and blood clotting-, but also a mechanism to dissolve the

thrombus, fibrinolysis (figure 1). *In vivo*, these processes are associated and the interplay defines the thrombotic capacity of the blood.

Table 2. Relationship between increasing levels or activities of fasting lipid or hemostatic factors with cardiovascular risk: evidence from epidemiological studies.

	CVD risk	Remarks
Lipid factors		
LDL cholesterol	+	
HDL cholesterol	-	
Triacylglycerol	+?	
Lipoprotein(a)	+	
Apolipoprotein B	+	
Apolipoprotein A-I	-	
Total:HDL cholesterol	+	
ApoA-I:ApoB	-	
Hemostatic factors		
Platelet aggregation	+?	(ADP-induced whole blood ADP-induced platelet-rich plasma)
Coagulation		
Factor VIIc activity	+	(fatal events)
Women	?	
Men	+	(fatal events)
Fibrinogen	+	
Antithrombin activity	- or + ?	(U-shaped risk profile)
Fibrinolysis		
PAI activity	+?	

The presence of stable thrombi in advanced atherosclerotic plaques suggests an important contribution of thrombogenesis in CVD (24). In addition, hypercoagulability and impaired fibrinolytic function, forming occlusive thrombi, after an atherosclerotic plaque has been fissured, has been proposed to play a role in the pathogenesis of CVD (25).

Clinical trials indeed revealed that the treatment of CVD-patients with anticoagulants or fibrinolytic therapy reduces the incidence of recurrent infarction and decrease the progression of new lesion formation (25). However, the evidence for the role of hemostatic factors in CVD from prospective studies, which link these factors

with CVD, are scarce. In addition, the few prospective studies on platelet aggregation (26, 27), coagulation (28, 29) and fibrinolytic factors (30, 31) (see below for further discussion) have only been carried out in males. Whether the associations for females are similar awaits confirmation.

Hemostatic factors are difficult to measure. Due to the venipuncture and subsequent blood sampling and plasma preparation, platelets and coagulation factors may become activated which makes it very difficult to obtain a true reflection of the *in vivo* situation. In addition, many different methods are used, which makes comparisons between studies difficult.

Figure 1. Processes involved in thrombus formation as discussed in this thesis. The hemostatic system involves interacting processes for the formation of a stable thrombus -platelet aggregation, blood clotting and endothelial function-, but also a mechanism to dissolve the thrombus, fibrinolysis.

Platelet aggregation

Blood platelets have a central role in thrombus formation. Circulating platelets display on their surfaces passive and activation dependent receptors, which can interact with many agonists, including small molecules, plasma proteins and proteins from the extracellular matrix. Receptor mediated reactions then initiate complex series of intercellular activation events which lead to excretion of substances important for the overall hemostatic response. Receptor mediated reactions lead to platelet adhesion and aggregation of platelets to the injured blood vessel to form a hemostatic plug (32). Platelets excrete substances like calcium, stimulate the

formation of thrombin, carry a fibrinogen receptor, and provide the phospholipid surface that is important for blood coagulation.

Measurement of platelet aggregation

A broad scale of methods is available to measure platelet aggregation *in vitro*. First of all, the blood sample needs to be anticoagulated to avoid clotting of the blood in the test tube or in the aggregometer. Different anticoagulants are used like citrate, which depletes the sample from calcium, heparin, which bind to antithrombin as a result of which the conversion of prothrombin to thrombin is subsequently inhibited, and hirudin, which is a specific thrombin inhibitor. Platelet aggregation *in vitro* can be measured in whole blood, in platelet rich plasma, or -to remove the influence of possible interfering constituents from the plasma- in washed platelet suspensions. Also, the aggregation reaction in the test tube can be triggered with many different compounds, like collagen, ADP, arachidonic acid, and thrombin.

Platelet aggregation and CVD

Although platelets and platelet activity undoubtedly play an important role in thrombogenesis and tests of platelet function have been helpful clinically for detecting platelet hemostatic defects, limited evidence for the association between increased *in vitro* platelet aggregation and CVD is available from prospective studies. In one study, subjects with increased ADP-induced platelet rich plasma aggregation had a higher risk to develop fatal cardiovascular events (26). Collagen- or adrenaline-induced aggregation, however, was not related with CVD mortality (26). Another study, in addition, demonstrated higher CVD prevalence subjects with high ADP-induced compared with those having low ADP-induced whole blood aggregation (27). Results were, however, not adjusted for other risk indicators associated with increased CVD risk.

Coagulation factors

Several pathways for blood coagulation exist. Most important *in vivo* is the tissue factor pathway of blood coagulation, previously known as the extrinsic pathway of blood coagulation (33). The factors involved in the tissue factor pathway of blood coagulation are depicted in a simplified scheme in figure 2. The coagulation factors are present in the plasma in inactivated form. Of factor VII, however, 1% is present in the activated state (factor VIIa) (34). Figure 2 shows, mainly the activated coagulation factors.

Blood coagulation *in vivo* is initiated when factor VII or factor VIIa contacts a tissue factor, for example after vessel injury or inflammation. A tissue factor, an integral membrane glycoprotein, that is expressed by the subendothelial components of the vessel wall, acts as a procoagulant. Once bound, factor VII is rapidly activated into factor VIIa. The complex of factor VIIa with tissue factor then initiates a cascade of reactions, which ultimately results in the conversion of factor X into factor Xa and the generation of thrombin from prothrombin. Thrombin cleaves fibrinogen and finally an insoluble fibrin clot is formed, that stabilizes a thrombus. Thrombin is one of the key

factors in blood coagulation. Besides many other actions such as stimulation of platelet aggregation and back-activation of coagulation factors, thrombin also activates the anticoagulant pathways.

Figure 2. Schematic representation of the coagulation cascade (simplified). When factor VII contacts tissue factor, expressed for example after vessel injury or inflammation, factor VII is rapidly activated into factor VIIa. The complex of factor VIIa with tissue factor initiates a cascade of reactions, which ultimately results - after cleavage of fragment 1+2 - in the conversion of prothrombin into thrombin. Thrombin cleaves fibrinogen into fibrin, that stabilizes a thrombus. Antithrombin and tissue factor pathway inhibitor (TFPI) inhibit the coagulation cascade via complex formation with activated coagulation factors. Activated protein C in complex with protein S inactivates factor Va and factor VIIIa.

Important inhibitors of coagulation are antithrombin and tissue pathway inhibitor (TFPI). Antithrombin inhibits most of the activated coagulation factors. The main targets of antithrombin are probably thrombin and factor Xa (32). TFPI binds to factor Xa and induces feedback inhibition of coagulation by complex formation with the tissue factor/factor VIIa complex. This prevents further activation of the coagulation

cascade (33). Another anticoagulant pathway is via protein C. Protein C is activated when thrombin interacts with thrombomodulin. A complex of activated protein C with protein S then inactivates factors Va and VIIIa.

Measurement of coagulation

Most assays measure the total amount (e.g. factor VII antigen or fibrinogen concentrations) or the activity (e.g. factor VII-coagulant activity) of circulating coagulation factors. However, these factors are normally present in large excess in the blood, and only a small percentage is converted into active enzymes under *in vivo* situations. Nowadays, other assays are also available, which reflect actual *in vivo* coagulation. The plasma fragment 1+2 concentration reflects the amount of prothrombin conversion into thrombin, while fibrinopeptides A and B concentrations reflect the conversion of fibrinogen into fibrin. However, in healthy subjects, concentrations of these markers are very low and just above detection limits, which makes it sometimes difficult to implement their measurements in dietary studies.

Many methods are available to measure factor VII. In most studies, factor VII is measured with a coagulant assay (factor VIIc). Factor VII amidolytic (factor VIIam) activity can be measured with a two step chromogenic assay, which depends on the rate of generation of factor Xa from factor X by activated factor VII (factor VIIa). Both the factor VIIc and the factor VIIam activity assay do not differentiate between factor VIIa and factor VII antigen (factor VIIag) concentrations (34).

Fibrinogen concentrations are usually measured with the method of Clauss (35) or a functional assay modified after Clauss. A fixed surplus of thrombin is added to diluted platelet poor plasma samples, the clotting time recorded, and the fibrinogen concentration read from a calibration curve.

Coagulation and CVD

Several long-term prospective epidemiological studies have reported that in healthy males factor VIIc activity and fibrinogen concentrations were higher in subjects who developed cardiovascular diseases at a later stage of the study (28, 29). Factor VIIc activity was in particular associated with an increased risk of dying of cardiovascular disease (28, 29). It was also noted that low fibrinogen levels were associated with low CVD risk even when LDL cholesterol concentrations were raised (28). Other factors of the coagulation system that might be related to CVD risk is high factor VIII activity (36) and high factor VIIag (37).

Data from the Northwick Park Heart Study (NPHS) showed that low and, unexpectedly, also high concentrations of antithrombin were associated with increased deaths from CVD (38). In view of results of several cross-sectional studies (39-41) the relation of high antithrombin concentrations and high CVD risk was unexpected. In a clinical study, high activity of TFPI has been demonstrated in patients with CVD (42), but this might be related to raised levels of lipoprotein-bound TFPI, which probably has lesser anticoagulant capacity than free TFPI (43).

Fibrinolytic factors

The process involved in thrombus dissolution, and thus the conversion of fibrin in fibrin degradation products, is called fibrinolysis. A simplified scheme of fibrinolysis is given in figure 3. The central reaction in the fibrinolytic process is the conversion of plasminogen into plasmin, which is regulated by the action of tissue plasminogen activator (t-PA). The fibrinolytic capacity of blood is regulated by inhibiting t-PA activity by the action of Plasminogen Activator Inhibitor type-1 (PAI), while plasmin is inhibited mainly by α_2 -antiplasmin (44).

Measurement of fibrinolysis

The fibrinolytic capacity of plasma can be measured by global tests or more specific assays. The global tests include the dilute clot lysis time, euglobulin, clot lysis time and the fibrin plate assay. The dilute and euglobulin clot lysis time measure total fibrinolytic capacity. The total blood sample, or the insoluble protein fraction (euglobulin fraction) is diluted with a buffer, clotted, and then the lysis time of the clot is recorded. In the fibrin plate assay, a standard volume of the euglobulin fraction of plasma is added to standardized plasminogen-rich fibrin plates and the amount of lysis is recorded. In more specific assays total plasma antigen concentration of t-PA and PAI can be determined with enzyme-linked immunosorbent assay methods. Plasma t-PA and PAI activities can be assessed with chromogenic assays.

Figure 3. Schematic representation of the fibrinolytic pathway (simplified). Fibrin is degraded by plasmin. The conversion of plasmin from plasminogen is regulated by the action of tissue plasminogen activator (t-PA). t-PA activity is suppressed by plasminogen activator inhibitor type-1 (PAI), while plasmin is inhibited by α_2 -antiplasmin.

Fibrinolysis and CVD

The Northwick Park Heart Study demonstrated that low fibrinolytic capacity of plasma, measured as clot lysis time was significantly associated with increased CVD risk in middle-aged men (30). Prospective studies in healthy subjects, including determinations of the individual components of the fibrinolytic system, t-PA and PAI, however, are lacking. Hamsten et al (31) reported that higher PAI concentrations were associated with increased risk of reoccurrence of coronary events. The evidence for a role of t-PA in CVD is conflicting. In general, t-PA activity is inversely related to PAI activity, the association of t-PA with CVD risk therefore is generally opposite to that for PAI. Hamsten et al (45), in agreement, found lower t-PA activities in patients with myocardial infarction compared with control subjects. Other studies, however, showed the opposite and demonstrated that patients with atherosclerotic disease have higher concentrations of t-PA suggesting increased fibrinolytic capacity of plasma (46, 47). In addition, a prospective follow-up of the Physicians Health Study showed significantly higher t-PA concentrations in subjects with future stroke compared with those who remained free of cardiovascular disease (48).

SATURATED FATTY ACIDS AND CVD RISK MARKERS

More than 40 years ago, the Seven Countries Study already demonstrated that countries with a higher average intake of total fat, and in particular of saturated fatty acids, experienced a higher mortality from CVD after 15 years of follow up (1). The strong correlation between saturated fat intake and CVD mortality rates extended through the 25 yr. follow up (49). Of the individual saturated fatty acids, lauric, myristic, palmitic and stearic acids intakes correlated strongly with CVD risk ($r=0.84$ for lauric acid, $r=0.86$ for myristic acid, $r=0.81$ for palmitic acid, $r=0.84$ for stearic acid) (49).

The results from the Seven Countries Study (1) strongly suggested that the saturated fatty acid content of the diet affected processes involved in the development of cardiovascular diseases. Of these processes increased cholesterol concentrations in those men dying of CVD were demonstrated, and serum total cholesterol levels were strongly correlated with saturated fat intake (1). More recent data indicate that this correlation is slightly stronger for lauric and myristic acids than for palmitic and stearic acids (49). Several clinical trials, in addition, confirmed that lowering cholesterol levels reduces the risk for CVD and can retard the progression of atherosclerosis (50).

Recent data (2) highlighted that the correlation between saturated fatty acids and CVD risk persists even after adjustment for other CVD factors such as cholesterol concentrations. Saturated fatty acids may thus also exert their effects on CVD risk via other processes than those involved in cholesterol metabolism. Among them, factors involved in hemostasis might be important, as evidence exists for an effect of dietary fatty acid composition on factors involved in platelet aggregation (51, 52) coagulation (6, 7) and fibrinolysis (6, 7). The effects of saturated fatty acids and certainly those of

individual saturated fatty acids on hemostatic components, however, have been studied less extensively than those on lipid and lipoprotein concentrations.

INDIVIDUAL SATURATED FATTY ACIDS AND CVD RISK MARKERS

Lipid factors

Controlled dietary trials have demonstrated that serum lipid and lipoprotein levels are affected by the total saturated fat content and the type of saturated fatty acid in the diet. Keys and co-workers (4) already observed that cocoa butter did not raise total cholesterol concentrations compared with palm oil. Later studies indicated that stearic acid, the main saturated fatty acid of cocoa butter decreases serum total cholesterol concentrations compared with palmitic acid from palm oil (53). This different serum cholesterol-raising potency of two fats high in saturated fatty acids showed that not all saturated fatty acids have equal cholesterolemic effects.

The effects of MCFA have been thought to be neutral compared with carbohydrates (54). Like carbohydrates, MCFA have been demonstrated to increase fasting triacylglycerol concentrations (55, 56). However, studies on the effects of MCFA were carried out with limited number of subjects, with short dietary periods, or with experimental diets in which MCFA were not the only variable among the diets. Thus, precise effects of MCFA are not known.

Also the effects of lauric acid are not yet conclusive, because only a small number of studies have been carried out indicating either lowering (57), increasing (58) or similar effects (59, 60) compared with palmitic acid. Of the saturates, myristic acid might have the strongest potency to increase serum total (3) and LDL cholesterol concentrations. Hegsted et al (3) estimated that the cholesterol raising effect of myristic acid is about 4.8 times that of lauric acid and 1.3 times that of palmitic acid. However, as mentioned by the authors, this estimate varied with the regression equation used. Fats rich in lauric acid naturally contain also large amounts of myristic acid. Therefore, it is difficult to study lauric acid separately. If lauric acid, for example, was not included in the equation, myristic acid was about 3.8 times as cholesterol raising as palmitic acid. Other authors also combined the results of several experiments by using multiple regression analysis, but concluded that myristic acid was about equally hypercholesterolemic as palmitic acid (55, 61).

The cholesterol raising properties of palmitic acid, the major dietary saturated fatty acid have been studied extensively. However, results on the effects of palmitic acid are not yet conclusive. Many studies (53, 57, 62, 63) have indicated that palmitic acid raises serum total and LDL cholesterol, but only slightly increases HDL cholesterol relative to carbohydrates. Other studies (64, 65) indicated that palmitic acid not raises total and LDL cholesterol concentrations as compared with oleic acid under special circumstances that not down-regulate the LDL receptor activity. These circumstances include adequate dietary linoleic acid and low cholesterol content of the diet in normocholesterolemic, nonobese subjects.

Adverse effects of lowering cholesterol concentrations on mortality of non-illness related causes (violence, suicides and accidents) have also been reported from epidemiological studies. The hypothesized mechanism associates declining serum cholesterol concentrations with a more negative mood state (66). The latter might be related to the low plasma serotonin (67) or low tryptophan concentrations (68), found in subjects with low cholesterol concentrations as compared with a reference group.

Hemostatic factors

Limited studies have investigated the effects on hemostatic factors of individual saturated fatty acids in healthy human subjects. The interest for the effects for individual saturated fatty acids on platelet aggregation, coagulation and fibrinolytic factors has increased lately. Separate effects of MCFA and lauric acid, however, have never been studied. In addition, studies in other subjects than young and lean men are scarce. The effects of diets rich in lauric plus myristic acids (8), myristic acid (69), palmitic acid (8, 69) and stearic acid (8) on coagulation and fibrinolytic factors have been investigated. These studies (8, 69) suggested that particular saturated fatty acids alter factor VIIc activity in a different way. Fats rich in lauric plus myristic acid or palmitic acid raise factor VIIc activity compared with fats rich in stearic acid (8). In addition, fats rich in myristic acid increase factor VII activity slightly stronger than fats rich in palmitic acid (69).

Food intake

Maintaining a healthy weight is another factor that is important to prevent CVD. Food intake therefore needs to be balanced with the energy need. In the etiology of the development of obesity, factors that affect feelings of appetite might play a role. Results of Rolls et al (70) suggest that differences in satiety mechanisms exist between different fatty acids as preloads of medium chain triglycerides reduced food intake shortly after the preload had been given, compared with preloads of corn oil. No other data, however, are available on the effects of fatty acid composition on feelings of appetite.

OUTLINE OF THE THESIS

This thesis describes two studies on the effects of individual saturated fatty acids on risk markers for cardiovascular diseases in healthy women and men. In the first study effects of lauric and palmitic acids were investigated and the second study focused on the effects of MCFA and myristic acid. In both studies the reference fatty acid was oleic acid.

I: Lipid factors

In **Chapter 2** we have compared the effects of lauric acid and palmitic acid with the effects of oleic acid on lipid and lipoprotein concentrations. The effects of these fatty acids were studied under circumstances that were expected not to down-regulate the LDL receptor activity: diets contained sufficient linoleic acid and low cholesterol levels, while subjects were normocholesterolemic and non-obese. **Chapter 3** addresses the effects of MCFA and myristic acid. Because natural fats only rich in MCFA or myristic acid do not exist, synthetic fats were used in this second study. To know to what extent the triglyceride structure of these fats differs from natural fats, the triglyceride structure of the experimental fats was also determined. **Chapter 4** focuses on dietary induced changes in lipoproteins and effects on mood.

II. Hemostatic factors

Chapter 5 discusses the effects of MCFA, lauric acid, myristic acid and palmitic acid on whole blood aggregation. The effects on coagulation and fibrinolytic parameters are discussed in **Chapter 6** for lauric and palmitic acid and in **Chapter 7** for MCFA and myristic acid.

III. Appetite and proposed regulators of food intake.

Chapter 8 focuses on the effects of dietary fatty acid composition (MCFA, myristic or oleic acid rich diets) on feelings of appetite. In addition, concentrations of some proposed regulators of food intake -apolipoprotein A-IV and leptin- are studied.

Finally, in **Chapter 9** the main results of both studies will be summarized and its implications will be discussed.

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Comparison of the effects of diets enriched in lauric, palmitic or oleic acids on serum lipids and lipoproteins in healthy women and men

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ABSTRACT

The degree to which different saturated fatty acids exert their cholesterol-raising effects is still unknown. We, therefore, studied the effect on serum lipid and lipoproteins of diets rich in lauric, palmitic or oleic acids. Eighteen women and fourteen men consumed in random order three experimental diets, each for six weeks. The diets consisted of solid foods, and contained 40 energy percent (En%) fat of which 28 En% was supplied by the experimental fats. The fat high in lauric acid was a mixture of palm kernel (75%) and a high oleic acid sunflower oil (25%), the fat high in palmitic acid consisted of dairy fat (55%), palmstearin (36%) and sunflower oil (9%) and the fat high in oleic acid of dairy fat (37%) and the sunflower oil (63%). The calculated nutrient composition was the same in each diet except for about 8.5 En% which was provided by either lauric, palmitic or oleic acids. On the lauric acid diet the subjects' serum total cholesterol concentration increased by 0.22 mmol/L ($P=0.0121$; 95% confidence interval (CI), 0.02 to 0.41 mmol/L) as compared with the palmitic acid diet and by 0.48 mmol/L ($P<0.0001$; 95% CI, 0.29 to 0.67 mmol/L) versus the oleic acid diet. Total cholesterol concentrations on the palmitic acid diet were 0.26 mmol/L ($P=0.0012$; 95% CI, 0.07 to 0.46 mmol/L) higher than on the oleic acid diet. High-density lipoprotein (HDL) cholesterol concentrations increased by 0.12 mmol/L ($P=0.006$; 95% CI, 0.04 to 0.20 mmol/L) on the lauric acid versus the palmitic acid diet and by 0.14 mmol/L ($P<0.001$; 95% CI, 0.07 to 0.22 mmol/L) versus the oleic acid diet. HDL cholesterol concentrations on the palmitic acid and the oleic acid diet were the same. No effects were seen on serum triacylglycerol and lipoprotein(a) concentrations. We conclude that both lauric and palmitic acids are hypercholesterolemic as compared with oleic acid. Lauric acid raises total cholesterol concentrations more than palmitic acid, which is partly due to a stronger rise in HDL cholesterol.

INTRODUCTION

The serum cholesterol-raising effect of the saturated fatty acids in the diet appears to be limited to lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) (1, 2). Several studies suggest that the degree to which these saturated fatty acids increase serum cholesterol concentrations is different. Coconut and palm kernel oils, rich in lauric acid, have been shown to raise serum total, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol concentrations compared with palm oil, high in palmitic acid (3, 4). These effects are not necessarily due to increased intakes of lauric acid, as these fats also contain relatively high concentrations of myristic acid, which is probably a very potent serum cholesterol-raising fatty acid (2, 5). Recent studies have investigated the specific effects of lauric acid relative to those of other fatty acids, using either mixtures of natural fats (6, abstr) or synthetic fats (7). Results, however, are still inconclusive. In one study lauric acid was found to lower (7) serum cholesterol concentrations relative to palmitic acid, but in the other study no differences between these two fatty acids were found (6, abstr). A possible explanation for this discrepancy is that effects of synthetic fats, rich in lauric acid, differ from those of natural fats.

Many previous studies have shown that palmitic acid raises serum total and LDL cholesterol concentrations compared with oleic acid (7-10). Recently, however, it has been suggested that palmitic acid may be neutral in normocholesterolemic subjects, if the diet contains little cholesterol and the linoleic acid intake is adequate (11). Indeed it has been reported that under these conditions palmitic and oleic acids exert similar effects on serum total, LDL and HDL cholesterol concentrations (12, 13). We, therefore, decided to examine, at adequate intakes of linoleic acid, the effects on serum lipids and lipoproteins of lauric, palmitic and oleic acids in healthy volunteers. The experimental fats, which were incorporated into solid foods, were natural and contained little or no myristic acid and relatively small amounts of cholesterol.

SUBJECTS AND METHODS

Subjects

Volunteers were recruited via advertisements in local newspapers and posters in public buildings. After they had been thoroughly informed about the purpose and protocol of the study, 62 subjects from Maastricht and surroundings entered the selection procedure. Forty-three people were selected for participation since they met all our eligibility criteria: aged between 20 and 60 years, not on a prescribed or a slimming diet, no anemia, no hypertension and serum total cholesterol concentrations below 6.5 mmol/L. The protocol of the study had been approved by the local ethics committee and was explained to the volunteers. All subjects had given their written informed consent.

Forty volunteers completed the first dietary period, 35 the first and the second period and 32 subjects completed all three dietary periods. Subjects withdrew

because of reasons specifically related to the strict study protocol (6 subjects), job or study commitments (2 subjects), family circumstances (2 subjects) and illness (one subject).

Thus, 18 women and 14 men, aged between 20 and 60 years (mean, 41 years old) completed the trial. The women weighed 51 to 96 kg (mean, 69 kg), were between 156 and 183 cm (mean 166 cm) while their body mass indexes varied from 19 to 30 kg/m² (mean 25 kg/m²). The men weighed 71 to 101 kg (mean, 81 kg), were between 175 and 188 cm (mean 179 cm), while their body mass indexes varied from 22 to 31 kg/m² (mean 25 kg/m²). Four women and 5 men were smokers. None of the selected volunteers received medications known to affect serum lipids. Two women used oral contraceptives and 6 women were postmenopausal. Fasting concentrations of serum lipids ranged from 3.41 to 6.48 mmol/L (mean, 5.13 mmol/L or 198 mg/dL) for total cholesterol, 0.86 to 2.48 mmol/L (mean, 1.51 mmol/L or 58 mg/dL) for HDL cholesterol, and 0.26 to 2.53 mmol/L (mean, 0.93 mmol/L or 82 mg/dL) for serum triacylglycerols. These characteristics did not differ between the people who withdrew and the people who completed the trial.

Design and statistical analyses

Each participant consumed each of the three different diets during three periods of six weeks. One diet was high in lauric acid, another high in palmitic acid and a third high in oleic acid. Before the study started, subjects were categorized according to sex and then randomly divided into six groups. Each group received the diets in one of the six possible treatment orders. The three dietary periods were interrupted by wash-out periods of two and three weeks, respectively. During these periods the participants consumed their habitual diets.

Data were analyzed with diet, period, and diet and sex interaction as independent variables. When the analyses indicated a significant effect ($P < 0.05$) of the diet, the Tukey method was used to compare the diets pairwise (14). The data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analyses System (15). Only data of the thirty two subjects, who completed three dietary periods were included in the analyses. Because lipoprotein-(a) (Lp(a)) concentrations were not normally distributed, the untransformed individual changes of Lp(a) were analyzed with the non-parametric Friedman test (16).

Diets

Before the start of the study, subjects recorded their habitual food intake for two working days and one weekend day, in order to enable us to estimate each subjects' actual energy intake. The food records were coded and the composition of the diets was calculated using the Dutch food composition table (17). As this method underestimates actual energy intake by on average 10% (18), the calculated amount of energy was multiplied by 1.1. Diets used in the study consisted of products in which the normal fat was replaced by the experimental fats which were mixtures of natural oils and fats. The fat high in lauric acid was a mixture of palm kernel (75%) (Wesco; Karlsamns, Zaandijk, The Netherlands) and a high oleic acid sunflower oil (Trisun;

SVO enterprises, Wickliffe, USA) (25%), the fat high in palmitic acid consisted of dairy fat (55%) (FDF, Leeuwarden, The Netherlands), palmstearin (36%) (Aarhus oil, Aarhus, Denmark) and sunflower oil (9%) and the fat high in oleic acid of dairy fat (37%) and sunflower oil (63%). Cholesterol was added to the fats high in lauric and oleic acids, so that all fats contained 145 mg of cholesterol per 100 g of fat. The composition of the experimental fats is given in table 1. In addition, we supplied a high linoleic margarine for preparing dinner meals. In this way we increased the subjects' linoleic acid intake to between four and five energy percent. Subjects did not get any payment for the study, except that the products were free. These products included margarines, dairy products (cheese and custards) and bakery products (bread, cake, biscuits and pie).

The calculated fatty acid composition was converted into a list of the available products. Lists were formulated at fifteen levels of energy ranging from 5 to 22 MJ per day. Diets consisted of 40 energy percent of fat (En%). The experimental fats provided about 70 percent of total fat energy, which equals 28 En%.

The subjects had to eat a certain amount of the experimental products corresponding with their energy intake. The remaining thirty percent of total fat intake was free and provided by the "free choice" fat containing products. These products were listed and given points according to their fat contents.

Table 1. Fatty acid composition of the experimental fats (g/100g fatty acids).

	Lauric Acid Fat	Palmitic Acid Fat	Oleic Acid Fat
≤C10:0	3.7	5.3	3.4
C12:0	43.8	1.9	1.2
C14:0	15.4	6.1	3.6
C16:0	6.8	45.1	12.1
C18:0	2.4	8.7	6.5
C18:1	24.4	25.2	61.9
C18:2	3.1	1.8	7.1
Others	0.3	5.9	4.1

One point was equal to 1 gram of fat. Each subject was required to eat daily a number of points, again corresponding with his or her energy intake. The free choice items were listed by the subject in a diary. Detailed diet instructions, menu suggestions, suggestions for free choice items and recipes were provided during personal interviews by a registered dietitian and in a three-weekly newspaper.

The planned fatty acid composition of the three diets was the same except for 8.5 En% which was provided by either lauric acid, palmitic acid or oleic acid. However, the lauric acid diet contained also 2.2 En% more myristic acid than the other two diets.

The volunteers came at least once a week to a special room at the department to receive a new supply of products. All products, coded with a blue, red or yellow label to blind the subject, were handed out to the volunteers on an individual basis. The subjects were asked to maintain their usual pattern of activity and not to change their smoking habits or use of contraceptives. They recorded in diaries any signs of illnesses, medication used, alcoholic consumption, menstrual phase, the free choice items selected and any deviations from the study protocol.

Body weight was recorded every week and individual allowances were adjusted to minimize weight changes. In each period subjects recorded their food intake for two working days and two weekend days and records were checked by a dietitian. For computing the fatty acid composition of the diets, the various individual saturated fatty acids were added to the Dutch food composition table (17) manually.

Blood sampling and analyses

Blood was sampled after an overnight fast and after abstinence from drinking alcohol the preceding day and smoking on the morning before blood sampling. Blood was sampled twice at the end of a dietary period (week 5 and week 6) with minimum stasis and using a 1.2 mm needle (Strauß Kanule, Luer) with the volunteer in a recumbent position. About 15 mL blood was taken for additional measurements (to be reported elsewhere) and finally, 10 mL blood was drawn in a 10 mL clotting-tube to obtain serum. All venipunctures were performed by the same person, at the same location, and for each subject generally at the same time of the same day of the week.

Serum was obtained by low-speed centrifugation one hour after venipuncture and stored at -80 °C. Total cholesterol (CHOD-PAP method; Monotest cholesterol, Boehringer Mannheim, Mannheim, Germany), HDL cholesterol (precipitation method; Monotest cholesterol, Boehringer Mannheim, Mannheim, Germany) and triacylglycerols (GPO-Trinder; Sigma Diagnostics, St Louis, USA) were analyzed enzymatically. The coefficient of variation within runs was 1.1 % for total cholesterol, 0.4 % for HDL cholesterol and 1.2 % for triacylglycerols. LDL cholesterol was calculated using the Friedewald equation (19).

Apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) were measured in serum, using an immunoturbidimetric reaction (UNI-KIT apoA-I and UNI-KIT apoB, Roche, Basel, Switzerland) and antiserum raised in sheep and rabbits, respectively. The coefficients of variation within runs were 1.9 % for apoA-I and 2.9 % for apoB.

Plasma lipoprotein(a) concentrations were analyzed by Enzyme Linked Immuno Sorbent Assay (ELISA) using a commercial kit (Apo-TEK, Organon Teknika, Maryland, USA). The samples were incubated in microelisa wells coated with a monoclonal antibody that specifically recognizes apolipoprotein(a). Unbound antigen is washed away and the immune complex is further incubated with a horseradish peroxidase coupled to a polyclonal antibody that recognizes apoB. The coefficient of

variation was 3.9 % within runs. All samples from one subject were analyzed within one run.

The lipid and lipoprotein values obtained for the two sampling days at the end of each dietary period were averaged for data analyses. Samples for apolipoprotein and lipoprotein(a) analyses from these two sampling days, however, were pooled and analyzed as such.

RESULTS

Diets and dietary adherence

The mean daily energy intake and the composition of the three different diets, as determined by the food records, agreed well with the calculated diet composition (table 2). The intake of oleic acid decreased from 19.0 to 10.3 En% on the lauric acid diet and to 10.6 En% on the palmitic acid diet. It was exchanged for extra lauric acid or for palmitic acid. The lauric acid diet, however, also provided some more myristic acid (mean 2.2 En%) than the palmitic and oleic acid diets.

The mean amount of fat consumed via "free choice" fat items, as listed daily in the subjects' diary, agreed with the calculated amount and was about 30% of total fat energy.

The effectiveness of blinding was measured using a questionnaire. Four subjects made a correct guess and recognized all three diets while four other subjects recognized the oleic acid diet only. The taste of experimental products on the three diets, as revealed by the same questionnaire, was almost identical. The palmitic acid margarine and palmitic acid cheese, however, were appreciated less than the margarines and cheeses of the lauric acid and oleic acid diets. Body weights did not differ between the three diets and were 74 ± 12 kg (mean $\pm$ SD) on the lauric, 75 ± 12 kg on the palmitic and 74 ± 11 kg on the oleic acid diet.

Serum lipid and lipoproteins

Table 3 gives the serum lipid and lipoprotein concentrations at the end of each dietary period. Compared with the oleic acid diet, serum total cholesterol concentrations increased by 0.48 mmol/L or 19 mg/dL ($P < 0.0001$; 95% confidence interval (CI), 0.29 to 0.67 mmol/L) on the lauric acid diet and by 0.26 mmol/L or 10 mg/dL ($P = 0.0012$; 95% CI, 0.07 to 0.46 mmol/L) on the palmitic acid diet. Serum total cholesterol concentrations on the lauric acid diet were also significantly higher (0.22 mmol/L or 9 mg/dL) than on the palmitic acid diet ($P = 0.0120$; 95% CI, 0.02 to 0.41 mmol/L). LDL cholesterol rose by 0.35 mmol/L or 14 mg/dL ($P < 0.0001$; 95% CI, 0.17 to 0.52 mmol/L) on the lauric acid diet and by 0.22 mmol/L or 9 mg/dL ($P = 0.0026$; 95% CI, 0.05 to 0.39 mmol/L) on the palmitic acid diet; the latter value was not significantly different from the increase on the lauric acid diet ($P = 0.0912$; 95% CI, -0.04 to 0.20 mmol/L). Changes in LDL cholesterol concentrations on the two saturated fat diets were not dependent on subjects' LDL cholesterol concentrations on the oleic acid diet (figure 1).

Table 2. Mean daily intake of nutrients by the 32 subjects while on the lauric acid diet, the palmitic acid diet and the oleic acid diet, as calculated from the recorded food intakes during two working days and two weekend days per period.*

	Lauric Acid Diet	Palmitic Acid Diet	Oleic Acid Diet
Energy			
MJ/day	9.3±2.7	9.8±2.8	9.4±2.4
kcal/day	2204±653	2322±662	2232±581
Protein (En%)	14.3±3.0	14.3±2.8	14.0±3.3
Fat (En%)	41.5±5.5	41.0±5.4	41.8±5.5
Saturated FA	24.3±3.6	22.5±3.5	14.5±3.0
Lauric acid (C12:0)	10.6±2.4	1.9±0.8	2.5±1.7
Myristic acid (C14:0)	4.2±0.8	2.2±0.5	1.9±0.6
Palmitic acid (C16:0)	5.9±1.5	13.2±2.3	5.7±1.1
Monounsaturated FA	11.1±2.1	12.0±2.1	20.2±3.0
Oleic acid (cis-C18:1)	10.3±1.9	10.6±1.9	19.0±2.8
Polyunsaturated FA	4.6±2.1	4.8±2.1	5.5±1.9
Linoleic acid (cis,cis-C18:2)	4.4±2.0	4.4±2.0	5.2±1.9
Carbohydrates (En%)	43.9±5.6	44.7±6.1	44.3±6.1
Alcohol (En%)	1.3±2.6	0.9±2.1	0.9±2.5
Cholesterol (mg/MJ)	23.9±10.8	22.8±8.5	24.9±10.3
Dietary fiber (g/MJ)	2.9±0.9	2.8±0.8	2.8±0.7

* Values are means±standard deviations.

HDL cholesterol concentrations increased by 0.14 mmol/L or 5.4 mg/dL ($P<0.0001$; 95% CI, 0.07 to 0.22 mmol/L) on the lauric acid diet as compared with the oleic acid diet. The lauric acid diet increased HDL cholesterol (0.12 mmol/L or 4.6 mg/dL) as compared with the palmitic acid diet ($P=0.0006$; 95% CI, 0.04 to 0.20 mmol/L). HDL cholesterol concentrations were the same on the palmitic acid diet and the oleic acid diet. The HDL to LDL cholesterol ratio and triacylglycerols did not differ between any of the three diets.

When the analyses included two women and one men who completed only two dietary periods, results were similar.

Table 3. Serum lipid and lipoprotein cholesterol levels on the three diets studied.*

	Lauric Acid Diet	Palmitic Acid Diet	Oleic Acid Diet
Total cholesterol (mmol/L)			
All	5.90±1.02 †‡	5.69±0.93 †	5.42±1.02
Women	5.91±1.11 †	5.68±1.05 †	5.35±1.12
Men	5.89±0.94 †	5.70±0.79	5.51±0.90
LDL cholesterol (mmol/L)			
All	3.84±0.99 †	3.71±0.91 †	3.49±0.94
Women	3.82±1.02 †	3.67±0.99 †	3.35±0.97
Men	3.87±0.98	3.76±0.83	3.67±0.90
HDL cholesterol (mmol/L)			
All	1.59±0.45 †‡	1.47±0.40	1.44±0.38
Women	1.67±0.41 †‡	1.58±0.33	1.58±0.37
Men	1.47±0.48 †‡	1.32±0.44	1.26±0.32
Triacylglycerols (mmol/L)			
All	1.05±0.50	1.11±0.54	1.07±0.42
Women	0.93±0.33	0.93±0.35	0.92±0.32
Men	1.21±0.63	1.35±0.65	1.27±0.46
HDL to LDL ratio			
All	0.46±0.25	0.44±0.21	0.46±0.23
Women	0.48±0.21	0.47±0.19	0.51±0.20
Men	0.44±0.31	0.39±0.24	0.39±0.26

* Values are means±standard deviations. Eighteen women and fourteen men consumed all three diets, for six weeks each, in random order. To convert values for total, LDL, and HDL cholesterol to milligrams per deciliter, multiply by 38.67. To convert values for triacylglycerols to milligrams per deciliter, multiply by 88.54.

† Significantly different from the oleic acid diet, $P<0.02$.

‡ Significantly different from the palmitic acid diet, $P<0.02$.

Table 4. Serum apolipoprotein A-I and B levels on the three diets studied.*

	Lauric Acid Diet	Palmitic Acid Diet	Oleic Acid Diet
Apolipoprotein A-I (mg/L)			
All	1826±395 †‡	1735±368	1670±304
Women	1879±320 †	1811±306	1752±318
Men	1757±478 †	1638±427	1563±257
Apolipoprotein B (mg/L)			
All	1206±343 †	1181±299 †	1123±316
Women	1178±338 †	1170±305 †	1069±317
Men	1242±358	1195±302	1192±314
ApoA-I to ApoB ratio			
All	1.7±0.7	1.6±0.6	1.6±0.6
Women	1.8±0.7	1.7±0.5	1.8±0.6
Men	1.6±0.8	1.5±0.7	1.4±0.6

* Values are means±standard deviations. Eighteen women and fourteen men consumed all three diets, for six weeks each, in random order.

† Significantly different from the oleic acid diet, $P<0.02$.

‡ Significantly different from the palmitic acid diet, $P<0.02$.

ApoA-I and B concentrations are given in table 4. ApoA-I concentrations were increased on the lauric acid diet compared with the oleic acid (156 mg/L, $P<0.0001$; 95% CI, 82 to 231 mg/L) and palmitic acid diets (90 mg/L, $P=0.0178$; 95% CI, 16 to 165 mg/L). The mean ratio of apoA-I to HDL cholesterol was 1181±169 mg/mmol on the lauric acid diet, 1212±184 mg/mmol on the palmitic acid diet, and 1188±162 mg/mmol on the oleic acid diet and did not differ significantly between the three diets. ApoB concentrations on the lauric and palmitic acid diets were both higher than on the oleic acid diet (83 mg/L, $P=0.0015$; 95% CI, 26 to 139 mg/L and 58 mg/L, $P=0.0142$; 95% CI, 1 to 114 mg/L) but did not differ between the two saturated-fat diets. The apoB to LDL cholesterol ratio was 317±59 mg/mmol on the lauric acid, 322±63 mg/mmol on the palmitic acid and 323±36 mg/mmol on the oleic acid diet and did not differ between the diets. The apoA-I to apoB ratio (table 4) and Lp(a) concentrations (table 5) did not differ between the three diets.

Responses to the diets did not differ significantly between men and women.

Table 5. Plasma lipoprotein(a) levels on the three diets studied.*

	Lauric Acid Diet	Palmitic Acid Diet	Oleic Acid Diet
Lipoprotein(a) (mg/L)			
All	32 (0 to 929)	37 (0 to 1049)	32 (0 to 726)
Women	40 (0 to 929)	40 (0 to 1049)	32 (0 to 726)
Men	19 (0 to 765)	34 (0 to 627)	32 (0 to 712)

* Values are median levels (ranges). Eighteen women and fourteen men consumed all three diets, for six weeks each, in random order.

Figure 1. Individual changes in LDL cholesterol levels on diets high in lauric acid (C12:0) or palmitic acid (C16:0), relative to a diet high in oleic acid (C18:1). Subjects are sorted, from left to right, according to LDL cholesterol levels on the oleic acid diet (range 1.51 to 5.28 mmol/L).

DISCUSSION

Lauric acid versus palmitic acid

In this study with mixtures of natural fats, we found that a diet high in lauric acid increased serum total cholesterol concentrations compared with a diet high in palmitic acid. The cholesterol raising effect of lauric acid was due to a rise in both LDL and HDL cholesterol concentrations. In studies with synthetic lauric acid fats incorporated into liquid formula diets, contradictory results were found. Vergroesen and de Boer (20) reported increased serum total cholesterol concentrations of glyceryl trilaurate compared with synthetic tristearate or tripalmitate. Another study (7) showed that lauric acid transesterified with a high-oleic sunflower oil was less potent in raising total and LDL cholesterol concentrations than a natural fat high in palmitic acid. Earlier data of McGandy et al (21) were inconsistent. When trilaurate and tripalmitate were transesterified with a high-linoleic acid safflower oil, serum total and LDL cholesterol concentrations were the lowest on the diet high in lauric acid. Transesterification with olive oil, however, did not demonstrate a difference in the cholesterol-raising potency of lauric and palmitic acid. It is possible that the use of synthetic fats has contributed to these inconsistent results. In a synthetic fat the fatty acids are randomly distributed over the glycerol backbone, whereas in natural fats the distribution depends on the fat source and is not similar among the three positions. Studies on effects of fats with different positional distributions of palmitic and stearic acids, however, do suggest comparable effects on serum lipids and lipoproteins (22, 23). The position of palmitic and stearic acids on the triglyceride molecule does not change their effects on serum lipid concentrations (22, 23). Whether these effects can be extrapolated to lauric acid, is not known.

A possible confounding factor in our study might be the higher intake of myristic acid (2 En%) on the lauric acid diet compared with the other two diets. The regression equations developed by Hegsted et al (2) suggested that myristic acid is the most potent cholesterol raising saturated fatty acid. Recent studies (9, 24) that compared side-by-side effects of myristic acid with those of palmitic acid yielded contradictory results. Zock et al (9) found that myristic acid raised serum total cholesterol about 1.5 and LDL cholesterol concentrations 1.3 times more than palmitic acid, which is less than predicted by the regression equations of Hegsted and co-workers (2). Tholstrup et al (24), on the contrary, found similar effects of myristic and palmitic acids on total and LDL cholesterol concentrations. Finally, Vergroesen and de Boer reported comparable effects of lauric and myristic acids on serum cholesterol concentrations (20). However, in our study lauric acid is still hypercholesterolemic relative to palmitic acid, even if we adjust our data for the possible potent cholesterolemic effect of myristic acid (9).

The effects of natural fats high in lauric acid have further been examined with coconut and palm kernel fat or mixtures of these two fats. These studies show, in general, increased total, LDL and HDL concentrations compared with palm oil (3, 4, 12, 25).

About half of the cholesterol-raising effect of the lauric acid diet was due to increased HDL cholesterol concentrations (table 3). Elevated HDL cholesterol concentrations were also found by Denke and Grundy (7), although the difference did not reach statistical significance. Raised HDL concentrations are furthermore reported in studies on coconut fat versus palm oil (3) and in studies that specifically investigated mixtures of lauric and myristic acids versus palmitic acid (12, 24, 25). Also myristic acid increased HDL cholesterol compared with palmitic acid (9).

Palmitic acid versus oleic acid

In this study, a 7.5 En% exchange from oleic acid for palmitic acid increased serum total cholesterol concentrations by 0.26 mmol/L. This response was caused by increased LDL cholesterol without a concomitant rise in HDL cholesterol concentrations. Other studies on the effects of palmitic acid relative to oleic acid have reported similar findings (7-10). Some investigators, however, have hypothesized that palmitic acid is only hypercholesterolemic in situations where the LDL receptors are down-regulated (11). Thus, in normo-cholesterolemic lean subjects consuming diets with relatively small amounts of cholesterol and adequate linoleic acid concentrations, palmitic and oleic acids are supposed to be neutral as has been reported by Ng et al (12) and Choudhury et al (13). In the present study a down-regulation of the LDL receptors is not expected because the subjects were normocholesterolemic while the diets were low in cholesterol and contained adequate amounts of linoleic acid. Nevertheless, palmitic acid was cholesterolemic relative to oleic acid. In addition, figure 1 does not suggest that changes in LDL cholesterol concentration are dependent on the subjects' concentration on the oleic acid diet. Also in other studies palmitic acid raised total and LDL cholesterol concentrations in normocholesterolemic subjects (7, 9, 10), even when the diets were cholesterol free (7) or when subjects were young and lean (9). An important difference between these studies (7-10, 12, 13) is total daily fat intake of the diets. Daily fat intake was 40 En% in studies that demonstrated hypercholesterolemic effects of palmitic acid (7-10) and around 32 En% in the studies that found similar effects of palmitic and oleic acids (12, 13). Therefore, it should be examined if total daily fat intake might moderate the cholesterolemic effect of palmitic acid.

Apolipoproteins and lipoprotein(a)

Changes in apolipoproteins B and A-I concentrations reflected those of LDL and HDL cholesterol, respectively. Differences between diets did not result in altered apoB to LDL cholesterol or apoA-I to HDL cholesterol ratios. It has been suggested that increased apoB to LDL cholesterol ratio and thus small LDL particles rich in apoB are associated with increased atherogenicity of the LDL particles (26). Mensink et al (27) have reported that a mix of saturated fatty acids decreased the apoB to LDL cholesterol ratio of saturated fatty acids compared with both *trans*- and *cis*-isomers of oleic acid. Results of Zock et al (9) indicated lower ratios on myristic and palmitic acid diets compared with oleic acid. The present results, on the contrary, do not indicate differences in apoB to LDL ratio on the two diets high in saturated fatty acids. Also in

the guinea pig no effects were found of different saturated fatty acids from palm kernel oil or palm oil on LDL particle size (28).

No differences were found in the concentrations of Lp(a) on the three diets. This finding agrees with the results of a recent study (29, abstr) which compared the effects of lauric plus myristic acid versus palmitic acid. In that study, however, stearic acid increased Lp(a) concentrations, which suggests that the different saturated fatty acids do not have similar effects on Lp(a). Results of another study from our lab suggested that a mixture of saturated fatty acids with 12 to 16 carbon atoms decreases Lp(a) concentrations compared with oleic acid (30). Other (31) and the present results do not indicate an Lp(a) lowering effect of saturates. We do not have an explanation for this apparent discrepancy.

Results from the four days food records and the daily records of the "free choice" fat products gave good confidence that the prescribed diets were actually eaten. Our findings therefore suggest that, at 40 En% of total fat intake, both lauric acid and palmitic acid are hypercholesterolemic compared with oleic acid. Lauric acid raises serum total cholesterol concentrations more than palmitic acid, which is partly due to a stronger rise in HDL cholesterol.

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Effects of medium chain fatty acids (MCFA), myristic acid, and oleic acid on serum lipoproteins in healthy subjects

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ABSTRACT

In this study we investigated the effects on lipoproteins of medium chain fatty acids (MCFA) and myristic acid relative to those of oleic acid. Thirty-seven women and 23 men consumed a 3 wk run-in diet enriched in oleic acid followed by a 6 wk test diet rich in MCFA (n=21), myristic (n=20), or oleic acid (n=19). Experimental fats were incorporated into solid foods. Total fat intake was 40 En% fat. The dietary compositions were the same except for 10 En%, which was provided by MCFA, myristic or oleic acids, respectively. With the myristic acid diet, LDL cholesterol was 0.37 mmol/L higher compared with the oleic acid diet ($P=0.0064$ for difference in changes). The MCFA diet increased LDL cholesterol, though not significantly, with 0.23 mmol/L relative to the oleic acid diet ($P=0.0752$). Compared with the oleic acid diet, HDL cholesterol concentrations increased with the myristic acid diet by 0.10 mmol/L ($P=0.0273$) but not with the MCFA diet. The MCFA diet slightly elevated triacylglycerol concentrations, but responses did not significantly differ between the diets. The MCFA diet significantly decreased the apoA-I to apoB ratio compared with both other diets ($P<0.02$). We conclude that MCFA raise LDL cholesterol concentrations slightly and affect the apoA-I to apoB ratio unfavorably compared with oleic acid. Myristic acid is hypercholesterolemic, although less than predicted earlier, and raises both LDL and HDL cholesterol concentrations compared with oleic acid.

INTRODUCTION

Medium Chain Fatty Acids (MCFA) that have between six and ten carbon atoms are not only used in diets for patients with malabsorption disorders, but also in structured lipids like caprenin (50% caprylic acid (C8:0) plus capric acid (C10:0), and 45% behenic acid (C22:0)). After absorption, the transport of MCFA through the blood differs from longer chain fatty acids. MCFA are not incorporated into triacylglycerols packaged with chylomicrons as longer chain fatty acids are, but are transported primarily through the portal vein to the liver and provide the body with a rapid source of energy (1). It has been suggested that effects of MCFA on serum cholesterol are similar to those of carbohydrates (2), but whether the effects of MCFA on different lipoproteins are indeed comparable remains to be determined. Studies carried out to investigate effects of MCFA on lipoproteins found total cholesterol levels similar to concentrations on western diets (3-5), increased triacylglycerol concentrations (3, 4), and decreased HDL concentrations (3, 5). These studies, however, were of short duration (3) and were carried out with a limited number of subjects (4, 6). In addition, the amount of MCFA was not the only variable among the experimental diets (3-7).

Certain edible fats like coconut oil, palm kernel oil and butterfat do not only contain considerable amounts of MCFA, but are also rich in myristic acid. Myristic acid might be the most potent cholesterol-raising fatty acid (8, 9). Recent studies in healthy volunteers consuming diets enriched in myristic acid, however, yielded contradictory results (10, 11). Compared to palmitic acid, myristic acid raised total, LDL and HDL cholesterol in one study (10) but only increased HDL cholesterol concentrations in another (11). In both studies, however, myristic acid was less cholesterolemic than predicted by the formula developed by Hegsted et al (8). We, therefore, decided to compare in healthy volunteers the effects on serum lipids and lipoproteins of an MCFA and a myristic acid enriched diet with those of a diet enriched in oleic acid.

SUBJECTS AND METHODS

Subjects

The volunteers were recruited via advertisements in local newspapers and university newsletters, via posters in university buildings and other public buildings and announcements on local radio and television. People responding to the advertisements were informed about the study purposes and requirements. 120 volunteers underwent the selection procedure which consisted of two fasting blood samples taken one week apart to determine serum lipids and lipoproteins, measurement of blood pressure, collection of a urine sample for determination of glucose and protein and a medical questionnaire. Seventy volunteers met our selection criteria. All had serum total cholesterol concentrations below 6.7 mmol/L, blood pressures below 140/80 mm Hg, no glucosuria, no proteinuria and did not use any medication known to affect blood lipids, coagulation, fibrinolysis or platelet aggregation. All selected volunteers gave their written informed consent.

During the study period seven subjects withdrew. One subject because of job commitments, two subjects because of illness and four subjects because of reasons specifically related to the strict study protocol. Results of three subjects were excluded from analyses. Two subjects, after being enrolled, started to use medications known to affect blood lipids. The third subject was excluded because her results were highly deviant for most variables possibly due to stressful personal circumstances. LDL cholesterol concentrations of this subject were 3.86 mmol/L after the oleic acid run-in period and 4.91 mmol/L after the oleic acid test period.

Thus, analyses included 37 women and 23 men. Women were aged 22-60 y (mean 40 y), weighed 48-101 kg (mean 67 kg), were between 155 and 183 cm in height (mean 167 cm), while body mass indexes ranged between 20-30 kg/m² (mean 24 kg/m²). The men were aged between 23-59 y (mean 43 y), weighed 56-88 kg (mean 78 kg), were between 163 and 190 cm in height (mean 178 cm), and had body mass indexes from 17 to 29 kg/m² (mean 25 kg/m²). Nine women were postmenopausal and 10 women used oral contraceptives. Six women and 5 men smoked. In women fasting concentrations of serum lipids ranged from 3.64 to 6.38 mmol/L (mean 5.28 mmol/L) for total cholesterol, 1.12 to 2.98 mmol/L (mean 1.65 mmol/L) for HDL cholesterol and 0.17 to 1.94 mmol/L (mean 0.74 mmol/L) for triacylglycerols. In men fasting concentrations of serum lipids ranged from 4.02 to 6.60 mmol/L (mean 5.43 mmol/L) for total cholesterol, 0.77 to 1.65 mmol/L (mean 1.23 mmol/L) for HDL cholesterol and 0.07 to 2.72 mmol/L (mean 1.05 mmol/L) for triacylglycerols.

Design and statistical analyses

The trial, which was approved by the medical ethics committee of Maastricht University, had a parallel design. During the run-in period all volunteers consumed a diet high in oleic acid for three weeks. They were then divided into three groups. For the next six weeks (the test period), one group consumed the MCFA diet, a second group the myristic acid diet and a third group continued on the oleic acid diet. The groups were stratified for initial serum cholesterol concentrations and sex. The response to the experimental diet was calculated per subject as the change from the end of the run-in period to the end of the MCFA, myristic acid or oleic acid test period. Differences in responses of lipid and lipoprotein concentrations were examined with diet, sex and diet-and-sex interaction as independent variables. The data were analyzed with the General Linear Models (GLM) procedure of the SAS Program (12). When the analyses indicated a significant effect of diet ($P < 0.05$), the diets were compared pair-wise. In addition, 95% confidence intervals were calculated for the differences between the diets. The results were corrected for 3-group comparisons by the Bonferroni correction. Because changes in lipoprotein(a) (Lp(a)) concentrations were not normally distributed, the untransformed individual changes of Lp(a) were analyzed with the non-parametric Kruskal-Wallis test (13). The a priori power to detect a true difference in total cholesterol concentrations of 8% between two diets was 80%.

Diets

Before the study started, the subjects recorded their habitual food intake for two working days and one weekend day. From these food records, each subjects' actual energy intake was calculated by using the Dutch food-composition table (14). The study diets were formulated at 13 levels of energy ranging from 5 MJ to 22 MJ, so that each subject received a diet that met his or her energy needs. Diets used in the study consisted of products in which the normal fat was replaced by the experimental fats. The products included margarines and bakery products (bread, cookies, pies and cakes). According to the subjects' energy level, lists were computed that stated the amount of experimental products the subject had to eat each day (margarine, bread, and cookies) and each week (cake and pie). These solid foods supplied 63% (25 En%) of the total fat energy. The remaining 37% (15 En%) of the total fat intake had to be chosen from a list of "free-choice" fat-containing products. These products were given points according to their fat contents. One point was equal to one gram of fat. Each subject was required to eat a certain number of points daily, again corresponding with his or her energy intake, and to list the products chosen daily on a special form. The fat composition of the calculated diets was similar except for approximately 10% of total energy intake, which was provided by MCFA, myristic acid or oleic acid, respectively.

Products were not labeled in the run-in period and were coded with a yellow, red or blue label in the test period to blind the subjects as to the nature of the diets. Products were handed out on an individual basis and were free of charge. Subjects came at least once a week to the university to receive a new supply of products, to be weighed, and to receive new forms to list the free-choice items.

Subjects recorded their food intake on one weekend and two weekdays in the last week of both the run-in and the test period. They were also asked to maintain the same activity level, and smoking and drinking habits throughout the study. They recorded in diaries any signs of illness, medication used, alcohol consumption, menstrual cycle and any deviations from the study protocol.

Experimental fats

Special experimental fats were developed (Grünau GMBH, Illertissen, Germany). The fat high in MCFA was made by interesterification of 34.0% palm stearin, 17.6% high oleic sunflower oil, 8.4% sunflower oil, and 40% medium chain fatty acid (MCT) oil. The composition of the MCT oil was 33.3% C6:0, 25.3% C8:0, and 41.5% C10:0. The myristic acid fat was made by interesterification of a blend of 34% palm stearin, 17% high oleic sunflower oil, 9% sunflower oil and 40% trimyristin. The oleic acid fat consisted of a blend of 30% palm stearin and 70% high oleic sunflower oil. Margarines were made from the fats and consisted of 83% fat and 17% water.

Sn-2 fatty acid composition of the margarines was determined using the Grignard reagent -allyl magnesium- to partially deacylate triacylglycerols leading to a representative mixture of *sn*-2 monoacylglycerols (15). In addition, the fatty acid composition of total triacylglycerols was determined (see table 1). The percent of each fatty acid at the *sn*-2 position was calculated from the total and the *sn*-2 fatty acid

compositions. For example, the percent of myristic acid present at the *sn*-2 position in the experimental margarine as a percent of total proportion myristic acid is:

$$sn\text{-}2 \text{ myristic acid} / (3 \times \text{total myristic acid}) \times 100.$$

Blood sampling and analyses

Blood was sampled after an overnight fast and after subjects abstained from drinking alcohol the preceding day and from smoking on the morning before blood sampling. Blood was sampled at the end of the run-in period (week 2 and week 3) and in weeks 6, 8 and 9 of the test period. Blood was drawn with minimum stasis using a 1.2 mm needle (Strauß Kanüle, Luer, Wächtersbach, Germany) with the volunteer in a recumbent position. First, 15 mL blood was taken for additional measurements (to be reported elsewhere), while the last 10 mL blood was drawn into a 10 mL clotting-tube for serum lipid and lipoprotein measurements. All venipunctures were performed by the same person, at the same location, and for each subject generally at the same time of the same day of the week.

Total cholesterol (CHOD-PAP method; Monotest cholesterol, Boehringer Mannheim, Mannheim, Germany), HDL cholesterol (precipitation method; Monotest cholesterol, Boehringer Mannheim, Mannheim, Germany) and triacylglycerols (GPO-Trinder; Sigma Diagnostics, St Louis, MO) were analyzed enzymatically. The coefficient of variation within runs was 1.0% for total cholesterol, 2.6% for HDL cholesterol and 1.5% for triacylglycerols. LDL cholesterol was calculated using the Friedewald equation (16).

Apolipoprotein (apo) A-I and apoB were measured in serum by using an immunoturbidimetric reaction (UNI-KIT apoA-I and UNI-KIT apoB, Roche, Basel, Switzerland) and antiserum raised in sheep and rabbits, respectively. The coefficients of variation within runs were 1.1% for apoA-I and 1.5% for apoB.

Lipoprotein(a) (Lp(a)) was measured in serum by an enzyme linked immunosorbent assay (ELISA) (TintElyseLp(a), Biopool, Umea, Sweden). The Lp(a) from the sample was bound to the Lp(a) antibodies in the wells. Peroxidase conjugated Lp(a)-antibodies were then used to tag the bound Lp(a). After washing away unbound antibodies, the peroxidase substrates were added. The extinction of the yellow color that developed was directly proportional to the amount of Lp(a) present in the sample. The coefficient of variation was 6.3% within runs.

Table 1. Total and *sn*-2 fatty acid composition and the percentage of each fatty acid at the *sn*-2 position (%*sn*-2)* in triacylglycerols of the experimental margarines.

Fatty acid	MCFA margarine			Myristic acid margarine			Oleic acid margarine		
	Total	<i>sn</i> -2	% <i>sn</i> -2	Total	<i>sn</i> -2	% <i>sn</i> -2	Total	<i>sn</i> -2	% <i>sn</i> -2
Saturated	76.7	65.1	28.3	64.4	67.8	35.1	23.6	8.4	11.9
MCFA	59.6	38.7	21.6	1.4	0.5	11.9	0.0	0.0	0.0
C12:0	0.2	0.4	66.7	0.5	0.7	46.7	0.1	0.1	33.3
C14:0	0.5	0.7	46.7	40.2	41.8	34.7	0.5	0.4	26.7
C16:0	14.3	22.1	51.5	18.6	20.7	37.1	17.5	7.1	13.5
C18:0	1.9	3.2	56.1	3.5	4.0	38.1	4.8	0.8	5.6
Monounsaturated	17.4	26.5	50.8	26.2	23.9	30.4	70.1	83.4	39.7
<i>cis</i> C18:1	16.9	26.6	52.5	25.6	23.9	31.1	69.9	83.4	39.8
Polyunsaturated	5.9	8.4	47.5	9.4	8.3	29.4	6.3	8.2	43.4
<i>cis,cis</i> C18:2	5.7	8.4	49.1	9.1	8.3	30.4	5.8	8.0	46.0

* Calculated as (*sn*-2 fatty acids / 3×total fatty acids)×100. In random distributions this value would equal 33%.

Serum was obtained by centrifugation at 2000 g (10 min, 4 °C) one hour after venipuncture and stored at -80 °C. The lipid and lipoprotein concentrations obtained for the two sampling days at the end of the run-in (week 2 and week 3) and the test period (week 8 and week 9) were averaged for data analyses. Samples of apoA-I, apoB and Lp(a) from these two sampling days, however, were pooled and analyzed as such. All samples from one subject were analyzed within one run.

Fatty acid composition of serum total lipids at the end of the run-in and at the end of the test period was determined in samples pooled according to diet and sex. Serum lipids were extracted with a modified Folch extraction (17), and transmethylated to their corresponding methyl esters (18), which were then quantified on a gas chromatograph, with a polar capillary column (CP Sil 88, Chrompack, Middelburg, The Netherlands) as described before (19).

RESULTS

Diets and dietary adherence

Table 1 shows the total and *sn*-2 fatty acid composition of the experimental margarines and the percentage of each fatty acid at the *sn*-2 position. In the MCFA margarine, 22% of MCFA was attached to the *sn*-2 position and the remaining 78% to either the *sn*-1 or the *sn*-3 position. Palmitic acid, oleic acid and linoleic acid, the other major fatty acids in this fat were mainly on the *sn*-2 position. In the myristic acid margarine, myristic acid and the other major fatty acids (palmitic, oleic and linoleic acids) were equally distributed among each position of the glycerol molecule.

The composition of the diets, as determined by a three days food record, is given in table 2. The average intake of oleic acid was 15.1 En% during the run-in period and decreased in the test period to 6.6 En% with the MCFA and to 6.3 En% with the myristic acid diet. It was exchanged for extra MCFA or myristic acid. Oleic acid intake during the oleic acid diet did not change from the run-in to the test period. Changes of other nutrients, except for En% saturated and monounsaturated fatty acids, did not significantly differ among the diets.

Dietary adherence was confirmed by determination of serum total fatty acid composition. Serum fatty acids with the MCFA diet group showed minor increases in the proportions of total fatty acids in capric acid (from 0.1% to 0.2%), myristic acid (from 1.7% to 2.0%) and stearic acid (from 5.6% to 6.2%). With the myristic acid diet the proportion of myristic acid increased from 2.1% to 3.2% of total fatty acids. The proportion of oleic acid with the oleic acid diet was similar after the run-in and test period (23.0% and 24.0%) but decreased with both the MCFA (from 22.2% to 18.3%) and myristic acid diets (from 25.1% to 18.8%).

Records of "free choice" fat-containing items during the experiment showed minimum deviation from the prescribed grams of "free choice" fat (mean $\pm$ standard deviation: $0 \pm 6\%$). The largest discrepancies were recorded during the first week of the run-in period, when the subjects had to familiarize themselves with the protocol.

Table 2. Mean daily intake of nutrients as calculated from the recorded food intakes during two weekdays and one weekend day in the run-in and the test period with diets enriched in MCFA (n=21), myristic acid (n=20) or oleic acid (n=19).*

	MCFA Diet	Myristic Acid Diet	Oleic Acid Diet
Energy (MJ/day)			
Run-in	9.2±3.0	11.0±3.6	9.8±2.2
Test period	9.1±2.8	10.9±2.9	9.5±2.0
Change	-0.1±1.1	-0.2±1.6	-0.4±1.4
Fat (En%)			
Run-in	38.7±6.5	38.4±4.6	39.7±3.2
Test period	40.4±3.7	39.7±4.5	40.0±3.9
Change	1.7±5.1	1.3±4.3	0.3±4.1
Saturates			
Run-in	11.3±2.0	11.3±1.4	11.7±1.4
Test period	21.3±2.4	21.0±2.2	11.6±1.9
Change	10.0±2.2	9.6±2.1	-0.1±2.0
MCFA †			
Run-in	0.0±0.0	0.0±0.0	0.0±0.0
Test period	9.9±1.9	0.0±0.0	0.0±0.1
Change	9.9±1.9	0.0±0.0	0.0±0.1
Myristic acid †			
Run-in	0.1±0.2	0.1±0.1	0.0±0.0
Test period	0.0±0.0	9.7±1.6	0.1±0.0
Change	-0.1±0.1	9.6±1.6	0.0±0.0
Oleic acid †			
Run-in	15.0±4.2	14.8±2.3	15.7±1.6
Test period	6.6±1.3	6.3±1.0	16.7±2.8
Change	-8.5±4.0	-8.5±2.1	0.9±2.3
Linoleic acid			
Run-in	4.0±0.8	4.0±0.9	3.7±0.5
Test period	4.0±0.6	4.0±0.8	4.0±0.5
Change	0.0±0.7	0.0±0.8	0.3±0.8

Table 2 continued.

	MCFA Diet	Myristic Acid Diet	Oleic Acid Diet
Carbohydrates			
Run-in	47.4±5.5	46.4±5.2	46.3±4.0
Test period	44.0±5.5	45.4±5.6	44.6±4.9
Change	-3.4±4.9	-1.0±5.5	-1.7±4.3
Protein			
Run-in	11.8±2.8	11.9±1.3	12.4±1.9
Test period	13.3±3.8	12.2±2.4	12.5±2.6
Change	1.5±2.2	0.3±1.3	0.2±2.1
Alcohol (mg/MJ)			
Run-in	2.0±2.2	3.1±3.6	1.6±2.6
Test period	2.2±3.7	2.6±4.0	2.8±5.5
Change	0.2±2.5	-0.5±2.5	1.2±3.9
Cholesterol (mg/MJ)			
Run-in	16.3±4.1	14.4±3.1	15.8±5.0
Test period	17.0±4.7	14.8±5.8	14.3±4.7
Change	0.7±5.5	0.4±5.3	-1.5±4.4
Fiber (g/MJ)			
Run-in	2.4±0.3	2.2±0.4	2.4±0.4
Test period	2.3±0.3	2.5±0.5	2.4±0.5
Change	-0.1±0.3	0.3±0.5	-0.1±0.7

* Values are means±standard deviations.

† As provided by the experimental fats only.

Mean body weight (means±standard deviations) in the MCFA group was 70±10 kg after the run-in and 70±10 kg after the MCFA diet, in the myristic acid group 72±9 kg after the run-in and 72±10 kg after the myristic acid diet and in the oleic acid group 71±12 kg after the run-in and 71±12 kg after the oleic acid diet. Body weights within the diet groups did not differ from the run-in to the end of the test periods.

Serum lipid and lipoproteins

Mean changes of serum total, LDL and HDL cholesterol concentrations are given in table 3 and in figure 1. Serum total cholesterol concentration increased by 0.55 mmol/L when the subjects switched to the myristic acid diet. This change was significantly different from the change with the oleic acid diet (difference in changes of 0.40 mmol/L; $P=0.0057$; 95% confidence interval (CI), 0.10 to 0.69 mmol/L). The difference in changes between the MCFA and the oleic acid diet group was 0.23 mmol/L ($P=0.0912$; 95% CI, -0.06 to 0.52 mmol/L). LDL cholesterol concentrations

were 0.37 mmol/L higher with the myristic acid diet compared with the oleic acid diet ($P=0.0064$; 95% CI, 0.09 to 0.64 mmol/L). The change of LDL cholesterol with the MCFA diet did not significantly differ from the change with the oleic acid diet (difference in changes of 0.23 mmol/L; $P=0.0752$; 95% CI, -0.04 to 0.50 mmol/L) and the change with the myristic acid diet (difference in changes of -0.14 mmol/L; $P=0.284$; 95% CI, -0.41 to 0.13 mmol/L).

Figure 1. Relative concentrations of serum total, LDL and HDL cholesterol concentrations on diets enriched in MCFA ($n=21$), myristic ($n=20$) or oleic acids ($n=19$). Concentrations of lipids and lipoproteins in week 3 were defined as 100%.

The HDL cholesterol concentration was higher with the myristic acid diet compared with the MCFA diet (difference in changes of 0.11 mmol/L; $P=0.0086$; 95% CI, 0.02 to 0.21 mmol/L), and compared with the oleic acid diet (difference in changes of 0.10 mmol/L; $P=0.0273$; 95% CI, 0.00 to 0.19 mmol/L). The MCFA diet did not raise HDL cholesterol concentrations compared with the oleic acid diet. Although the change in triacylglycerol concentrations with the MCFA diet was greater compared with the myristic acid diet, effects were not significantly different (difference in changes of 0.19 mmol/L; $P=0.0873$; 95% CI, -0.05 to 0.44 mmol/L). The total cholesterol to HDL ratio increased with the MCFA diet. However, this increase was not significantly different from the change on the other diets.

Table 3. Serum lipids and lipoprotein concentrations on diets enriched in MCFA, myristic or oleic acids.*

	MCFA Diet	Myristic Acid Diet	Oleic Acid Diet
Total cholesterol (mmol/L)			
Run-in	5.26±0.58	5.15±0.70	5.03±0.88
Test period	5.65±0.42	5.77±0.88	5.19±0.87
Change	0.39±0.39	0.55±0.51 †	0.16±0.39
HDL cholesterol (mmol/L)			
Run-in	1.49±0.32	1.52±0.54	1.52±0.32
Test period	1.51±0.32	1.65±0.60	1.55±0.32
Change	0.02±0.11 ¥	0.13±0.16	0.04±0.14
LDL cholesterol (mmol/L)\$			
Run-in	3.35±0.67	3.13±0.76	3.07±0.81
Test period	3.67±0.63	3.59±0.88	3.16±0.83
Change	0.32±0.36	0.46±0.51 †	0.09±0.36
Triacylglycerols (mmol/L)			
Run-in	0.92±0.42	1.15±0.81	0.98±0.43
Test period	1.02±0.56	1.11±0.66	1.04±0.49
Change	0.11±0.29	-0.09±0.47	0.06±0.26
Total cholesterol:HDL cholesterol ratio			
Run-in	3.73±1.09	3.84±1.50	3.49±1.14
Test period	3.95±1.08	3.92±1.63	3.48±0.97
Change	0.21±0.34	0.08±0.48	0.00±0.44

* Values are means±standard deviations.

† Significantly different from the oleic acid diet, $P<0.02$.¥ Significantly different from the myristic acid diet, $P<0.02$.

\$ Calculated with Friedewald equation.

ApoA-I concentrations increased with the myristic acid diet compared with the MCFA diet (difference in changes of 119 mg/L; $P=0.0093$; 95% CI, 22 to 216 mg/L) (Table 4). Changes in apoB concentrations were not significantly different among the three diets. ApoA-I to ApoB ratios significantly decreased with the MCFA compared with the myristic (difference in changes of -0.15; $P=0.0127$; 95% CI, -0.27 to -0.02) and the oleic acid diet (difference in changes of -0.14; $P=0.0197$; 95% CI, -0.27 to -0.01).

Table 4. Apolipoprotein A-I and apolipoprotein B concentrations on diets enriched in MCFA, myristic or oleic acids.*

	MCFA Diet	Myristic Acid Diet	Oleic Acid Diet
ApoA-I (mg/L)			
Run-in	1508±265	1556±354	1523±226
Test period	1489±217	1656±396	1564±239
Change	-19±209 ‡	100±115	41± 92
ApoB (mg/L)			
Run-in	898±153	922±165	859±215
Test period	980±134	998±201	892±217
Change	82±106	77±124	32± 70
ApoA-I:ApoB ratio			
Run-in	1.74±0.47	1.76±0.59	1.89±0.60
Test period	1.57±0.42	1.74±0.58	1.86±0.56
Change	-0.17±0.24 †‡	-0.02±0.17	-0.03±0.16

* Values are means±standard deviations.

† Significantly different from the oleic acid diet, $P<0.02$.‡ Significantly different from the myristic acid diet, $P<0.02$.

Lp(a) concentrations decreased with the myristic acid diet compared with the oleic acid diet ($P=0.0083$ for difference in changes); (table 5). Changes in Lp(a) concentrations with the MCFA diet did not differ from those with the myristic acid and the oleic acid diets. Changes in Lp(a) concentrations were more pronounced in subjects with higher Lp(a) values with the oleic acid run-in period.

For all variables, responses to the diets were similar in women and men.

Table 5. Lipoprotein (a) concentrations on diets enriched in MCFA, myristic or oleic acids.*

	MCFA Diet	Myristic Acid Diet	Oleic Acid Diet
Lipoprotein(a) (mg/L)			
Run-in	43 (0 to 677)	89 (1 to 691)	105 (0 to 767)
Test period	95 (3 to 597)	54 (2 to 689)	118 (0 to 782)
Change	0 (-297 to 95)	-4 (-164 to 202) †	13 (-34 to 190)

* Values are given as median levels (ranges)

† Significantly different from the oleic acid diet, $P<0.02$.

DISCUSSION

In this project we studied the effects on serum lipids and lipoproteins of MCFA, myristic and oleic acids. Results of the food records, serum total fatty acid composition and the daily records of the "free choice" products used indicated good compliance to the strict protocol. The energy percentage of the studied fatty acids was planned to be the only difference among the diets. To achieve this, MCFA and myristic acid were given to the subjects with synthetic fats. As in dairy fat, MCFA from the MCFA fat were mainly attached to the *sn*-1 or *sn*-3 positions of the glycerol molecule. In the myristic acid fat, myristic acid and other saturates were equally distributed among the *sn*-2 and *sn*-1,3 positions. Myristic acid rich fat sources as palm kernel or coconut fat have myristic acid mainly at the *sn*-1,3 (20), whereas dairy fat carries half of the total proportion of myristic acid at the *sn*-2 position (21). So far, dietary experiments comparing fats rich in palmitic acid (22, 23) and stearic acid (24) suggested that the positional distribution of these saturates did not affect lipid and lipoprotein concentrations. One small study reported that randomized butter oil was less hypercholesterolemic than a non-randomized butter oil diet (25). Although this may suggest that our results underestimate the cholesterol-raising effects of natural fats rich in MCFA and myristic acid, the results of that study (25) are difficult to interpret as only eight subjects were involved. Larger studies are necessary to examine whether the positional distribution of MCFA and myristic acid influences their cholesterolemic effects.

The effects of MCFA

The exchange of oleic acid by MCFA increased serum total and LDL cholesterol concentrations. The responses, however, did not differ significantly from the responses in the oleic acid and myristic acid diet groups. Other studies have reported similar serum total or LDL cholesterol concentrations with MCFA and western experimental or western habitual diets (3-5). These latter diets were high in saturated fatty acids, which cause higher plasma cholesterol levels than high oleic acid diets (26). Therefore, these earlier studies can also be interpreted as demonstrating a moderate cholesterol-raising effect of MCFA compared with oleic acid. In agreement, a recent study (27) demonstrated plasma total and LDL cholesterol raising effects of both diets rich in MCFA or palmitic acid compared with a diet rich in a high oleic acid sunflower oil.

Although the MCFA diet slightly increased triacylglycerol concentrations compared with myristic acid, triacylglycerol responses were not significantly different among the three diets. Other authors (3, 4) reported considerably elevated serum triacylglycerol concentrations with MCFA diets. In these studies (3, 4), however, diets provided 32 En% as MCFA, whereas in the present study only 10 En% MCFA was given. MCFA probably raise triacylglycerols in a dose dependent manner as McGandy et al (4) demonstrated that a diet with 18 En% MCFA caused a smaller increase of serum triacylglycerol concentrations than a diet with 32 En% MCFA (4). In agreement with our results, diets with 6 En% MCFA did not significantly increase

serum triacylglycerol concentrations (5). However, in studies that applied diets with high energy percentages of MCFA (3, 27) increased triacylglycerol concentrations did not affect the composition of the VLDL particles (3) or the ratio of triacylglycerol to VLDL cholesterol concentrations (27).

Compared with the oleic acid diet, the MCFA diet did not change HDL cholesterol nor did it change apoA-I concentrations. Also Cater et al (27) demonstrated similar HDL cholesterol concentrations on diets rich in MCFA or oleic acid. Diets enriched in carbohydrates, however, showed significantly decreased HDL cholesterol and apoA-I concentrations and similar LDL cholesterol concentrations compared with oleic acid (28). Thus, effects of MCFA on HDL cholesterol and apoA-I concentrations are likely to differ from those of carbohydrates, but this hypothesis needs to be confirmed in studies that compare the effects of MCFA and carbohydrates side-by-side.

In addition to slightly raised LDL cholesterol concentrations after the MCFA diet, other potent lipid predictors of coronary heart disease (29, 30) -the ratio of apoA-I to apoB and the total to HDL cholesterol ratio-, were unfavorably changed by this diet, although the latter ratio was not significantly different among the three diets.

The effects of myristic acid

Serum total cholesterol concentrations with the myristic acid diet increased with 0.55 mmol/L when 10 En% from oleic acid was replaced by myristic acid. This change was the result of both an increase in both LDL and HDL cholesterol concentrations. Zock et al (10) found an increase in total cholesterol of 0.66 mmol/L when the intake of myristic acid increased by 10 En% at the expense of oleic acid. Changes are smaller than predicted from the regression equations of Hegsted et al (8). This equation estimates that total cholesterol concentrations would increase with 2.19 mmol/L when 10 En% from oleic acid is replaced by myristic acid.

The HDL cholesterol raising effect of myristic acid was also found by other investigators (10, 11). In our earlier study (31), a comparable increase of 10% was found when 8 En% of lauric acid was exchanged for oleic acid. Raised HDL cholesterol concentrations were also reported in studies on coconut versus palm oil (32) and in studies that specifically investigated mixtures of lauric and myristic acids versus palmitic or oleic acids (33, 34). In addition, higher HDL cholesterol concentrations on western diets compared with MCFA diets (3, 5) might also be due to increased intakes of lauric or myristic acids with the western diets. It is possible that a lower plasma cholesterol ester transfer protein (CETP) activity may have contributed to the higher HDL cholesterol concentration on the myristic acid diet. An *in vitro* study indeed indicated a lower rate of CETP-mediated transfer of lauric or myristic acid rich cholesteryl esters from synthetic HDL compared with longer chain saturated fatty acids (35). The inverse relation between CETP activity and HDL cholesterol concentrations was also demonstrated in human studies comparing diets rich in *trans* fatty acids with those rich in linoleic or oleic acids (36, 37). However, as compared with a safflower oil diet two myristic acid rich diets - one high in butter fat and the other high in coconut fat-, induced similar HDL cholesterol concentrations, but the cholesterol ester transfer activity was increased on the butter and not on the

coconut fat diet (38). Clearly, more studies are needed to clarify the regulatory mechanism behind changes in HDL cholesterol concentrations.

Myristic acid decreased Lp(a) compared with oleic acid which emphasized that Lp(a) concentrations were regulated independently from LDL cholesterol concentrations. From another study (39) it was concluded that oleic acid raised Lp(a) levels relative to a mixture of saturated fatty acids. In our previous study we did not observe decreased Lp(a) concentrations when comparing lauric or palmitic acids with oleic acid (31). The suggestion, however, that myristic acid in particular decreases Lp(a) concentrations is not supported by a recent study reporting similar Lp(a) concentrations after diets rich in myristic or palmitic acids (40).

To summarize, the MCFA diet increased total and LDL cholesterol slightly, though not significantly, but not HDL cholesterol concentrations compared with the oleic acid diet. The MCFA diet unfavorably affected the apoA-I to apoB ratio compared with both other diets. Myristic acid is a hypercholesterolemic saturated fatty acid although responses on serum total cholesterol concentrations are smaller than has been suggested earlier (8). Part of the cholesterolemic effect of myristic acid is due to increased HDL cholesterol concentrations.

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Dietary induced changes in lipoproteins and mood

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ABSTRACT

Adverse effects of lowering cholesterol concentrations on mortality of non-illness related diseases have been reported from epidemiological studies. The hypothesized mechanism associates decreased serum cholesterol concentrations with a more negative mood state. Little information, however, is available on the effects of dietary-induced changes in lipoprotein concentrations on mood. In this study, sixty normocholesterolemic subjects (37 women and 23 men) first consumed a 3 wk run-in diet enriched in oleic acid. For the next 6 weeks, 19 subjects continued with the oleic acid diet, 21 subjects with an MCFA enriched diet, while 20 subjects received a diet high in myristic acid. Total diets contained 40 percent of energy from fat and the nutrient composition was the same in each diet except for 10 En%, which was provided by MCFA, myristic or oleic acids. Ten mood states were measured with 100 mm visual analog scales. In addition, scores of state and trait anxiety were determined with Spielbergers' state-trait anxiety inventory (STAI). Serum total cholesterol levels increased with 0.40 mmol/L on the myristic acid diet compared with the oleic acid diet ($P=0.0057$ for difference in changes). Changes in scores of mood did not differ between the three diets, and mood scores were not correlated with lipoprotein concentrations. Also changes in state anxiety or trait anxiety did not differ between the diets, but trait anxiety scores tended to associate with HDL concentrations ($r=0.24$; $P=0.0650$ after the run-in and $r=0.31$; $P=0.0148$ after the test period). In conclusion, our data indicate unchanged mood after changes in lipoproteins when a 6 wk oleic acid diet is compared with a myristic acid or MCFA rich diet.

INTRODUCTION

A meta-analysis of six randomized primary prevention trials demonstrated that lowering serum cholesterol concentrations was associated with increased deaths from violence, suicides or accidents (1). It was hypothesized that reduced serum cholesterol concentrations cause decreased serotonin levels in the brain or at synapses, which might be related with a more negative mood state (2). Indeed, low cholesterol concentrations have been associated with low concentrations of plasma serotonin (3) and tryptophan (4), the precursor for serotonin. In addition, the functioning of the synaptic membranes -i.e. release and uptake of neurotransmitters and receptor function- may be affected by membrane fluidity as affected by fatty acid composition and membrane cholesterol content, which alter after modifying the intake of certain dietary fatty acids (see for review (5)).

In human subjects, studies on diet-induced changes in cholesterol concentrations and mood are scarce. Recently, we carried out an intervention study in healthy normocholesterolemic women and men to investigate the effects of diets enriched in saturated fatty acids (Medium Chain Fatty Acids (MCFA) or myristic acid) relative to those of monounsaturated fatty acids (oleic acid). As changes in serum cholesterol concentrations through dietary fatty acid alterations were expected, the effects on mood were measured.

SUBJECTS AND METHODS

Subjects

Sixty subjects (37 women and 23 men) completed this controlled dietary experiment. Women were aged 22-60 y (mean 40 y), weighed 48-101 kg (mean 67 kg), were between 155 and 183 cm in height (mean 167 cm), and had body mass indexes from 20-30 kg/m² (mean 24 kg/m²). The men were aged between 23-59 y (mean 43 y), weighed 56-88 kg (mean 78 kg), were between 163 and 190 cm in height (mean 178 cm), and had body mass indexes from 17 to 29 kg/m² (mean 25 kg/m²). Nine women were postmenopausal and 10 women used oral contraceptives. Six women and 5 men smoked. In women fasting concentrations of serum lipids ranged from 3.64 to 6.38 mmol/L (mean 5.28 mmol/L) for total cholesterol, 1.12 to 2.98 mmol/L (mean 1.65 mmol/L) for HDL cholesterol and 0.17 to 1.94 mmol/L (mean 0.74 mmol/L) for triacylglycerols. In men fasting concentrations of serum lipids ranged from 4.02 to 6.60 mmol/L (mean 5.43 mmol/L) for total cholesterol, 0.77 to 1.65 mmol/L (mean 1.23 mmol/L) for HDL cholesterol and 0.07 to 2.72 mmol/L (mean 1.05 mmol/L) for triacylglycerols.

Diets and design

The main purpose of the experiment was to test the effects of diets enriched in MCFA, myristic or oleic acid on lipoprotein concentrations and hemostatic factors of the blood. Details of this study have been published previously (6). The trial had a

parallel design and was approved by the medical ethics committee of Maastricht University. During a 3 wk run-in period, all subjects consumed a diet high in oleic acid. They were then randomly divided into three groups. For the next six weeks, one group consumed the MCFA diet (n=21), a second group the myristic acid diet (n=20) and a third group continued with the oleic acid diet (n=19). The groups were stratified for initial serum cholesterol concentrations and sex.

Before the study started, the subjects recorded habitual food intake for two working days and one weekend day. From this food record each subjects' actual energy intake was calculated by using the Dutch food-composition table (7). The study diets were formulated at 13 levels of energy (5 MJ to 22 MJ), so that each subject received a diet that met his or her energy needs. Diets used in the study consisted of products in which the normal fat was replaced by the experimental fats. The products included margarines and bakery products (bread, cookies, pies and cakes). Based on the subjects' energy intake, the amount of experimental products the subject had to eat each day (margarine, bread, and cookies) and each week (cake and pie) were calculated and used. These solid foods supplied 63% (25 En%) of the total fat energy. The remaining 37% (15 En%) of the total fat intake had to be chosen from a list of "free-choice" fat-containing products. These products were given points according to their fat contents. One point was equal to one gram of fat. Each subject was required to eat a certain number of points daily, again corresponding with his or her energy intake, and to list the products chosen daily on a special form. The fat composition of the calculated diets was similar except for approximately 10% of total energy intake, which was provided by MCFA, myristic acid or oleic acid, respectively. The composition of the experimental fats has been described elsewhere (6).

Scores of mood

On each day of the final week of the run-in (week 3) and of the experimental period (week 9), subjects were asked to rate on 100 mm lines (visual analog scale (VAS)) ten items depicting ten different mood states. The ten items included annoyance, despair (mood state of depression/dejection), moodiness, irritation (mood state of anger/hostility), tiredness, boredom (mood states of fatigue/inertia), brightness, pleasure (mood state of vigor/activity), self confidence and tenseness (mood state of tension/anxiety). The 100 mm lines for each item were printed on separate sheets of paper. Each day, the items were presented in a different order. The items were balanced so that for half of the items the left hand side of the line reflected the positive mood, while this was reversed for the other half. For analyses, the last mentioned items were reversed, so that 0% corresponded with the most positive and 100% with the most negative mood. The subject rated the ten items at the end of each day just before retiring for the night.

State and trait anxiety inventories

On the last day of the run-in and the experimental periods the subjects came to the university for a fasting blood sample. Afterwards the subjects' state and trait anxiety scores were measured with the Dutch versions of the Spielberger State-Trait Anxiety Inventory (STAI) (8). State anxiety is defined as "a transient arousal state of anxiety; an emotional condition marked by subjective feelings of tension and stress, which vary in intensity and fluctuate in time and by increased activity of the nervous system. Trait anxiety, on the other hand, is the general disposition to anxiety; the tendency to react to situations experienced as threatening by increasing intensities of state anxiety" (9).

Each inventory consists of 20 quotes, that are used by people to describe themselves (for example "I am happy" or "I am nervous"). The subjects were asked to rate every quote on a scale ranging from 1 (hardly ever) to 4 (all the time) according to how they had been feeling at the specific moment that they filled out the inventory (state anxiety inventory) or in general (trait anxiety inventory). Total scores for the state anxiety or trait anxiety were calculated by summing the scores for the quotes; the total score therefore ranged from 20 to 80. The test-retest correlations for the trait scale were on average 0.82 (for retesting after 5 to 202 days) for psychiatric outpatients and 0.75 for students (for retesting after 118 days) (9). The state scale, which is supposed to be inconsistent with time, indeed has a low test-retest reliability (0.40 (for retesting after 5 to 202 days in psychiatric outpatients) and 0.28 (for retesting after 118 days in students) (9). The STAI has been validated in students undergoing examinations, who had higher state anxiety and similar trait anxiety (44 for state and 35 for trait anxiety) compared with control students (34 for state and 35 for trait anxiety). The trait anxiety scores have been validated in patients diagnosed with (48 for state and 52 for trait anxiety) or without (41 for state and 45 for trait anxiety) anxiety problems, and in frequent (37 for state and 37 for trait anxiety) or occasional visitors (35 for state and 34 for trait anxiety) of the general practitioner (9). Strong correlations exist between anxiety and depression scores (9).

Statistical analysis

Daily VAS on mood were averaged per item, per week. For each item, changes from the run-in to the test periods were calculated. Differences in responses of mood scores were examined with diet as independent variable. Responses of scores of self confidence, boredom and despair were not normally distributed and were evaluated with the non-parametric Kruskal-Wallis test (10). Other scores were analyzed with the General Linear Models (GLM) procedure of the SAS Program (11). Of the total of 9240 scores of mood, 97 (=1%) were missing. The missing items were left out from the calculated means.

For the state and the trait inventory, respectively, the scores on the 20 quotes were summed. If questions were not answered (8 of 2400 questions), a score of 2 was used as suggested (9). One subject did not complete the trait questionnaire after the run-in period. Trait of the test and run-in period for this subject have been assumed to be similar. Spearman correlation coefficients were calculated between

changes in lipid and lipoprotein concentrations, and changes in mood and state and trait anxiety scores. To investigate whether the subjects' general disposition to anxiety was associated with lipoprotein concentrations we, in addition, calculated Spearman correlation coefficients between trait anxiety scores and lipid and lipoprotein concentrations after both the run-in and the test period.

RESULTS

Serum total cholesterol levels increased by 0.55 mmol/L or 11% with the myristic acid diet and by 0.39 mmol/L or 7% with the MCFA diet during the test period. With the oleic acid diet, the serum total cholesterol concentrations also slightly increased by 5%. The response with the myristic acid diet was significantly different from the response with the oleic acid diet (difference in changes of 0.40 mmol/L; $P=0.0057$). HDL cholesterol concentrations increased with the myristic acid as compared with the MCFA diet (difference in changes of 0.11 mmol/L; $P=0.0086$) and the oleic acid diet (difference in changes of 0.10 mmol/L; $P=0.0273$). Changes in HDL cholesterol concentrations with the MCFA diet and the oleic acid diet did not differ. Triacylglycerol concentrations were not significantly changed by the three diets.

Scores of mood at the end of the run-in and changes from run-in to test period are given in table 1. No differences were observed between the changes on the three different diets. Of the mood scores, scores of moodiness after the run-in period were associated with triacylglycerol concentrations ($r=0.26$; $P=0.0443$). Other mood scores did not significantly correlate with lipid or lipoprotein concentrations (data not shown).

State and trait anxiety scores are given in table 2. Changes in state and trait anxiety did not differ between the three diets. Trait anxiety after the run-in was strongly correlated to trait anxiety after the experimental period ($\rho=0.82$, $P<0.0001$; results of all subjects combined). With state anxiety this correlation was smaller ($\rho=0.60$, $P<0.0001$; results of all subjects combined). Trait anxiety scores after the test period correlated positively with HDL concentrations ($r=0.31$; $P=0.0148$) and negatively with total to HDL cholesterol ($r=-0.28$; $P=0.0320$) and LDL to HDL cholesterol ratios ($r=-0.29$; $P=0.0272$) (table 3). Correlations were less pronounced after the run-in period; $r=0.24$, $P=0.0650$ for HDL concentrations; $r=-0.22$, $P=0.0787$ for the total to HDL cholesterol ratio; and $r=-0.25$, $P=0.0524$ for the LDL to HDL cholesterol ratio.

Table 1. Scores of mood after diets enriched in MCFA (n=21), myristic acid (n=20) or oleic acid (n=19).*

	MCFA Diet		Myristic Acid Diet		Oleic Acid Diet	
	Run-in	Change	Run-in	Change	Run-in	Change
<i>Depression/Dejection</i>						
Annoyance	21±18	3±9	23±21	1±7	23±16	4±9
Despair	19±20	0±7	21±21	1±10	23±16	1±9
<i>Anger/Hostility</i>						
Moodiness	23±20	1±6	29±17	0±7	25±16	-1±6
Irritation	25±20	1±10	31±17	-1±10	25±14	1±7
<i>Fatigue/Inertia</i>						
Tiredness	43±17	0±9	41±19	0±7	38±15	1±8
Boredom	17±16	-1±8	18±20	1±6	11± 7	2±9
<i>Vigor/Activity</i>						
Brightness	32±20	0±8	33±19	1±6	30±17	-3±8
Pleasure	32±18	-0±7	34±15	1±6	33±16	0±6
<i>Tension/Anxiety</i>						
Self Confidence	28±21	0±6	29±18	2±5	30±17	0±7
Tenseness	28±20	1±12	35±16	1±11	31±17	-2±10

* Values are means±standard deviations (%). 0% corresponds with the most positive mood, 100% with the most negative mood.

Table 2. State and trait anxiety scores after run-in (week 3) and changes from run-in to test period (week 9).*

	MCFA Diet		Myristic Acid Diet		Oleic Acid Diet	
	Run-in	Change	Run-in	Change	Run-in	Change
State anxiety	33.0±8.0	-1.0±7.4	32.4±6.2	-0.7±4.8	35.0±11.7	-0.9±8.9
Trait anxiety	31.9±6.9	1.0±6.8	35.0±7.0	-0.1±5.0	35.4±11.1	-0.5±4.2

* Values are means±standard deviations.

Table 3. Spearman correlation coefficients between trait anxiety and serum lipids and lipoprotein concentrations at the end of the run-in period and test period.

	Trait anxiety Run-in	Trait anxiety Test Period
Total cholesterol	-0.01	-0.02
LDL cholesterol	-0.12	-0.12
HDL cholesterol	0.24	0.31 †
Triacylglycerols	0.03	0.00
Total to HDL cholesterol ratio	-0.22	-0.28 †
LDL to HDL cholesterol ratio	-0.25	-0.29 †

† $P < 0.05$.

DISCUSSION

This study found lower serum total cholesterol concentrations in subjects on a diet, of six weeks duration, rich in oleic acid compared with a myristic acid rich diet. The diet-induced changes in serum cholesterol were not associated with altered scores of mood or anxiety. In our study, a difference of 6% in total cholesterol concentrations was found between the myristic and the oleic acid groups. This is modest compared with reductions of 13% in two long term dietary interventions with normocholesterolemic subjects, included in the meta-analysis that found an association between total cholesterol lowering and deaths not related to illness (1).

Other studies that especially focused on cholesterol-lowering and mood after drug (12, 13) or diet (14) interventions found similar results as we did. Long-term treatment during one (13) or three years (15) with cholesterol-lowering drugs did not affect emotional well-being as assessed by a quality of life questionnaire (13) or the shortened profile of mood states questionnaire (15). Also only four weeks administration of cholesterol-lowering drugs did not indicate effects on mood measured with the hospital anxiety scale (16). Results of a serum cholesterol-lowering education program, in which measurements of psychological well-being (with Hopkins Symptom Checklist) were included decreased feelings of hostility and depression in the treated compared with the control group were found (14). In that study (14), however, the control subjects lacked the attention given to the treated subjects, which might have influenced the results. In addition, the only small changes (2.7%) in serum cholesterol concentrations that were found between groups (14) were not very likely to have major effects on psychological well-being through changes in serotonin concentrations. In the study that found low plasma serotonin in

men with low serum cholesterol concentrations, serum cholesterol levels were 42% lower compared with the reference group (3).

The association between trait anxiety and HDL cholesterol concentrations needs to be confirmed in further studies. So far, few studies in this field have determined complete lipid profiles. Higher HDL cholesterol, besides lower total cholesterol concentrations, have been reported from patients with major depression disorders compared with controls (17). On the other hand Maes et al (18) reported lower HDL cholesterol concentrations in subjects with major depression compared with controls.

In conclusion, our data indicate unchanged mood after changes in lipoprotein concentrations, when an 6 wk oleic acid diet is compared with a myristic acid or MCFA rich diet in normocholesterolemic subjects. Data from these type of studies on the effects of fatty acid composition on serum lipids and mood, however, are still very limited and need to be extended with future research.

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5

Individual saturated fatty acids and effects on whole blood aggregation *in vitro*.

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ABSTRACT

In two studies we have compared the effects of four different saturated fat diets (Medium Chain Fatty Acids (MCFA), lauric acid, myristic and palmitic acid) with the effect of a monounsaturated oleic acid diet on *in vitro* whole blood aggregation in healthy women and men. STUDY 1 had a cross-over study design with three diet periods of each six weeks, and examined the effects of diets enriched in lauric, palmitic or oleic acids. STUDY 2 had a parallel study design. After a 3 week oleic acid run-in diet, three groups were formed which consumed either an MCFA, myristic acid or oleic acid rich diet for six weeks. Eighteen women and 14 men were included in STUDY 1 and 37 women and 23 men in STUDY 2. All subjects were healthy and aged 20-60 yr. The experimental diets were the same in nutrient composition except for on average 8 En% (STUDY 1) or 10 En% (STUDY 2) which was provided by either MCFA, lauric acid, myristic acid, palmitic acid or oleic acid. Blood samples were taken at the end of each dietary period. Whole blood platelet aggregation, anti-coagulated with recombinant hirudin was assessed after administration of collagen (final concentration (fc): 0.38 µg/mL) in STUDY 1 and collagen (fc: 0.22 µg/mL) or ADP (fc: 1.25 µmol/L) in STUDY 2. Collagen-induced formation of thromboxane (Tx)A₂, measured as TxB₂, was evaluated in STUDY 1 only. The aggregation velocity between the saturated fatty acid diets and the monounsaturated fatty acid diet did not differ. TxB₂ concentrations measured in collagen activated blood samples, which correlated significantly with aggregation velocity, did not differ between the lauric or the palmitic compared with the oleic acid diet. A stepwise regression analysis indicated that red blood cell count was negatively related with collagen- and ADP-induced aggregations. We conclude that the exchange of 8-10 En% from oleic acid for MCFA, lauric, myristic or palmitic acid does not affect *in vitro* whole blood aggregation induced by collagen. ADP-induced aggregation is not affected when 10 En% from oleic acid is exchanged for MCFA or myristic acid.

INTRODUCTION

Blood platelets play an important role in the etiology of coronary heart disease. Activated platelets provide tissue factor, membrane phospholipid surface, and other regulatory compounds which are necessary for the coagulation process. Increased platelet reactivity for ADP was demonstrated in middle aged men at risk for coronary heart disease (1) and in patients with coronary heart disease (2). Also, the protective effect of aspirin against myocardial infarction suggests a major role of platelets in the development of coronary heart disease (3, 4), as it inhibits platelet synthesis of thromboxane A₂ (TxA₂).

Different types of dietary fatty acids have been demonstrated to change the reactivity of platelets. Renaud and coworkers (5) found increased thrombin-induced, and decreased ADP-induced platelet rich plasma (PRP) aggregation when farmers switched from high saturated fat diets to high polyunsaturated fat diets for one year. Mutanen and coworkers (6) demonstrated lower ADP- and collagen-induced PRP aggregation on a dairy fat diet compared with diets rich in unsaturated fatty acids from rapeseed or sunflower oil. Ng et al (7), in addition, found increased thromboxane B₂ (TxB₂) in collagen-activated whole blood comparing diets rich in coconut with palm oil. This observation (7) suggested that the various saturated fatty acids have different effects on platelet activity and that thromboxane dependent aggregation increases after diets rich in fatty acids from coconut oil (i.e. MCFA, lauric and myristic acid). However, effects of specific saturated fatty acids on platelet aggregation have hardly been investigated systematically.

Platelet aggregation is frequently measured with the optical density technique using citrated PRP and activators of aggregation such as ADP, collagen or thrombin. This technique might not always be preferable, since it does not take into account the possible role of short-lived plasmatic components and of red and white blood cells. In this present study, therefore, the effects of diets enriched in MCFA, lauric, myristic and palmitic acid on aggregation in whole blood *in vitro* were investigated and compared with those of oleic acid.

METHODS

Design and statistical analysis

The project consisted of two different studies also designed to investigate the effects of separate saturated fatty acids on lipoproteins, coagulation and fibrinolysis as reported elsewhere (8, 9). The protocols of both studies had been approved by the ethical committee of Maastricht University. All subjects had given their written informed consent.

In the first study (STUDY 1) the effects of lauric acid, palmitic acid and oleic acid were investigated with a cross-over study design. The three different diets were consumed by 32 healthy subjects, 18 women and 14 men, for six weeks separated by wash out periods from two or three weeks. The second study (STUDY 2) examined

the effects of MCFA and myristic acid versus oleic acid with a parallel study design. After a run-in period of three weeks, in which 60 volunteers, 37 women and 23 men, consumed an oleic acid diet, the subjects were divided into three groups; the first group received an MCFA diet ($n=21$), the second group a myristic acid diet ($n=20$) and the last group continued with the oleic acid diet ($n=19$).

Data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analyses System. In STUDY 1, the aggregation parameters at the end of each dietary period were analyzed with diet, period and diet-and-sex interaction as independent variables. Data of TxB_2 were logarithmic transformed before analysis, because they were not normally distributed. In STUDY 2, changes in aggregation parameters from the end of the run-in to the end of the experimental period were analyzed with diet, sex and diet-and-sex interaction as independent variables. Pearson correlation coefficients were calculated between aggregation velocity and hematologic parameters. Correlations were calculated when subjects consumed the oleic acid diet during the experimental period (STUDY 1) or the run-in period (STUDY 2). To determine the relationship between hematologic parameters and aggregation in whole blood, a stepwise regression procedure (forward selection) was performed with whole blood aggregation velocity as dependent and the main hematologic parameters -platelet count, red blood cell count and white blood cell count- as independent variables. The significance level for entry in the model was set at 0.05.

In both studies differences in responses between women and men were evaluated with unpaired t-tests.

Subjects

Details of both studies have been described in chapter 2 and chapter 3. Briefly, STUDY 1 included 32 subjects, 18 women and 14 men, aged between 20 and 60 years (mean, 41 years old). The women had body mass indexes from 19 to 30 kg/m^2 (mean 25 kg/m^2), the men had body mass indexes from 22 to 31 kg/m^2 (mean 25 kg/m^2). Four women and 5 men were smokers. Six women were postmenopausal and 2 women used oral contraceptives. STUDY 2 included 60 subjects, 37 women and 23 men, aged between 22 and 60 years (mean, 41 years old). Women had body mass indexes between 20 and 30 kg/m^2 (mean 24 kg/m^2) and the men between 17 and 29 kg/m^2 (mean 25 kg/m^2). Nine women were postmenopausal and 10 women used oral contraceptives. Six women and 5 men smoked.

Diets

The diets of both studies consisted of products in which the normal fat was replaced by experimental fats. Total fat energy was on average 40 percent of energy (En%). The experimental fats supplied 70% of the total fat energy in the first and 63% in the second study. The nutrient composition of the calculated diets was similar except that approximately 8 En% (STUDY 1) or 10 En% (STUDY 2) of oleic acid was exchanged for lauric acid or palmitic acid (STUDY 1) and for MCFA or myristic acid

(STUDY 2). These differences were achieved by the use of special experimental fats. The lauric acid fat was a mixture of palm kernel (75%) (Wesco; Karlshamns, Zaandijk, the Netherlands) and a high oleic acid sunflower oil (25%) (Trisun; SVO enterprises, Wickliffe, USA). The palmitic acid fat consisted of dairy fat (55%) (FDF, Leeuwarden, The Netherlands), palmstearin (36%) (Aarhus oil, Aarhus, Denmark) and sunflower oil (9%) and the fat high in oleic acid of dairy fat (37%) and high oleic acid sunflower oil (63%). Cholesterol was added to the fats high in lauric and oleic acid, so that all fats contained 145 mg of cholesterol per 100 g of fat. The fat high in MCFA was made by interesterification of 34.0% palm stearin, 17.6% high oleic acid sunflower oil, 8.4% sunflower oil, and 40% medium chain triglyceride (MCT) oil. The composition of the MCT oil was 33.3% C6:0, 25.3% C8:0, and 41.5% C10:0. The myristic acid fat was made by interesterification of a blend of 34% palm stearin, 17% high oleic acid sunflower oil, 9% sunflower oil and 40% trimyristin. The oleic acid fat consisted of a blend of 30% palm stearin and 70% high oleic acid sunflower oil (Grünau GMBH, Illertissen, Germany). Margarines were made from the fats and consisted of 83% fat and 17% water. The experimental margarines were given as such and were incorporated in bread, biscuits, pies, cakes (STUDY 1 and STUDY 2), cheeses and custards (STUDY 1 only).

The study diets were formulated at different levels of energy, so that each subject received a diet that met his or her energy needs. According to these energy levels lists were computed which stated the amount of experimental products the subject had to eat each day or each week. Products were handed out on an individual basis and were free of charge. 30% (STUDY 1) and 37% (STUDY 2) of the total fat intake was free of choice. The subject could choose these "free" products from a list of fat containing products. Subjects came at least once a week to a special room in the university to get a new supply of products and to be weighted. They were also asked to maintain the same activity level, smoking and drinking habits throughout the study. Any changes were written down in a special diary as well as medication used, illnesses, daily alcohol use and menstrual cycle.

Blood sampling and measurements

Blood was sampled after an overnight fast and after abstinence from drinking the preceding day and smoking on the morning before blood sampling. Blood for platelet aggregation was sampled at the end (week 6) of each period in STUDY 1 and at the end of the run-in (week 3) and the end of the experimental period (week 9) in STUDY 2. Blood was sampled with a minimum of stasis and using a 1.2 mm needle (Strauß Kanüle, Luer, Wächtersbach, Germany) with the subject in recumbent position. All venipunctures were performed by the same person, at the same location, and for each subject generally at the same time of the same day of the week.

The first 3-4 mL blood was collected in an EDTA tube to count the number of platelets (Hematology system, Technicon Instruments Corporation, Tarrytown, NY, USA) in STUDY 1 and to measure complete hematologic parameters in STUDY 2 (Coulter microdiff 18, Coulter Corporation, Miami, USA). Then, 1.5 mL of blood was collected in a tube containing 67 µg/mL recombinant desulphatohirudin variant 1

(TMRevasc; a gift from Novartis Pharma AG, Horsham, Great Britain). Whole blood aggregation was immediately measured after blood sampling using a whole blood aggregometer (Chrono-log whole blood aggregometers, model 500 (STUDY 1 and STUDY 2) and model 540 (STUDY 2)) (Chrono-log Corporation, Haverton, PA, USA). 1 mL of blood was transferred to a pre-warmed plastic cuvet and the electrodes were inserted in the cuvettes. The sample was stirred at 1000 rpm, kept at 37 °C and allowed to reach equilibrium. After calibrating the instrument, 10 µl of the aggregation inducer (collagen or ADP) was added. Aggregation curves were registered for 15 minutes and the velocity was expressed as the steepest slope of the aggregation curve (Ω/minute). When aggregation curves of ADP-induced aggregation consisted of two curves the steepest slope of the first curve was determined.

In STUDY 1, platelet aggregation was induced by addition of collagen (Collagen Horm, Hormon Chemie, Munich, Germany) at a final concentration of 0.38 µg/mL. In STUDY 2, collagen- and ADP- (Sigma Chemical Co, St Louis, MO, USA) induced aggregation were measured. Final concentrations were 0.22 µg/mL for collagen and 1.25 µmol/L for ADP. Samples of the subjects were measured at the same channel of the aggregometer along the study. Due to an occasional technical problem with the aggregometers, aggregation values of some subjects are missing, which has been emphasized in the tables.

After 15 minutes of activation, collagen-activated aggregation samples from STUDY 1 were centrifuged in an eppendorf centrifuge for 3 minutes to measure TxB₂ production. The plasma samples were then immediately snap-frozen and stored at -80 °C. The TxB₂ concentration was determined with an Enzyme Immunoassay (Cayman Chemical Company, Ann Harbor, MI, USA). Coefficient of variation within runs was 6.1%. Samples from one subject were analyzed within one run.

RESULTS

Dietary composition

The mean daily energy intake and the composition of the diets was determined from four days food records in STUDY 1 and three days food records in STUDY 2. In addition, duplicate portions of food and drinks consumed have been collected by the subjects for two days (STUDY 1). Lipids of these portions were extracted and fatty acid composition determined.

In STUDY 1, total fat intake was 41.5 En% with the lauric acid diet, 41.0 En% with the palmitic acid diet and 41.8 En% with the oleic acid diet. As determined from the duplicate portions, the difference in lauric acid was 7.3 En% between the lauric and the oleic acid diet, while the palmitic acid diet was 6.1 En% higher in palmitic than the oleic acid diet. The lauric acid diet contained also some (average 1.8 En%) more myristic acid than the other two diets. The diets did not differ with respect to linoleic acid or α-linolenic acid intakes (10).

In STUDY 2, the average intake of fat was 39 En%. The average intake of oleic acid was 15.1 En% during the run-in period and decreased in the test period to 6.6

En% with the MCFA and to 6.3 En% with the myristic acid diet. It was exchanged for extra MCFA or myristic acid. Oleic acid intake during the oleic acid diet did not change from the run-in to the test period. Changes of other nutrients, except for En% saturated and monounsaturated fatty acids, did not significantly differ between the diets.

Relative to the oleic acid diet, fatty acid composition of pooled serum samples in both studies (8, 10) did not indicate significant changes in the proportions of linoleic acid, α -linolenic acid, arachidonic acid or total polyunsaturated fatty acids.

Platelet aggregation and TxB_2

Table 1 shows that collagen-induced aggregation velocity and TxB_2 production after 6 weeks consumption of diets enriched in lauric, palmitic or oleic acids did not differ significantly between the diets. Also, diets rich in MCFA and myristic acid (STUDY 2) did not affect collagen- and ADP-induced aggregation velocity differently as compared with a diet rich in oleic acid (table 2).

In STUDY 1, logarithmic transformed values of TxB_2 concentrations correlated with aggregation velocity ($r=0.42$; $P=0.0165$, $r=0.48$; $P=0.0055$, $r=0.39$; $P=0.0274$, for subjects on the lauric, palmitic or oleic acid diet, respectively).

Collagen-induced aggregation velocity did not differ between men and women in STUDY 1. Collagen- and ADP-induced aggregation velocities after the run-in period in STUDY 2, however, were significantly higher in women compared with men ($P=0.0020$ and $P=0.0010$, respectively). The data of collagen-induced aggregation velocity of the females consuming the oleic acid diet (oleic acid diet period in STUDY 1 and oleic acid run-in period in STUDY 2) were combined to determine differences between pre- and postmenopausal women. Collagen-induced aggregation velocity was (means $\pm$ standard deviations) 5.2 ± 1.8 Ω/min in premenopausal women not on contraceptives ($n=27$, one missing value), 6.6 ± 1.4 Ω/min in premenopausal women on contraceptives ($n=11$, one missing value) and 5.9 ± 2.2 Ω/min in postmenopausal women ($n=15$) and did not differ significantly between these groups of women. ADP-induced aggregation velocity, which was only measured in STUDY 2, was 6.6 ± 1.9 Ω/min in premenopausal women not on contraceptives ($n=17$, one missing value), 5.6 ± 1.2 Ω/min in premenopausal women on contraceptives ($n=10$) and 5.7 ± 1.1 Ω/min in postmenopausal women ($n=9$) and did not differ in a significant different way between these women.

Table 1. Aggregation velocity (Ω/min) induced by collagen (fc: $0.38 \mu\text{g/mL}$) and plasma thromboxane B_2 concentrations of collagen activated blood in 32 healthy subjects consuming diets enriched in lauric acid, palmitic acid or oleic acid (STUDY 1).*

	Lauric Acid Diet	Palmitic Acid Diet	Oleic Acid Diet
Collagen-induced aggregation velocity (Ω/min)			
All	5.5 ± 1.4	5.2 ± 1.5	5.6 ± 1.7
Women	5.8 ± 1.2	5.0 ± 1.8	5.6 ± 1.7
Men	5.1 ± 1.7	5.0 ± 1.8	5.5 ± 1.8
Thromboxane B_2 (ng/mL)			
All	17.3 ± 5.4	17.7 ± 5.8	16.5 ± 5.5
Women	17.6 ± 6.2	16.9 ± 6.1	15.7 ± 6.1
Men	16.9 ± 4.5	18.7 ± 5.5	17.5 ± 4.7

* Values are given as means $\pm$ standard deviations**Table 2.** Aggregation velocity (Ω/min) induced by collagen ($0.22 \mu\text{g/mL}$) or by ADP ($1.25 \mu\text{mol/L}$) on diets enriched in MCFA ($n=18$), myristic acid ($n=20$) or oleic acid ($n=19$) (STUDY 2).*

	MCFA Diet		Myristic Acid Diet		Oleic Acid Diet	
	Run-in	Change	Run-in	Change	Run-in	Change
Collagen-induced aggregation velocity (Ω/min)						
All†	5.0 ± 2.2	0.4 ± 2.7	5.4 ± 1.8	0.3 ± 1.8	4.8 ± 1.9	0.1 ± 2.6
Women‡	6.1 ± 1.7	-0.5 ± 2.1	6.0 ± 1.9	0.3 ± 1.7	4.9 ± 2.4	0.1 ± 3.0
Men	2.9 ± 1.4	2.2 ± 2.9	4.5 ± 1.1	0.3 ± 2.0	4.6 ± 0.8	0.2 ± 1.9
ADP-induced aggregation velocity (Ω/min)						
All	5.6 ± 1.8	0.1 ± 2.0	5.2 ± 1.4	-0.2 ± 1.5	5.9 ± 1.3	-0.2 ± 1.5
Women‡	6.0 ± 2.0	-0.2 ± 1.7	6.0 ± 1.4	-0.4 ± 1.6	6.3 ± 1.4	0.0 ± 1.3
Men	5.0 ± 1.1	0.5 ± 2.6	4.1 ± 0.5	0.1 ± 1.3	5.3 ± 0.9	-0.6 ± 1.8

* Values are given as means $\pm$ standard deviations

† Values of one woman and one man are missing in the myristic acid group and one woman and one man in the oleic acid group.

‡ Values of women after the run-in period (all subjects combined) are significantly different from those of men, $P < 0.01$.

Hematologic parameters

Platelet count in STUDY 1 did not change throughout the study and did not differ between women and men (data not shown). Platelet counts correlated significantly with platelet aggregation velocity ($r=0.40$; $P=0.0217$, $r=0.47$; $P=0.0069$, $r=0.48$; $P=0.0049$, on the lauric, palmitic or oleic acid diet, respectively).

In STUDY 2 more hematologic parameters were measured (table 3). Although changes did not differ significantly between the three diets, a trend was observed for a decreased mean platelet volume with the myristic acid (mean response of -0.2 fL; $P=0.0006$) compared with the MCFA diet (difference of changes -0.3 fL; $P=0.0659$, 95% confidence interval -0.7 to 0.1 fL).

After the run-in period in STUDY 2, red blood cell concentrations were higher in men than in women ($4.9\pm0.3\times10^{12}/L$ versus $4.4\pm0.3\times10^{12}/L$; $P<0.0001$). Other red blood cell related parameters -hemoglobin and hematocrit- were also higher in men compared with women, whereas platelet count and mean platelet volume did not differ between the two sexes (see table 3). Whereas collagen-induced aggregation was not correlated with platelet count ($r=0.20$; $P=0.1368$), ADP-induced aggregation velocity was ($r=0.39$; $P=0.0026$). Both collagen- and ADP-induced aggregation velocity were inversely correlated with the concentration of red blood cells ($r=-0.38$; $P=0.0035$ for collagen-induced aggregation, $r=-0.48$; $P=0.0001$ for ADP-induced aggregation).

Table 3. Hematologic parameters on diets enriched in MCFA (n=21), myristic acid (n=20) or oleic acid (n=19) (STUDY 2).*

	MCFA Diet			Myristic Acid Diet			Oleic Acid Diet		
	Run-in	Change		Run-in	Change		Run-in	Change	
White blood cells ($\times 10^9/L$) [†]									
Women	5.9 $\pm$ 1.6	-0.1 $\pm$ 1.8		6.0 $\pm$ 1.3	0.2 $\pm$ 1.4		5.7 $\pm$ 1.1	0 $\pm$ 1.2	
Men	6.2 $\pm$ 1.7	-0.6 $\pm$ 1.7		5.9 $\pm$ 1.3	0.4 $\pm$ 1.5		5.6 $\pm$ 1.2	-0.5 $\pm$ 1.0	
	5.6 $\pm$ 1.7	0.9 $\pm$ 1.8		6.1 $\pm$ 1.3	-0.2 $\pm$ 1.1		5.9 $\pm$ 0.9	0.7 $\pm$ 1.1	
Red blood cells ($\times 10^{12}/L$)									
Women	4.6 $\pm$ 0.5	-0.1 $\pm$ 0.3		4.7 $\pm$ 0.3	-0.1 $\pm$ 0.2		4.5 $\pm$ 0.4	0 $\pm$ 0.3	
Men	4.4 $\pm$ 0.4 [‡]	-0.2 $\pm$ 0.4		4.5 $\pm$ 0.3 [‡]	-0.0 $\pm$ 0.2		4.4 $\pm$ 0.2 [‡]	0 $\pm$ 0.2	
	5.0 $\pm$ 0.2	-0.1 $\pm$ 0.2		5.0 $\pm$ 0.3	-0.1 $\pm$ 0.2		4.8 $\pm$ 0.5	-0.1 $\pm$ 0.4	
Hemoglobin (mmol/L)									
Women	8.3 $\pm$ 0.8	-0.2 $\pm$ 0.3		8.6 $\pm$ 0.8	-0.1 $\pm$ 0.3		8.4 $\pm$ 0.7	0 $\pm$ 0.4	
Men	7.9 $\pm$ 0.5 [‡]	-0.2 $\pm$ 0.4		8.2 $\pm$ 0.4 [‡]	0 $\pm$ 0.4		8.1 $\pm$ 0.3 [‡]	0 $\pm$ 0.4	
	9.1 $\pm$ 0.6	-0.1 $\pm$ 0.3		9.4 $\pm$ 0.5	-0.2 $\pm$ 0.2		9.1 $\pm$ 0.7	0 $\pm$ 0.4	
Hematocrit (L/L)									
Women	0.40 $\pm$ 0.04	-0.01 $\pm$ 0.02		0.42 $\pm$ 0.03	0.01 $\pm$ 0.02		0.41 $\pm$ 0.03	0 $\pm$ 0.02	
Men	0.38 $\pm$ 0.03 [‡]	-0.01 $\pm$ 0.02		0.40 $\pm$ 0.02 [‡]	-0.01 $\pm$ 0.02		0.39 $\pm$ 0.01 [‡]	0 $\pm$ 0.02	
	0.44 $\pm$ 0.02	-0.01 $\pm$ 0.02		0.45 $\pm$ 0.02	-0.01 $\pm$ 0.01		0.43 $\pm$ 0.04	0 $\pm$ 0.03	
Platelets ($\times 10^9/L$)									
Women	270 $\pm$ 67	3 $\pm$ 52		277 $\pm$ 43	3 $\pm$ 35		281 $\pm$ 62	1 $\pm$ 47	
Men	280 $\pm$ 69	-6 $\pm$ 53		273 $\pm$ 45	7 $\pm$ 34		286 $\pm$ 74	1 $\pm$ 46	
	252 $\pm$ 64	19 $\pm$ 49		284 $\pm$ 43	-2 $\pm$ 38		272 $\pm$ 33	2 $\pm$ 52	
Mean platelet volume (fL)									
Women	8.5 $\pm$ 0.6	0.1 $\pm$ 0.8		8.3 $\pm$ 0.5	-0.2 $\pm$ 0.2		8.3 $\pm$ 0.7	0 $\pm$ 0.3	
Men	8.5 $\pm$ 0.7	0.2 $\pm$ 1.0		8.3 $\pm$ 0.6	-0.2 $\pm$ 0.2		8.2 $\pm$ 0.9	0 $\pm$ 0.3	
	8.5 $\pm$ 0.6	0.1 $\pm$ 0.6		8.2 $\pm$ 0.5	-0.2 $\pm$ 0.3		8.3 $\pm$ 0.4	0 $\pm$ 0.3	

* Values are given as means $\pm$ standard deviations.[†] Values of two subjects are missing in the MCFA group, and the value of one subject is missing in the myristic acid group.[‡] Significantly different from men, $P < 0.01$.

Independent hematologic determinants of whole blood platelet aggregation velocity as measured after the run-in period in STUDY 2 were evaluated with a stepwise forward regression analysis and are shown in table 4. The red blood cell count was negatively related to collagen- and ADP-induced aggregations. ADP-induced aggregation was also, but to a lesser extent positively associated with platelet count.

Table 4. Independent hematologic determinants of whole blood aggregation velocity *in vitro* as determined from a forward stepwise regression procedure.

Dependent variable *	Parameter estimate	Partial	
		R ²	P
<i>Collagen-induced aggregation</i>			
Intercept	13.811		
Red blood cell count (×10 ¹² /L)	-1.896	0.15	0.0032
<i>ADP-induced aggregation</i>			
Intercept	10.404		
Red blood cell count (×10 ¹² /L)	-1.631	0.21	0.0003
Platelet count (×10 ⁹ /L)	0.009	0.13	0.0023

* Dependent variable =Platelet count and Red blood cell count and White blood cell count.

DISCUSSION

Compared with monounsaturated fatty acids (oleic acid), diets enriched in saturated fatty acids (MCFA, lauric acid, myristic acid or palmitic acid) did not change aggregation velocity *in vitro* in whole blood samples anticoagulated with hirudin. From measurements in PRP, Mutanen et al (6) reported lower ADP- and collagen-induced aggregations after consumption of diets rich in dairy fat compared with diets rich in rapeseed oil (oleic acid and α -linolenic acid) or sunflower oil (linoleic acid). It was suggested (6) that these effects were due to differences in the linoleic acid and α -linolenic acid ratio of diets and not to the exchange of saturated for monounsaturated fatty acids. In our study, the diets given did not differ with respect to linoleic acid (STUDY 1 and 2) or α -linolenic acid (STUDY 1) content as determined from the recorded food intakes (STUDY 2) (8) or the duplicate portions (STUDY 1) (10). Therefore, the present study does not necessarily contradict this suggestion (6), but can also not confirm because collagen- and ADP-induced aggregation have now been measured in whole blood samples. Earlier, Renaud and co-workers (5) also reported increased ADP-induced aggregation when farmers replaced their saturated

fat diets for one year with diets rich in linoleic acid. For the next three years, when they increased the intake of oleic and α -linolenic acid at the expense of linoleic acid, ADP-induced PRP aggregation was similar to levels on the saturated fat diet. Both replacements, however, reduced collagen- and thrombin-induced aggregation compared with the saturated fat diet. Therefore, it remains uncertain whether this ratio of dietary linoleic acid and α -linolenic acid might also be important for aggregations induced with other stimuli than ADP.

One of the mechanisms to alter platelet aggregation is via the production of TxA_2 , which is synthesized by platelets and promotes platelet aggregation, and of PGI_2 , which is produced by endothelial cells and inhibits platelet aggregation. Both eicosanoids are derived from arachidonic acid, an important membrane phospholipid, the concentrations of which can be altered by changes in dietary fatty acid composition. Our results did not indicate changes in the TxB_2 concentrations (a TxA_2 -metabolite) after diets rich in lauric or palmitic acids compared with oleic acid, in collagen activated samples. Others, however, showed that diets rich in coconut oil (lauric and myristic acids) compared with palm oil (palmitic acid) or olive oil (oleic acid) increased the concentrations of TxB_2 in collagen-activated whole blood samples, whereas the concentration of 6-keto $\text{PGF}_{1\alpha}$ (a PGI_2 -metabolite) decreased compared with palm oil (7). The experimental periods in our and their (7) study were of the same duration. The exchange of lauric acid for palmitic acid in our study, however, was smaller (7 En% instead of 12 En% exchanged), which might have influenced the results. Aggregation was not measured in that study (7).

Our results seem in agreement with those of Mustad et al (11), who did not indicate an altered excretion of eicosanoid metabolites (TxB_2 and 6-keto $\text{PGF}_{1\alpha}$) in urine after 26 days consumption of diets enriched in butter fat compared with cocoa butter or milk chocolate (4 En% lauric plus myristic acid was exchanged for stearic acid). This lack of changes was observed despite an increased in the proportion of arachidonic acid in platelet phospholipid membranes with the butter fat compared with the cocoa butter diet (11). Whether urinary TxB_2 really reflects platelet thromboxane biosynthesis, however, has been disputed (12).

The components of blood that were related to whole blood aggregation most were red blood cell counts and platelet counts, the latter for ADP-induced aggregation only. The observed inverse association between red blood cell count and whole blood aggregation was also present in the study that demonstrated a marked association between ADP-induced whole blood aggregation and prevalent ischemic heart disease (2, 13) and a cross sectional study in elderly subjects (14). However, for collagen-induced whole blood aggregation, this association was not observed (14). This inverse association might explain the sex differences in ADP-induced aggregation velocity, as women have lower red blood cell counts than men. Why the gender differences were less pronounced with the collagen-induced aggregation is not clear, as also for collagen-induced aggregation an inhibitory effect of red blood cells was demonstrated.

We conclude that the combined action of the blood components on *in vitro* whole blood aggregation induced by collagen is not affected by the exchange of 8-10 En%

from oleic acid for MCFA, lauric, myristic or palmitic acid. Also, a 10 En% exchange from oleic for MCFA or myristic acid does not affect ADP-induced aggregation.

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Effects of diets enriched in lauric, palmitic or oleic acids on blood coagulation and fibrinolysis

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ABSTRACT

In this study we compared the effects of specific saturated fatty acids (lauric acid and palmitic acid) with those of a monounsaturated fatty acid (oleic acid) on coagulation and fibrinolytic parameters in healthy women and men. Eighteen women and fourteen men consumed, in random order, three experimental diets, each for six weeks. The diets consisted of solid foods and approximately 70% (28 percent of energy (En%)) of the fat calories was supplied. As determined from the duplicate portions, in the lauric acid diet 7.3 En% and in the palmitic acid diet 6.1 En% of oleic acid was exchanged for lauric or palmitic acid, respectively. The lauric acid diet also contained some (average 1.8 En%) more myristic acid. Factor VII amidolytic (factor VIIam) activity responses to the saturated fat diets were significant only in women but not in men. Compared with the oleic acid diet, the female subjects showed a 9% increase with the lauric acid diet ($P=0.0036$; 95% CI, 3 to 14%) and a 10% increase with the palmitic acid diet ($P=0.0011$; 95% CI, 5 to 16%). Antithrombin activity tended to decrease with the lauric compared with the oleic acid diet (decrease of 7%), only in men. For all subjects combined, fibrinogen concentrations tended to increase to a comparable degree, with the lauric (17 mg/dL) and the palmitic acid diets (18 mg/dL). Fragment 1+2 concentrations did not differ between the diets.

Plasminogen Activator Inhibitor (PAI) activity was higher on the palmitic acid compared with the oleic acid diet (2.3 U/mL; $P=0.0098$; 95% CI, 0.4 to 4.3 U/mL) and the lauric acid diet (2.2 U/mL; $P=0.0123$; 95% CI, 0.2 to 4.1 U/mL).

From this study we conclude that a diet rich in lauric or palmitic acid, compared with a diet rich in oleic acid, unfavorably influences the plasma coagulant capacity, in a gender specific manner.

INTRODUCTION

The coagulant capacity of the blood is important in the evolution of cardiovascular diseases as is suggested from the results of epidemiological studies. Fibrinogen (1, 2) and factor VII coagulant activity (factor VIIc) (1, 2) are independent risk markers for the development of coronary heart disease. In addition, high plasminogen activator inhibitor (PAI) concentrations are positively associated with the reoccurrence of coronary events (3).

Controlled dietary experiments have now indicated that plasma activities of coagulation or fibrinolytic parameters may be affected by the fatty acid composition of the diet (4, 5). Mitropoulos et al (4) found increased factor VIIc activity after 4 wk consumption of a diet rich in saturated fatty acids compared with unsaturated fatty acids. In addition, Almendingen et al (5) reported increased fibrinogen concentrations after a diet enriched with butterfat compared with a partially hydrogenated fish oil diet, while PAI activity was decreased with a butterfat diet compared with a partially hydrogenated soybean oil diet.

Recent studies (6-8) also suggested that the individual saturated fatty acids may differently affect the coagulant capacity of plasma. The effects of lauric acid, however, were not assessed in these studies (6-8), while only young and lean males were enrolled. In the present study we, therefore, investigated, in a 6 week cross-over trial, the effects of diets enriched with lauric acid (C12:0) or palmitic acid (C16:0) with those of a diet enriched in oleic acid (C18:1) on plasma coagulation and fibrinolytic parameters in healthy women and men.

SUBJECTS AND METHODS

Design and statistical analysis

This project was also designed to investigate, with a cross-over study design, the effects of the diets enriched in lauric, palmitic or oleic acids on serum lipids and lipoproteins (9). Each participant consumed each of the three different diets during three periods, each of six weeks duration. Before the study, the subjects were categorized according to sex. Subjects were then randomly divided into six groups in such way that each group contained about the same number of subjects of both sexes. Each group received the diets in a different order. The dietary periods were interrupted by washout periods of 2 (between first and second diet-period) and 3 (between second and third diet-period) weeks, respectively. During these periods, the participants consumed their habitual diets. The protocol of the study had been approved by the local ethics committee and all volunteers had given their written informed consent.

Data were analyzed with diet, period and diet-and-sex interaction as independent variables using the general linear models (GLM) procedure of the Statistical Analysis System (10). When a significant diet or diet-and-sex interaction effect was observed

($P < 0.05$), the three diets were compared pairwise. Analysis were corrected for 3-group comparisons by the Tukey method (11). In addition, diet-and-period interaction was checked and did not reveal any significant carry-over effects of the coagulation and fibrinolytic parameters. Whether age was related with the response to the diets of coagulation and fibrinolytic factors was examined by including a diet and age interaction term into the statistical model. This interaction term never reached statistical significance, indicating that the changes in the coagulation or fibrinolytic parameters to the diets were not related to age.

Pearson correlations were calculated between significant changes in lipoproteins and significant changes in coagulation and fibrinolytic factors for all subjects and according to gender. Only correlations with $P < 0.02$ were considered relevant.

Subjects

Volunteers were recruited via advertisements in local newspaper and posters in public buildings. After they had been thoroughly informed about the purpose and protocol of the study, the subjects underwent the selection procedure. The 43 people selected for the study met all of our eligibility criteria: aged 20-60 year, not taking a prescribed or reducing diet, not anemic, blood pressure below 140/80 mmHg, serum total cholesterol concentrations lower than 6.5 mmol/L and not using medications known to affect lipoproteins and hemostatic parameters. Eleven subjects withdrew because of reasons specifically related to the strict study protocol (six subjects), job or study commitments (two subjects), family circumstances (two subjects) and illness (one subject). Thirty-two subjects, 18 women and 14 men, completed the study. The women were between 22 and 60 years old (mean 40 y), weighed 51-96 kg (mean 69 kg), were between 156 and 183 cm in height (mean 166 cm) and had body mass indexes from 19 to 30 kg/m² (mean 25 kg/m²). The men were between 23 and 59 years old (mean 43 y), weighed 71-101 kg (mean 81 kg), were between 175 and 188 cm (mean 179 cm) in height, and had body mass indexes from 22 to 31 kg/m² (mean 25 kg/m²). Four women and 5 men were smokers. Two women used oral contraceptives and 6 women were postmenopausal.

Diets

The experimental margarines supplied 70% of the total fat energy and were given with solid foods. Total fat intake, in this semi-controlled trial, was on average 41 energy percent (En%) fat. The fatty acid composition of the diets, as determined from the collected duplicate portion samples, was almost identical between diets with the main exception that in the lauric acid diet 7.3 En% acid and in the palmitic acid diet 6.1 En% of oleic acid was exchanged for lauric acid or palmitic acid, respectively. These differences were achieved by the use of special experimental fats. The fat high in lauric acid was a mixture of palm kernel (75%) (Wesco; Karlshamns, Zaandijk, The Netherlands) and a high oleic acid sunflower oil (Trisun; SVO enterprises, Wickliffe, USA) (25%), the fat high in palmitic acid consisted of dairy fat (55%) (FDF, Leeuwarden, The Netherlands), palmstearin (36%) (Aarhus oil, Aarhus, Denmark) and the high oleic acid sunflower oil (9%), and the fat high in oleic acid of dairy fat

(37%) and the high oleic acid sunflower oil (63%). The fatty acid composition of these fats has been described before (9). Cholesterol was added to the fats high in lauric and oleic acids, so that all fats contained 145 mg of cholesterol per 100 g of fat. Margarines were made from the fats and consisted of 83% fat and 17% water. The experimental margarines were given as such and were incorporated in bread, biscuits, pies, cakes, cheeses and custards.

Before the study started, the subjects recorded habitual food intake on two working days and one weekend day. From this record their energy intake was calculated. The study diets were formulated at different levels of energy, so that each subject received a diet that met his or her energy needs. According to these energy levels, lists were computed which stated the amount of experimental products the subject had to eat each day or each week. Products were coded with colored labels to blind the subjects as to the nature of the diets and were handed out on an individual basis. 30% of total fat intake was free and subjects could choose them from listed "free choice" fat containing products. Subjects came at least once a week to a special room at the university to get a new supply of products and to be weighed. They were also asked to maintain the same activity level, smoking and drinking habits throughout the study. Any changes as well as medication used, illnesses, daily alcohol use and menstrual cycle were written down in a special diary.

The subjects collected a duplicate portion of the food and drinks consumed for two days at the end of each dietary period. Duplicate portions were pooled according to treatment group. Lipids from the duplicate portions were extracted with the Soxhlet method. The fatty acids were transmethyalted to their corresponding methylesters, which were quantified on a gas chromatograph, with a polar capillary column (CP Sil 88, Chrompack, Middelburg, The Netherlands) as described before (12).

Blood sampling and measurements

Blood was sampled in week 5 and at the end (week 6) of each period after an overnight fast and after subjects abstained from drinking alcohol the preceding day and smoking on the morning before blood sampling. The samples of week 5 and 6 of each period were pooled prior to analyses. Blood was sampled with a minimum of stasis and by using a 1.2-mm needle (Strauß Kanule, Luer, Wächtersbach, Germany) with the subject in a recumbent position. All venipunctures were performed by the same person, at the same location, and for each subject generally at the same time of the same day of the week.

The first 5 mL blood was used for measurement of hematologic parameters and platelet aggregation, to be reported elsewhere. Then, two portions of 5 mL blood were collected in tubes containing a mixture of citrate, theophylline, adenosine and dipyridamole (CTAD). One tube was immediately placed on ice and centrifuged at 2000 g (10 minutes, 4 °C) and used for measurement of PAI activity. The second CTAD tube was centrifuged at 2000 g (10 minutes, 4 °C) followed by centrifugation at 11,500 g (30 minutes, 4 °C) and used for measurement of coagulation factors. Finally, a 10 mL clotting tube was filled to determine lipids and lipoproteins. All samples from

one subject were analyzed within one run. Standard pool plasmas were obtained from normolipidemic subjects, and prepared by the same methods as described above. Samples were stored at -80 °C before analyses.

Factor VII, Factor X, antithrombin, plasminogen, α_2 -antiplasmin activities were assessed with chromogenic assays (Coaset FVII, Coatest FX, Coamatic antithrombin, Coamatic Plasminogen, Coatest Antiplasmin, Chromogenix, Mölndal, Sweden). Plasma fibrinogen concentrations were measured by the method of Clauss (13). The factor VII amidolytic activity (factor VIIam) assay is based on the two stage principle, using human placenta thromboplastin. In stage one, factor VII in the test sample complexes with thromboplastin and Ca^{++} . This complex activates factor X, which is present in excess in the reagent mixture. Factor VII is completely converted into activated factor VII during this process and accordingly there is no interference in the assay of preactivated factor VII. In stage two the generated activated factor X hydrolyses the chromogenic substrate. The coefficients of variation within runs were 5.8% for factor VIIam, 4.8% for factor X, 5.2% for antithrombin, 5.9% for plasminogen, 5.3% for α_2 -antiplasmin and 8.8% for fibrinogen. Activities were expressed as percentage of a reference plasma. PAI activity was measured with a chromogenic assay (Spectrolyse®(pL) PAI, Biopool, Umea, Sweden). Coefficient of variation within runs was 4.0%. Prothrombin activation fragment 1+2 was measured with an enzyme-linked immunosorbent assay (ELISA) (Thrombonostika F1.2, Organon Teknika, Durnham, USA). 10 μL of the 10 nmol fragment 1+2 calibrator was added to the sample wells to obtain concentrations in the linear area of the calibration curve. Coefficient of variation within runs was 10.1%.

Fatty acid composition of serum total lipids at the end each dietary period was determined in samples pooled according to treatment group. In this way six pooled samples per diet were obtained. Serum lipids were extracted with a modified Folch extraction (14). The fatty acids were transmethylated to the corresponding methyl esters (15), which were quantified on a gas chromatograph, with a polar capillary column (CP Sil 88, Chrompack, Middelburg, The Netherlands) as previously described (12).

RESULTS

Subjects and dietary adherence

Body weights (means $\pm$ standard deviations) of the subjects did not differ between the three diets and were 74 $\pm$ 12 kg with the lauric, 75 $\pm$ 12 kg with the palmitic and 74 $\pm$ 11 kg with the oleic acid diet.

The mean daily energy intake and the fatty acid composition of the diets was determined with a four days food records (9) and from the lipids extracted from two days duplicate portions (table 1). Total fat intake did not differ between the three diets and was 41 En% on average. As determined from the duplicate portion samples, the

difference in lauric acid was 7.3 En% between the lauric and the oleic acid diet, while the palmitic acid diet was 6.1 En% higher in palmitic acid than the oleic acid diet. The lauric acid diet contained also some (average 1.8 En%) more myristic acid than the other two diets.

The mean amount of fat consumed as "free-choice" fat items as listed daily in the subjects' diaries, agreed with the calculated amount and was 1 ± 3 gram (mean $\pm$ standard deviation) less than prescribed.

Table 1. Fatty acid composition of the experimental diets rich in lauric, palmitic or oleic acids, as determined from GC fatty acid analysis of lipids extracted from two days duplicate portions.*

	Lauric Acid Diet	Palmitic Acid Diet	Oleic Acid Diet
	Percent of energy		
Fat †	41.5 $\pm$ 5.5	41.0 $\pm$ 5.4	41.8 $\pm$ 5.5
SAFA	25.2 $\pm$ 1.3	23.6 $\pm$ 0.9	16.7 $\pm$ 2.6
$\leq$ C10:0	0.8 $\pm$ 0.1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1
C12:0	10.8 $\pm$ 1.2	3.3 $\pm$ 0.9	3.5 $\pm$ 1.7
C14:0	4.3 $\pm$ 0.3	2.7 $\pm$ 0.2	2.3 $\pm$ 0.5
C16:0	6.5 $\pm$ 1.2	12.6 $\pm$ 1.3	6.5 $\pm$ 0.9
C18:0	2.3 $\pm$ 0.3	3.5 $\pm$ 0.2	3.1 $\pm$ 0.3
Others	0.4 $\pm$ 0.2	0.8 $\pm$ 0.4	0.6 $\pm$ 0.1
MUFA	12.0 $\pm$ 0.9	13.1 $\pm$ 0.9	19.3 $\pm$ 2.9
C18:1(n-9)	11.0 $\pm$ 1.0	11.6 $\pm$ 1.0	18.4 $\pm$ 2.9
C18:1(n-9tr)	0.3 $\pm$ 0.1	0.8 $\pm$ 0.2	0.4 $\pm$ 0.1
Others	0.8 $\pm$ 0.2	0.7 $\pm$ 0.4	0.5 $\pm$ 0.2
PUFA	4.3 $\pm$ 0.7	4.7 $\pm$ 1.6	5.4 $\pm$ 0.5
C18:2(n-6)	3.2 $\pm$ 0.3	3.2 $\pm$ 1.6	4.2 $\pm$ 0.4
C18:3(n-3)	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1
Others	0.7 $\pm$ 0.6	1.1 $\pm$ 0.5	0.9 $\pm$ 0.4

* Values are means $\pm$ standard deviations. Duplicate portions were pooled according to treatment group and analyzed as such. Values are the mean of six pooled samples.

† As determined from the four days food records.

SAFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

The fatty acid composition of serum total lipids is shown in table 2. The percentages of lauric (C12:0) and myristic (C14:0) acids significantly increased with the lauric acid diet compared with both the palmitic acid (C16:0) and the oleic acid (C18:1(n-9)) diets. Palmitic acid increased with the palmitic acid diet compared with the oleic and the lauric acid diets. Serum oleic acid levels were lower, but palmitoleic acid (C16:1(n-7)) levels were higher with the saturated fatty acid diets compared with the oleic acid diet. The three diets did not affect the serum content of polyunsaturated fatty acids in a significant different way.

Table 2. Fatty acid composition of serum total lipids on diets enriched in lauric, palmitic or oleic acids.*

	Lauric Acid Diet	Palmitic Acid Diet	Oleic Acid Diet
% of total fatty acids			
SAFA	32.7±0.7	33.0±1.7	31.5±0.9
12:0	0.5±0.2 †‡	0.2±0.0	0.2±0.0
14:0	2.9±0.4 †‡	2.3±0.2	2.4±0.4
16:0	21.1±0.6 ‡	22.4±1.2 †	20.4±0.6
18:0	6.3±0.4	6.4±0.3	6.5±0.4
MUFA	23.0±1.3 †	23.4±1.5 †	26.5±2.3
16:1 (n-7)	1.9±0.1 †	2.1±0.3 †	1.6±0.1
18:1 (n-9)	18.1±1.2 †	18.1±1.0 †	21.0±2.3
PUFA (n-6)	41.6±1.4	40.9±2.0	39.2±1.8
18:2 (n-6)	32.9±1.4	32.6±2.4	30.6±1.8
20:4 (n-6)	6.7±0.7	6.3±0.7	6.7±0.6
PUFA (n-3)	2.7±0.2	2.7±0.2	2.7±0.9
18:3 (n-3)	0.6±0.2	0.5±0.1	0.4±0.0
20:5 (n-3)	0.6±0.1	0.6±0.1	0.7±0.7
22:6 (n-3)	1.6±0.2	1.5±0.2	1.6±0.3

* Fatty acids are determined in six pooled serum samples. Values are given as means±standard deviation.

† Significantly different from the oleic acid diet, $P<0.02$.

‡ Significantly different from the palmitic acid diet, $P<0.02$.

SAFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Blood coagulation and fibrinolytic parameters

Table 3 and 4 show the levels of the coagulation and fibrinolytic parameters at the end of each dietary period. Factor VIIam activity responses to the diets differed between women and men, as indicated by the highly significant diet-and-sex

interaction term ($P=0.0071$). Women showed a 9% increase with the lauric acid diet ($P=0.0036$; 95% CI, 3 to 14%) and a 10% increase with the palmitic acid diet ($P=0.0011$; 95% CI, 5 to 16%), both compared with the oleic acid diet. In men, no significant differences in factor VIIam activities were found. On the oleic acid diet, factor VIIam activities (mean and range) in premenopausal women not using contraceptives ($n=10$), pre-menopausal women on contraceptives ($n=2$) and postmenopausal women ($n=6$) were 101% (53%-135%), 117% (83%-151%) and 107% (91%-133%) respectively. In figure 1 individual changes in factor VIIam after the diets are shown, when subjects are sorted according to factor VIIam activity on the oleic acid diet. The responses to the diets did not differ with higher factor VIIam activity on the oleic acid diet. In addition, responses to the saturated fat diets of contraceptive users or postmenopausal women were not clearly higher compared with the responses of premenopausal women not using contraceptives.

Table 3. Coagulation parameters on diets enriched in lauric, palmitic or oleic acids.*

	Lauric Acid Diet	Palmitic Acid Diet	Oleic Acid Diet
Factor VIIam activity (% of standard)			
All	109±24 †	108±25 †	103±21
Women	114±27 †	115±26 †	105±23
Men	102±19	99±21	100±19
Factor X activity (% of standard)			
All	115±19	115±22	116±20
Women	110±16	108±20	112±17
Men	121±20	125±20	121±22
Antithrombin activity (% of standard)			
All	111±12	113±13	114±14
Women	110±11	110±12	111±11
Men	112±13	117±14	118±17
Fibrinogen (mg/dL)			
All	291±76	291±71	274±66
Women	288±80	288±69	270±62
Men	294±72	295±76	279±72
Fragment 1+2 (nmol/L)			
All	1.28±0.68	1.33±0.70	1.30±0.64
Women	1.28±0.59	1.29±0.64	1.30±0.70
Men	1.28±0.79	1.38±0.80	1.30±0.57

* Values are means±standard deviations. Eighteen women and fourteen men consumed all three diets, for six weeks each, in random order.

† Significantly different from the oleic acid diet, $P<0.02$.

Antithrombin activity, although not significantly, decreased by 4% ($P=0.0235$; 95% CI, -7 to -0.3%) with the lauric compared with the oleic acid diet. This effect was more apparent in men (decrease of 7%; $P=0.0350$; 95% CI, -13 to -0.5%) than in women (decrease of 1%; $P=0.2063$; 95% CI, -4 to 2 %), but the diet-and-sex interaction term was not significant ($P=0.2407$).

Fibrinogen concentrations increased not significantly by 17 mg/dL ($P=0.0302$; 95% CI, 1 to 33 mg/dL) with the lauric acid diet and by 18 mg/dL ($P=0.0219$; 95% CI, 1 to 34 mg/dL) with the palmitic acid diet. The responses to the saturated fat diets did just not reach statistical significance when the Tukey correction was applied. Responses to the diets were similar for women and men.

Factor X activities and fragment 1+2 concentrations did not change differently with the three diets.

For the fibrinolytic parameters, plasminogen and α_2 -antiplasmin activity did not differ between the three diets either. PAI activity, however, increased with the palmitic acid diet compared with both the lauric acid (difference of 2.2 U/mL; $P=0.0123$; 95% CI, 0.2 to 4.1 U/mL) and the oleic acid diet (difference of 2.3 U/mL; $P=0.0098$; 95% CI, 0.4 to 4.3 U/mL). The effects of PAI activity did not differ between women and men and were not related to the values measured after the oleic acid period (figure 2).

Table 4. Fibrinolytic parameters on diets enriched in lauric, palmitic or oleic acids.*

	Lauric Acid Diet	Palmitic Acid Diet	Oleic Acid Diet
Plasminogen activity (% of standard)			
All	124±17	125±16	122±14
Women	124±20	127±17	123±16
Men	123±14	122±14	120±13
α_2 -antiplasmin activity (% of standard)			
All	110±20	115±19	112±16
Women	107±20	110±20	110±19
Men	113±19	121±18	115± 8
PAI activity (U/mL)			
All	13.6±6.9 ‡	15.7±6.2 †	13.4±5.1
Women	13.9±6.2	14.8±5.7	12.6±5.1
Men	13.2±7.9	17.0±6.8	14.4±5.0

* Values are means±standard deviations. Eighteen women and fourteen men consumed all three diets, for six weeks each, in random order.

† Significantly different from the oleic acid diet, $P<0.02$.

‡ Significantly different from the palmitic acid diet, $P<0.02$.

Figure 1. Individual changes in factor VIIam activity on diets high in lauric acid (C12:0) or palmitic acid (C16:0), relative to a diet high in oleic acid (C18:1). Subjects are sorted, from the left to right, according to factor VIIam activity on the oleic acid diet.

Figure 2. Individual changes in plasminogen activator inhibitor (PAI) activity on diets high in lauric acid (C12:0) or palmitic acid (C16:0), relative to a diet high in oleic acid (C18:1). Subjects are sorted, from the left to right, according to PAI activity on the oleic acid diet.

The effects of the three diets on serum lipids and lipoprotein concentrations have been reported earlier (9). When all subjects together were taken into consideration, no strong associations were found between changes in coagulation or fibrinolytic parameters with changes in lipoproteins on the diets. In addition, no consistent correlations were demonstrated when separate correlation analyses were conducted according to gender. Intercorrelations of changes in coagulations and fibrinolytic factors demonstrated no significant associations when data of all subjects were combined, and no consistent correlations when data were analyzed according to gender.

DISCUSSION

In this study we investigated the effects on plasma activities of coagulation and fibrinolytic factors of diets enriched in lauric and palmitic acids compared with a diet enriched in oleic acid.

Marked differences were noted in factor VIIam responses between our female and male subjects. Increased factor VIIam activity after the diets rich in saturated fatty acids was only apparent in women, which suggested an interaction between diet and hormonal or genetic backgrounds. Endogenous estrogen levels have been linked to factor VII activity before as, in agreement with our results, factor VIIam activity was higher in postmenopausal women (16, 17) and in contraceptive users (18) than in premenopausal women not using contraceptives. Differences in estrogen concentrations in these three groups of women, however, were not likely to explain different factor VIIam responses to the saturated fat diets as changes in factor VIIam were not more pronounced in one of the groups (figure 1). However, this might also be related to the relative small numbers of women in the different groups. A recent study (19) showed that contraceptive use affects factor VIIc responses to changes in dietary fatty acid composition. Factor VIIc activity increased after a diet rich in *trans* fatty acid compared with a stearic acid diet in females using contraceptives, while no change was found among the non-users (19).

Another major determinant of factor VII activity is a polymorphism of the gene coding for factor VII (20, 21). Since similar frequencies of the different factor VII genotypes are found in women and men (20, 21), we speculate on the basis of our findings that a similar genotype does not preclude factor VII responses in a gender-specific way. An example of gender-specific influences on factor VII activity between women and men with similar genotypes was recently reported for the association between non-fasting plasma triacylglycerols and factor VIIc activity (22), but not for factor VII responses after changes in dietary fat or fatty acids. Clearly, more research is needed to define the possible gender-dependent responses of dietary fatty acids on coagulation factors and their underlying mechanisms.

Tholstrup et al (6, 7) demonstrated, in young and lean men, that diets rich in lauric plus myristic acids or palmitic acid increase factor VIIc activity compared with a stearic

acid diet (6). In a second experiment, myristic acid alone increased factor VIIc slightly (2%) stronger than palmitic acid (7). Since different reference diets were consumed in both studies (6, 7), compared with the oleic acid diet in the present study, a valid comparison of the results of both studies is difficult. Relative to oleic acid, the absence of effects of lauric and palmitic acid on factors VIIam activity in our male subjects seems to contrast their results (6, 7). However, in line with our results, except for stearic acid, no (6) or only small (7) differences in factor VIIc were observed between saturated fatty acids, in men.

In both women and men, the combined effects of the saturated fat diets on coagulation factor activities and the slightly increased fibrinogen concentrations, indicate that these diets increase the coagulant capacity of plasma relative to oleic acid. The unchanged fragment 1+2 concentrations, however, suggest that the saturated fat diets did not alter the *in vivo* activation of the coagulation system. Increased fragment 1+2 concentrations have been reported in male subjects after diets rich in lauric plus myristic acid compared with stearic acid. (8). Whether diets with higher levels of dietary myristic acid induces a more potent fragment 1+2 response is not known.

Compared with both the oleic and the lauric acid diet, palmitic acid increased PAI activity. Previous studies did not support this potentially unfavorable effect of palmitic acid on PAI. Tholstrup et al (6, 7) reported unchanged PAI antigen content and euglobulin fibrinolytic capacity after diets rich in saturated fatty acids with 12 to 18 carbon atoms. A diet rich in butterfat decreased PAI activity compared with a diet with partially hydrogenated soybean oil (5). The butterfat diet was rich in a mixture of saturated fatty acids, mainly palmitic acid, but the lowering effects could also be attributed to the lower 18:1 *trans* fatty acid content of this diet.

To summarize, both saturated fatty acids increased factor VIIam activity, only in women. The lauric acid diet tended to decrease antithrombin activity, only in men. Fibrinogen concentrations were slightly, though not significantly, elevated in both women and men. The PAI activity of plasma was negatively affected with the palmitic acid diet. From this study we, therefore, conclude that the lauric and the palmitic acid diets, compared with the oleic acid diet, had a unfavorable effect on the coagulant capacity of the blood, in a gender specific manner.

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7

Effects of diets rich in medium chain fatty acids (MCFA), myristic acid or oleic acid on coagulation and fibrinolysis

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ABSTRACT

In this study we investigated the effects on coagulation and fibrinolytic parameters of diets rich in medium chain fatty acids (MCFA) or myristic acid relative to those of oleic acid. Thirty-seven women and 23 men first consumed a 3 wk run-in diet enriched in oleic acid. For the next 6 weeks, 19 subjects continued with the oleic acid diet, 21 subjects with an MCFA enriched diet, while 20 subjects received a diet high in myristic acid. Experimental fats were incorporated into solid foods. The MCFA and the myristic acid fat were synthetic fats. Total diets contained 40 En% fats and the nutrient composition was the same in each diet except for 10 En%, which was provided by MCFA, myristic or oleic acids. In the females, but not in the males, overall plasma coagulability as reflected by the tissue factor (TF)-induced thrombin potential increased with the myristic acid diet compared with the oleic acid diet (difference in changes: 7%, $P=0.0022$). For all subjects combined, analysis of separate factors indicated increased factor VII amidolytic (factor VIIam) activity (difference in changes: 7%, $P=0.0106$) and decreased tissue factor pathway inhibitor (TFPI) activity (difference in changes: -3%, $P=0.0162$) on the myristic acid diet, whereas the MCFA diet did not significantly change factor VIIam activity and slightly decreased TFPI activity (difference in changes: -3%, $P=0.0384$) compared with the oleic acid diet. Changes in factor VIIam were more pronounced in women than in men. The diets did not affect fibrinogen concentrations, antithrombin activity and the fibrinolytic capacity of plasma as determined by plasminogen activator inhibitor type 1 (PAI) activity. In conclusion the present investigation showed that the plasma coagulant capacity is unfavorably affected by a myristic acid diet compared with an oleic acid, especially in women.

INTRODUCTION

Previous studies have shown that mixtures of saturated fatty acids not only unfavorably affect serum lipoprotein concentrations (1), but also have a negative impact on certain coagulation or fibrinolytic factors (2, 3), which may differ for the various saturated fatty acids (4-7). Diets enriched in lauric (C12:0) (4), myristic (C14:0) (6) or palmitic acids (C16:0) (4-6) have been reported to increase factor VII compared with diets rich in oleic acid (C18:1(n-9)) (4), habitual diets (6), or stearic acid (C18:0) (5). Myristic acid, on its turn, increased factor VII coagulant (factor VIIc) activity slightly stronger than palmitic acid (6) did, and might therefore be a more potent pro-thrombotic saturated fatty acid. However, the effects of myristic acid diets have only been studied once in young male subjects (6).

Fats rich in myristic acid (e.g. coconut fat, palm kernel fat, or dairy fat) also contain medium chain fatty acids (MCFA (C $\leq$ 10:0)). Diets rich in MCFA induced lower LDL and HDL cholesterol levels compared with a diet rich in myristic acid (8) and also slightly reduced low density lipoprotein (LDL) cholesterol compared with palmitic acid (9). Whether effects of MCFA on coagulation and fibrinolytic parameters are also less strong than those of myristic acid is not known. In this study we, therefore, examined the effects of diets enriched in MCFA or myristic acid compared with oleic acid on blood coagulation and fibrinolysis.

METHODS

Design and statistical analysis

In this parallel designed study, the effects on serum lipids and lipoproteins were also investigated (8). The protocol had been approved by the local medical ethics committee and all volunteers had given their written informed consent. After a run-in period of three weeks in which the volunteers consumed an oleic acid diet, 19 subjects continued with the oleic acid, 21 subjects consumed a diet enriched in MCFA and 20 subjects received a diet rich in myristic acid, each for six weeks.

Differences between the experimental diets were analyzed using the General Linear Models (GLM) procedure of the Statistical Analyses System (10). The values at the end of the experimental periods were used as dependent variables, diet as independent variable and values after the run-in period as covariate. When the analysis indicated a significant effect of diet ($P < 0.05$), the diets were compared pairwise. Because of the three-group comparison, statistical significance was set at a P value $< 0.05/3$ (or, 0.0167).

To determine whether the responses of the hemostatic variables were more pronounced in persons with higher run-in values, an interaction term between diet and run-in value was included in the model. Pearson correlation coefficients were calculated between those changes in coagulation or fibrinolytic factors and changes in lipoproteins that were significant. Correlation analyses were also carried out according to gender.

Subjects

Volunteers were recruited via advertisements in local newspaper and posters in public buildings. After they had been thoroughly informed about the purpose and protocol of the study, the subjects underwent the selection procedure. Subjects selected for the study were aged between 20 and 60 years, did not take a prescribed or reducing diet, had a blood pressure below 140/80 mmHg, serum total cholesterol concentrations below 6.7 mmol/L and did not use medications known to affect lipoproteins, hemostatic variables or platelet aggregation. The study included 60 subjects, 37 women and 23 men. Women were 22-60 y of age (mean 40 y), weighed 48-101 kg (mean 67 kg), measured between 155 and 183 cm in height (mean 167 cm), while body mass indexes ranged between 20-30 kg/m² (mean 24 kg/m²). The men were aged between 23-59 y (mean 43 y), weighed 56-88 kg (mean 78 kg), measured between 163 and 190 cm in height (mean 178 cm), and had body mass indexes from 17 to 29 kg/m² (mean 25 kg/m²). Nine women were postmenopausal and 10 women used oral contraceptives. Six women and 5 men smoked.

During the study period 7 subjects withdrew. One subject because of job commitments, two subjects because of illness and four subjects because of reasons specifically related to the strict study protocol. Results of an additional 3 subjects were excluded from analyses: 2 subjects started prescribed medication known to affect blood lipids during the experiment and one subject experienced very stressful personal circumstances, which may have influenced her response to the diets.

Diets

Before the study started, the subjects recorded their habitual food intake for two working days and one weekend day. From these food records, each subjects' actual energy intake was calculated by using the Dutch food-composition table (11). The study diets were formulated at 13 levels of energy ranging from 5 MJ to 22 MJ, so that each subject received a diet that met his or her energy needs. Diets used in the study consisted of products in which the normal fat was replaced by the experimental fats. The products included margarines and bakery products (bread, cookies, pies and cakes). According to the subjects' energy level, lists were computed which stated the amount of experimental products the subject had to eat each day (margarine, bread, and cookies) and each week (cake and pie). These solid foods supplied 63% (25 En%) of the total fat energy. The remaining 37% (15 En%) of the total fat intake had to be chosen from a list of "free-choice" fat-containing products. These products were given points according to their fat contents. One point was equal to one gram of fat. Each subject was required to eat a certain number of points daily, again corresponding with his or her energy intake, and to list the products chosen daily on a special form. The fat composition of the calculated diets was similar except for approximately 10% of total energy intake, which was provided by MCFA, myristic acid or oleic acid, respectively. The composition of the experimental fats has been described elsewhere (8).

Subjects came at least once a week to the university to receive a new supply of products, to be weighed, and to receive new forms to list the free-choice items. They

were also asked to maintain the same activity level, and smoking and drinking habits throughout the study. They recorded in diaries any signs of illness, medication used, alcohol consumption, menstrual cycle and any deviations from the study protocol.

Blood sampling and measurements

Blood for coagulation and fibrinolytic parameters was sampled at the end of the run-in (week 2 and week 3) and at the end of the experimental period (week 8 and week 9). The two samples at the end of each period were pooled and analyzed as such. Samples for fibrinogen were analyzed separately, but the results are given as the mean of the two samples. Blood was sampled after an overnight fast and after abstinence from alcohol the preceding day and smoking on the morning before blood sampling. Sampling was performed with a minimum of stasis and using a 1.2 mm needle (Strauß Kanüle, Luer, Wächtersbach, Germany) with the subject in recumbent position. All venipunctures were performed by the same person, at the same location, and for each subject generally at the same time of the same day of the week.

The first 5 mL blood was collected for measurement of hematologic parameters and platelet aggregation, to be reported elsewhere. Then, two 5 mL blood were collected in tubes containing citrate theophylline, adenosine and dipyridamole (CTAD). One tube was immediately placed on ice and centrifuged at 2000 g (4 °C, 10 minutes), and used for measurement of plasminogen activator inhibitor type 1 (PAI) activity. The second CTAD tube was centrifuged at 2000 g (4 °C, 10 minutes) followed by centrifugation at 11,500 g (4 °C, 30 minutes) and used for measurement of coagulation factors. Finally, a 10 mL tube was filled for serum preparation for lipid and lipoprotein analysis. samples were stored at -80°C before analyses. A standard pool plasma was obtained from healthy donors, and prepared by the same methods as described. Activities of tissue factor (TF)- and actin-induced thrombin potential (see below), factor VII, tissue factor pathway inhibitor (TFPI) and antithrombin activities were expressed as percentage of these standard plasmas. All samples from one subject were analyzed within one run.

A novel global coagulation assay (the endogenous thrombin potential) was used to assess the overall coagulability of plasma. Plasma thrombin potential was measured with a centrifugal analyzer as described (12). Briefly, plasma samples were first defibrinated by mixing plasma with a reptilase solution (Boehringer Mannheim, Mannheim, Germany) and the fibrin formed removed with a small spatula. Coagulation was triggered by either recombinant tissue factor (Dade®Innovin, Baxter Diagnostics Inc., Deerfield, IL, USA) for the extrinsic process (TF-induced thrombin potential) or by actin FS®, a suspension of ellagic acid and soy bean phosphatides (Baxter Deutschland GmbH, Unterschleissheim, Germany) for the intrinsic process (actin-induced thrombin potential). After 30 seconds of incubation, thrombin generation was started by adding a pre-warmed solution containing CaCl_2 and the chromogenic thrombin substrate S68 (Kordia, Leiden, The Netherlands). The final reaction mixture consisted of four parts of defibrinated plasma, one part of TF- or actin-solution and one part of CaCl_2 /S68 solution. After the start of the reaction, the

optical density was recorded at 405 nm at intervals of 30 seconds for 15 minutes. The thrombin potential values were calculated by a computerized system from the thrombin generation curves. The coefficient of variation within runs was 2.5% for TF-induced thrombin potential and 3.6% for actin-induced thrombin potential.

Factor VII amidolytic (factor VIIam) and antithrombin activities, were assessed with chromogenic assays (Coaset FVII, Coamatic antithrombin, Chromogenix, Mölndal, Sweden). The coefficients of variation within runs were 3.6% for factor VIIam and 2.3% for antithrombin. Functional TFPI activity was determined according to the method of Sandset et al (13). This assay measures total (free plus lipoprotein bound) TFPI activity. The coefficient of variation within runs was 2.4%. Plasma fibrinogen concentration (mg/dL) was assessed by the method of Clauss (14), and the coefficients of variation within runs was 2.8%. Plasminogen Activator Inhibitor-1 (PAI) activity was measured with a chromogenic assay (Spectrolyse®(pL)PAI, Biopool, Umea, Sweden). Coefficient of variation within runs was 3.6%.

RESULTS

Dietary composition

The composition of the diets has been described previously (8). The average intake of fat was 38.9 En% during the run-in period and 40.0 En% during the test period. The average intake of oleic acid was 15.1 En% during the run-in period and decreased in the test period to 6.6 En% with the MCFA and to 6.3 En% with the myristic acid diet. It was exchanged for MCFA or myristic acid. Oleic acid intake during the oleic acid diet did not change from the run-in to the test period. Changes in other nutrients, except for En% saturated and monounsaturated fatty acids, did not significantly differ between the diets.

Thrombin potential

Table 1 shows that, in the female subjects, the plasma thrombin potential induced by tissue factor (TF-induced thrombin potential) increased with the myristic acid diet compared with the oleic acid (difference in changes: 7%, $P=0.0022$), but not significantly with the MCFA diet (difference in changes: 5%, $P=0.0471$). The thrombin potential response induced by actin did not differ between the three diets. Changes in TF-induced thrombin potential correlated strongly with changes in actin-induced thrombin potential ($r=0.60$; $P<0.0001$) and with changes in factor VIIam activity ($r=0.28$; $P=0.0294$). The latter correlation was significant in women ($r=0.55$; $P=0.0004$), but not in men ($r=-0.19$; $P=0.4090$).

Table 1. Plasma thrombin potential after diets enriched in MCFA (n=21), myristic acid (n=20) or oleic acid (n=18).*

	MCFA Diet		Myristic Acid Diet		Oleic Acid Diet	
	Run-in	Change	Run-in	Change	Run-in	Change
TF-induced thrombin potential (% standard)						
All	93±13	3±7	104±13	3±6	100±13	-1±7
Women	92±10	3±5	101±12	5±5 †	98±15	-2±7
Men	94±17	2±10	109±13	0±6	105±9	1±6
Actin-induced thrombin potential (% standard)						
All	96±11	1±5	109±14	-1±11	106±14	-1±9
Women	94±10	2±4	108±12	1±10	104±14	-4±8
Men	98±14	1±7	110±17	-5±11	110±15	2±10

* Values are given as means±standard deviations. Values of one subject on the oleic acid diet were omitted from the analysis because of a clotted blood sample.

† P<0.01, Significantly different from the oleic acid diet.

TF: tissue factor.

Activities of coagulation and fibrinolytic factors in plasma

Plasma activities of coagulation and fibrinolytic factors are given in table 2. Factor VIIam activity increased by 6% when the subjects switched to the myristic acid diet. This change, which was more pronounced in women than in men, was significantly different from the change in the volunteers who continued on the oleic acid diet (difference in changes: 7%, P=0.0106). For women this effect (10%; P=0.0069) was more pronounced than in men (3%; P=0.2456). Factor VIIam activity responses within the MCFA diet group did not differ significantly from those within the myristic acid diet or the oleic acid diet groups.

Compared with the oleic acid diet, TFPI activity decreased with both the myristic acid diet (difference in changes: -3%, P=0.0162) and the MCFA diet (difference in changes: -3%, P=0.0384). Changes in TFPI activities did not correlate with changes in lipoproteins. Responses of antithrombin activity, fibrinogen, and PAI activity did not significantly differ between the three diets.

For women, significant interaction terms were observed between run-in values of TF-induced thrombin potential-and-diet (P=0.05 for interaction) and between run-in values of factor VIIam activity-and-diet (P=0.05 for interaction). This indicates that women with higher TF-induced thrombin potential values or factor VIIam activities after the oleic acid run-in diet responded stronger to the different experimental diets, than women with lower values. Premenopausal subjects using contraceptives demonstrated a significantly higher TF-induced thrombin potential than the premenopausal women not using contraceptives (P<0.0001) and slightly, though not

significantly, higher values than the postmenopausal women ($P=0.0307$) (table 3). Factor VIIam activity showed a trend to be higher in the premenopausal women using contraceptives compared with the premenopausal women not using contraceptives ($P=0.0267$).

Table 2. Coagulation and fibrinolytic factors after diets enriched in MCFA ($n=21$), myristic acid ($n=20$) or oleic acid ($n=19$).^{*}

	MCFA Diet		Myristic Acid Diet		Oleic Acid Diet	
	Run-in	Change	Run-in	Change	Run-in	Change
Factor VIIam activity (% of standard)						
All	75±19	2±6	80±27	6±9 †	83±26	-1±10
Women	81±20	1±6	87±32	7±10 †	84±32	-3±9
Men	65±11	3±5	71±15	4±6	82±10	1±12
Tissue Factor Pathway Inhibitor activity (% of standard)						
All	95±5	-1±6	93±5	-1±6 †	96±8	2±12
Women	94±5	-2±5	94±5	-2±6	96±8	1±11
Men	96±6	-1±6	92±5	1±8	96±10	3±13
Antithrombin activity (% of standard) [§]						
All	96±5	1±5	98±8	0±6	99±8	2±4
Women	95±5	-0±4	99±8	-2±7	97±9	3±5
Men	98±6	3±6	98±8	4±5	102±5	1±2
Fibrinogen (mg/dL) [§]						
All	365±26	-10±20	358±31	-6±33	362±27	2±27
Women	363±24	-5±19	355±34	-2±30	364±30	0±27
Men	369±31	-18±21	363±29	-11±37	358±22	6±29
PAI activity (U/mL)						
All	7.4±3.3	1.9±3.9	8.7±4.6	-0.5±3.1	9.1±5.0	0.4±3.8
Women	7.3±3.4	1.2±3.7	7.2±3.1	-0.4±3.4	7.6±5.4	0.8±4.2
Men	7.4±3.2	3.0±4.1	10.9±5.8	-0.6±2.6	11.7±2.8	-0.2±3.2

* Values are given as means±standard deviations.

§ Values of one subject on the oleic acid diet were omitted from the analysis because of a clotted blood sample.

† $P<0.02$, Significantly different from the oleic acid diet.

PAI: plasminogen activator inhibitor, U: arbitrary units.

Table 3. Hemostatic variables in premenopausal women not using oral contraceptives (n=18), premenopausal women using contraceptives (n=10), and postmenopausal women (n=9).*

	Premenopausal not on contraceptives	Premenopausal on contraceptives	Postmenopausal
		% of standard	
TF-induced thrombin potential	90±11	108±9 †	98±8
Actin-induced thrombin potential	96±12	110±14 †	105±8
Factor VIIam	77±19	101±42	78±12
Antithrombin	97±6	94±8	100±9
TFPI	94±7	95±5	96±3
Fibrinogen (mg/dL)	363±29	357±18	358±39
PAI (U/mL)	7.7±4.5	6.1±2.7	8.1±4.1

* Values are given as means±standard deviations after the oleic acid run-in period.

† Significantly different from the premenopausal women not on contraceptives: P<0.01.

TF: tissue factor, TFPI: tissue factor pathway inhibitor, PAI: plasminogen activator inhibitor, U: arbitrary units.

DISCUSSION

Prospective studies in middle aged men have stressed that the coagulant capacity of plasma, which can be affected by the fatty acid composition of the diet (2, 3), is an important marker for the development of coronary heart disease (15, 16). The present study, therefore, examined the effects of diets enriched in MCFA or myristic acid compared with oleic acid on separate coagulation factors (factor VIIam activity and fibrinogen) and inhibitors of coagulation (antithrombin and TFPI activities). However, changes in these separate clotting activities do not necessarily implicate an effect on the full clotting mechanism that is eventually important for the coagulant capacity of the blood. We, therefore, also, studied the effects of the diets on the plasma thrombin potential. This coagulation assay measures the total amount of thrombin formed, when all plasma factors that influence the coagulation process are taken into account, and is a sensitive assay to detect small changes in coagulability (12).

Compared with the oleic acid diet, the TF-induced thrombin potential increased with the myristic acid, but only in the female subjects. We are, to our knowledge, the first to report in human subjects changes in plasma coagulability as measured by

thrombin potential, after dietary fatty acid alterations. Plasma hypercoagulability, as determined by increased thrombin potential has been reported before in patients with an active thrombotic process (12), and also in women using contraceptives but not suffering from thrombosis (17). The latter observation was confirmed in the present study (table 3). Decreased thrombin potential, on the other hand, has been demonstrated after heparin injection (18) or in orally anticoagulated patients (19).

Of the separate coagulation factors, factor VIIam activity significantly increased with the myristic compared with the oleic acid diet. Diets enriched in MCFA, however, did not change factor VIIam activity. Recent studies on the effects of specific saturated fatty acids on factor VII activity also demonstrated that those saturated fatty acids that increased LDL cholesterol concentrations (lauric, myristic and palmitic acids) also increased factor VII activity (4-6). The magnitude of the factor VIIam response with the myristic acid compared with the oleic acid diet was comparable to increases reported for diets enriched in lauric or palmitic acids (4) and does not suggest a more potent factor VII response, as was observed in a myristic acid versus palmitic acid comparison (6).

The more pronounced coagulant activity response in women after saturated fat diets confirmed our earlier observations with diets enriched in lauric or palmitic compared with oleic acid (4). A stronger association between dietary saturated fat content and factor VII in women compared to men was also observed in a large prospective study (20). To explain factor VII differences between women and men, we earlier suggested a role for differences in hormonal backgrounds. In agreement with the earlier study and other reports (21, 22), the present results show that factor VIIam was especially high in premenopausal women using contraceptives, although their factor VII values vary widely. Both dietary responses of factor VIIam and TF-induced thrombin potential in women were stronger with higher run-in values, indicating that especially contraceptive users and to a lesser degree postmenopausal women are more prone to coagulation responses following dietary fatty acid alterations. The interactions, however, could not be demonstrated in our previous study, which might be related to the small numbers of contraceptive users in that study. Also, Mutanen et al (23) showed that in women using contraceptives factor VIIc increased after a *trans* fatty acid diet whereas in women not using contraceptives this effect was not observed.

Both the myristic acid and the MCFA diet decreased TFPI activity compared with the oleic acid diet indicating a lower capacity of the plasma to inhibit TF-induced coagulation (24). Since changes in TFPI activity are opposite to, and not associated with changes in LDL cholesterol or other lipoproteins, this suggested that these changes reside in the carrier free pool rather than the lipoprotein bound pool of TFPI. A recent study (25) also indicated that both pools, are probably regulated independently as cholesterol-lowering by Lovastatin® did not affect the pool of free TFPI, but only lowered the LDL-TFPI complexes (25). However, no other conditions are known that cause increased LDL cholesterol levels and decreased TFPI activity as is presently reported for the saturated fat diets. It has been proposed (25) that free

TFPI has a stronger anticoagulant activity than lipoprotein bound-TFPI, which may emphasize the importance of our findings. Future research on the effects of dietary fatty acid composition on TFPI should therefore also aim to differentiate between the two forms.

To summarize, the present investigation shows that in females, but not in males, the overall plasma clotting capacity as determined by TF-induced thrombin potential is unfavorably affected by a myristic acid and to a lesser extent by an MCFA compared with an oleic acid diet. For all subjects combined, analysis of separate factors indicate increased factor VIIam activity and decreased TFPI activity on the myristic acid diet, whereas the MCFA diet did not change factor VIIam activity and slightly decreased TFPI activity compared with the oleic acid diet. The diets did not affect the fibrinolytic capacity of plasma as measured by PAI activity. Diets low in myristic acid not only have beneficial effects on LDL lipoprotein concentrations, but also on the coagulation capacity of blood, the latter especially in women.

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8

Dietary fatty acid composition and feelings of appetite

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ABSTRACT

Little is known on the effects of dietary fatty acid composition on satiety mechanisms. This study compared the effects of diets enriched in medium chain fatty acids (MCFA), myristic acid or oleic acid on feelings of appetite and concentrations of some proposed regulators of food intake: apolipoprotein A-IV (apoA-IV) and leptin. Thirty-seven women and 23 men first consumed a 3 wk run-in diet enriched in oleic acid. For the next 6 weeks, 19 subjects continued with the oleic acid diet, 21 subjects with the MCFA enriched diet, while 20 subjects received a diet high in myristic acid. Total diets contained 40 percent of energy from fat and the nutrient composition was the same in each diet except for 10 En%, which was provided by MCFA, myristic or oleic acids. Feelings of hunger were determined with 100 mm visual analog scales and were higher with the myristic acid diet compared with the oleic acid diet (difference in changes of 7.3 mm; $P=0.0036$). The MCFA acid diet did not change feelings of hunger compared with the oleic acid diet and a trend for lower hunger-feelings was demonstrated compared with the myristic acid diet (difference in changes of -3.3 mm; $P=0.0546$). Of the proposed post-absorptive regulators of food intake apoA-IV concentrations did not significantly differ between the diets and were not associated with feelings of hunger. The diet high in MCFA showed a trend for higher leptin concentrations compared with the diet rich in myristic (difference in changes of 1.1 ng/mL; $P=0.0293$) and in oleic acid (difference in changes of 1.1 ng/mL; 0.0904). We conclude that a diet enriched in myristic acid increases feelings of hunger compared with a diet enriched in oleic acid. The mechanism of regulation remains obscure since changes in fasting apoA-IV or leptin concentrations were not observed for this comparison.

INTRODUCTION

Maintaining a healthy body weight is important for the prevention of health problems like coronary heart disease, diabetes mellitus and hypertension (1-3). A high intake of dietary fat is thought to be a key factor in the etiology of obesity (4). Dietary guidelines, therefore, stress the importance to reduce fat intake and to replace fats with (complex) carbohydrates. One of the arguments for this advice is that carbohydrates have a more satiating effect than iso-energetic amounts of fat in lean (5) and obese subjects (5, 6).

Iso-energetic replacement of fats for carbohydrates, however, unfavorably lowers the anti-atherogenic HDL cholesterol concentrations (7). Another suggested approach (8), therefore, is to replace dietary saturated by unsaturated fatty acids. Effects of the type of dietary fatty acids on feelings of hunger and satiety, however, are largely unknown. Results of Rolls et al (9) suggest that differences on satiety mechanisms between different fatty acid classes exist, because preloads of medium chain triglycerides (MCT with C8:0 and C10:0) reduced food intake shortly after the preload had been given, compared with preloads of corn oil, rich in linoleic acid (9). Whether these effects are also valid on the longer term (i.e. for intakes later on the day) and whether they are specific for MCT or also count for other saturated fatty acids than MCT is not known. MCT, namely, is composed of medium chain fatty acids (MCFA; $C_{\leq 10:0}$) with a unique metabolism. Compared with longer chain fatty acids their absorption, transportation via the portal vein, to the liver and oxidation in the tissues is very rapid (10). Fats rich in MCFA (coconut oil, palm kernel oil or dairy fat) also contain other saturated fatty acids, like myristic acid (C14:0), which are transported to the liver via the, more common and slower, lymphatic system and the uptake of which involves chylomicron formation. A similar transport route applies to oleic acid (*cis*C18:1(n-9)), the major unsaturated fatty acid in these fats.

So far, little is known as to why different fatty acids should modulate feelings of satiety or hunger. It has been suggested that apolipoprotein A-IV (apoA-IV), which is synthesized in the small intestines, acts as a fat-amount (11, 12) and fat-source (13) dependent post-absorptive signal for satiety. Fujimoto and colleagues (11) observed that intravenous infusions of mesenteric lymph with apoA-IV suppressed food intake in rat. This effect was mimicked by the infusion of purified apoA-IV and prevented by feeding an inhibitor of the chylomicron formation and by immunoprecipitation of apoA-IV by a monospecific antibody. Further experiments showed that apoA-IV acts directly on the central nervous system (12).

Another potent regulator of food intake might be leptin, excreted from adipose tissue cells (14). Campfield et al (15, 16) showed that mice treated with leptin decreased food intake. So far, only the mass effects of the adipose tissue have been described as an important regulator of the production of leptin (14). First reports on the effects of dietary energy source do not indicate that leptin concentrations are modulated by the replacement of fat for carbohydrates (17, 18) or vice versa (19). However, effects of dietary fatty acid composition on serum leptin concentrations have not been reported, yet.

We, therefore, have now studied the effects of saturated fat diets either rich in medium chain fatty acids (MCFA; C6:0, C8:0 and C10:0) or in myristic acid (C14:0) and a diet rich in the major unsaturated fatty acid, oleic acid (C18:1), on feelings of hunger and on fasting apoA-IV and leptin concentrations.

SUBJECTS AND METHODS

Subjects

Sixty subjects (37 women and 23 men) completed this controlled dietary experiment. Women were aged 22-60 y (mean 40 y), weighed 48-101 kg (mean 67 kg), were between 155 and 183 cm in height (mean 167 cm), and had body mass indexes from 20-30 kg/m² (mean 24 kg/m²). The men were aged between 23-59 y (mean 43 y), weighed 56-88 kg (mean 78 kg), were between 163 and 190 cm in height (mean 178 cm), and had body mass indexes from 17 to 29 kg/m² (mean 25 kg/m²). Nine women were postmenopausal and 10 women used oral contraceptives. Six women and 5 men smoked.

Design and diets

The main purposes of this experiment was to test the effects of diets enriched in MCFA, myristic or oleic acid on lipoproteins and hemostatic parameters. Details of the study have been published elsewhere (20). The trial had a parallel design and was approved by the medical ethics committee of Maastricht University. During a 3 wk run-in period all subjects consumed a diet high in oleic acid. They were then divided into three groups. One group consumed the MCFA diet (n=21), a second group the myristic acid diet (n=20) and a third group continued on the oleic acid diet (n=19) each for six weeks. The groups were stratified for initial serum cholesterol concentrations and sex.

Before the study started, the subjects recorded their habitual food intake for two working days and one weekend day. From these food records, each subjects' actual energy intake was calculated by using the Dutch food-composition table (21). The study diets were formulated at 13 levels of energy ranging from 5 MJ to 22 MJ per day, so that each subject received a diet that met his or her energy needs. Diets used in the study consisted of products in which the normal fat was replaced by the experimental fats. The products included margarines and bakery products (bread, cookies, pies and cakes). Based on the subjects' energy intake, the amount of experimental products the subject had to eat each day (margarine, bread, and cookies) and each week (cake and pie) were calculated and used. These solid foods supplied 63% (25 En%) of the total fat energy. The remaining 37% (15 En%) of the total fat intake had to be chosen from a list of "free-choice" fat-containing products. These products were given points according to their fat contents. One point was equal to one gram of fat. Each subject was required to eat a certain number of points daily, again corresponding with his or her energy intake, and to list the products chosen

daily on a special form. The fat composition of the calculated diets was similar except for approximately 10% of total energy intake, which was provided by MCFA, myristic acid or oleic acid, respectively. The composition of the experimental fats has been described elsewhere (20).

Subjects were asked to record food intakes in a diary on one weekend and two weekdays in both the run-in and the test period. They were also asked to maintain the same activity level, smoking and drinking habits throughout the study. They recorded in diaries any signs of illness, medication used, alcohol consumption, menstrual cycle and any deviations from the study protocol. The effectiveness of blinding and the palatability of the diets was measured using a questionnaire at the end of the experiment.

Hunger rating

The subjects rated feelings of hunger at the end of the run-in period (week 3) and at the end of the test period (week 9) daily for one week on 100 mm visual analog scales (VAS) of hunger. The hunger rating was part of a more extensive series of VAS on mood. The sentence "Today I was at all not hungry" was anchored on the right and "Today I was extremely hungry" on the right side of the 100 mm scale. Subjects were asked to rate the feeling of hunger of that day just before retiring for the night. Hunger ratings were averaged per week and the response to the experimental diet was calculated per subject as the change from the end of the run-in period to the end of the MCFA, myristic acid or oleic acid diet period.

Apolipoprotein A-IV and leptin analyses

Serum apoA-IV concentrations were measured according to the "sandwich"-type enzyme-linked immunosorbent assay as described by Rosseneu et al (22). The coefficient of variation within runs was 3.6 %.

Serum leptin concentrations were measured using a double-antibody "sandwich" ELISA assay using a monoclonal antibody specific for human leptin. This assay measures total (free and bound) leptin concentrations. The lowest concentration of detection was 0.5 ng/mL and the upper limit was 50 ng/mL. The coefficient of variation within runs was 9%. Leptin concentrations of normal weight subjects range from 1 to 12 ng/mL.

Statistical analysis

Differences between the experimental diets were analyzed with the General Linear Models (GLM) procedure of the SAS Program (23) with diet as independent variable and values after the oleic acid run-in period as covariate. When the analysis indicated a significant effect of diet ($P < 0.05$), the diets were compared pair-wise. Because of the three-group comparison the Bonferonni correction was applied and statistical significance was reached at a P value $< 0.05/3$ (or, 0.0167). Because leptin values and changes in leptin values were not normally distributed, the changes were analyzed with the non-parametric Kruskal-Wallis test (24).

RESULTS

Table 1 shows the composition of the diets during the run-in and the test period as calculated from the three day food records. Mean macronutrients composition (fat, carbohydrates and proteins) of the three diets, which included the consumed fat-free products, did not change from run-in to test period.

As expected, the energy percentages of total and specific saturated fatty acids (MCFA and myristic acid) increased at the expense of those from the monounsaturated fatty acids. The reported intakes of MCFA, myristic acid or oleic acid include only the contribution of the experimental fats used during the study. Fiber intake increased slightly, though not significantly, in the myristic acid diet group compared with both the MCFA and the oleic acid groups.

Table 1. Composition of the diets as calculated from 3 days food records.*

	MCFA Diet		Myristic Acid Diet		Oleic Acid Diet	
	Run-in	Change	Run-in	Change	Run-in	Change
Energy (MJ/day)	9.2±3.0	-0.1±1.1	11.0±3.6	-0.2±1.6	9.8±2.2	-0.4±1.4
	Percentage of energy					
Fat	38.7±6.5	1.7±5.1	38.4±4.6	1.3±4.3	39.7±3.2	0.3±4.1
SAFA	11.3±2.0	10.0±2.2	11.3±1.4	9.6±2.1	11.7±1.4	-0.1±2.0
MCFA†	0.0±0.0	9.9±1.9	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.1
Myristic acid†	0.1±0.2	-0.1±0.2	0.1±0.1	9.6±1.6	0.1±0.0	0.0±0.0
MUFA	20.3±4.1	-8.2±3.5	20.4±2.7	-8.6±2.2	21.2±1.9	0.3±2.7
Oleic acid†	15.0±4.2	-8.5±4.0	14.8±2.3	-8.5±2.1	15.7±1.6	0.9±2.3
PUFA	4.9±1.0	-0.1±1.0	4.6±1.1	0.2±1.0	4.5±0.6	0.1±0.7
Linoleic acid	4.0±0.8	0.0±0.7	4.0±0.9	0.0±0.8	3.7±0.5	0.3±0.8
Carbohydrates	47.4±5.5	-3.4±4.9	46.4±5.2	-1.0±5.5	46.3±4.0	-1.7±4.3
Protein	11.8±2.8	1.5±2.2	11.9±1.3	0.3±1.3	12.4±1.9	0.2±2.1
Alcohol	2.0±2.2	0.2±2.5	3.1±3.6	-0.5±2.5	1.6±2.6	1.2±3.9
Cholesterol						
(mg/MJ)	16.3±4.1	0.7±5.5	14.4±3.1	0.4±5.3	15.8±5.0	-1.5±4.4
Fiber (g/MJ)	2.4±0.3	-0.1±0.3	2.2±0.4	0.3±0.5	2.4±0.4	-0.1±0.7

* Values are means±standard deviations.

† As provided by the experimental fats only.

SAFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

The effectiveness of blinding was good. Twenty-three subjects (38%) recognized the run-in diet as the oleic acid diet. Six subjects (29%) recognized the MCFA diet, 9 subjects (45%) the myristic acid diet and 7 subjects (37%) the oleic acid diet. These numbers could be expected by chance. Palatability as measured with an anonymous questionnaire at the end of the study revealed that margarines, bread, cookies and pie from the MCFA and the myristic acid diet were less palatable than those from the oleic acid diet.

Mean body weight (means $\pm$ standard deviations) of the MCFA, myristic acid and the oleic acid group were after the run-in period 70 $\pm$ 10 kg, 72 $\pm$ 9 kg, 71 $\pm$ 12 kg, respectively and did not change over the test period.

Hunger ratings with the three diets are given in table 2 and individual changes of hunger ratings from the run-in to the test period are illustrated in figure 1. Feelings of hunger were higher with the myristic acid diet compared with the oleic acid diet (difference in changes of 7.3 mm; $P=0.0036$). With the MCFA acid diet a trend for lower hunger-feelings was demonstrated compared with the myristic acid diet (difference in changes of -3.3 mm; $P=0.0546$). The MCFA diet did not change feelings of hunger compared with the oleic acid diet (difference in changes 4.0 mm; $P=0.2960$).

Figure 1. Individual changes in feelings of hunger (mm of 100 mm visual analog scale) on diets rich in MCFA ($n=21$), myristic acid ($n=20$) or oleic acid diet ($n=19$) compared with a run-in diet rich in oleic acid.

Table 2. Hunger ratings (mm of 100 mm visual analog scale) in 60 healthy subjects consuming a MCFA (n=21), myristic acid (n=20) or oleic acid diet (n=19).*

	MCFA Diet		Myristic Acid Diet		Oleic Acid Diet	
	Run-in	Change	Run-in	Change	Run-in	Change
Hunger (mm)						
All	35±16	0±7	43±16	3±7 †	48±14	-4±7
Women	35±18	-1±7	42±17	4±8	48±11	-4±7
Men	34±13	2±8	45±16	2±4	47±17	-4±7

* Values are means±standard deviations.

† P<0.01, significantly different from the oleic acid diet.

Serum apoA-IV concentrations are shown in table 3. ApoA-IV concentrations were 1.4 mg/dL lower with the myristic acid diet compared with the oleic and the MCFA diet. The differences in changes between the diets did not reach statistical significance (P=0.1482 and P=0.2125, respectively for differences in changes).

Table 3. Apolipoprotein A-IV concentrations in 60 healthy subjects consuming diets enriched in MCFA (n=21), myristic acid (n=20) or oleic acid diet (n=19).*

	MCFA Diet		Myristic Acid Diet		Oleic Acid Diet	
	Run-in	Change	Run-in	Change	Run-in	Change
Apolipoprotein A-IV (mg/dL)						
All	12.1±2.8	0.2±2.4	13.7±4.0	-1.2±2.5	12.9±4.9	0.2±3.2
Women	11.3±2.8	0.6±2.7	12.0±3.8	-0.5±2.1	12.2±3.7	0.4±2.8
Men	13.4±2.4	-0.3±2.1	16.2±3.0	-2.3±2.7	14.1±6.6	-0.2±3.9

* Values are means±standard deviations.

Serum leptin concentrations are given in table 4. In subjects consuming the myristic acid or the oleic acid diet no changes in serum leptin concentrations were demonstrated. The MCFA diet induced higher leptin concentrations compared with both the myristic (difference in change of 1.1 ng/mL; P=0.0293) and the oleic acid diet (difference in change of 1.1 ng/mL; P=0.0904). The overall P-value for diet, however, did not reach significance (P=0.0752).

A negative Spearman correlation coefficient between changes in hunger-feelings and changes in leptin concentrations was demonstrated in subjects consuming the

MCFA diet ($r=-0.54$; $P=0.0115$), whereas a positive association was observed in subjects consuming the myristic acid diet ($r=0.54$; $P=0.0133$). No significant correlations were found between changes in apoA-IV and changes in hunger-feelings. A correlation was demonstrated between leptin concentrations and BMI ($r=0.27$; $P=0.0402$, all subjects combined after oleic acid run-in period).

Table 4. Leptin concentrations (ng/mL) in 60 healthy subjects consuming diets enriched in MCFA (n=21), myristic acid (n=20) or oleic acid diet (n=19).*

	MCFA Diet		Myristic Acid Diet		Oleic Acid Diet	
	Run-in	Change	Run-in	Change	Run-in	Change
Leptin (median, ng/mL)						
All	6.3 (0.3 to 18.4)	1.1 (-2.9 to 10.8)	5.2 (0.3 to 27.2)	0.0 (-10.5 to 5.5)	6.8 (0.3 to 49.2)	0.0 (-5.5 to 9.6)
Women	12.1 (1.2 to 18.4)	1.7 (-2.9 to 10.8)	8.1 (3.0 to 27.2)	-0.1 (-10.5 to 5.5)	11.7 (2.7 to 49.2)	-0.2 (-5.5 to 9.6)
Men	1.8 (0.3 to 4.2)	0.7 (-0.5 to 3.4)	1.3 (0.3 to 2.6)	0.0 (-0.6 to 0.8)	0.8 (0.3 to 6.8)	0.0 (-0.2 to 2.9)

* Values are medians and ranges.

DISCUSSION

The present results indicated that a diet rich in myristic acid increased feelings of hunger compared with an oleic acid diet. The increased feelings of hunger on the myristic acid compared with those on the MCFA diet were less pronounced and did not reach statistical significance.

In this study it was important that the diets did not differ in other aspects (for example, palatability) than fatty acid composition as we were interested post absorptive regulators of satiety mechanisms. If the observed differences in palatability would have affected feelings of hunger, the changes with the MCFA and the myristic acid diet would have been expected to occur in a similar direction relative to the oleic acid diet. The present results, however, rather indicate opposite effects of both saturated-fat diets.

Of the possible regulating mechanisms for satiety, differences in gastric emptying of the various diets are not very likely to explain the changed hunger feelings. Longer chain unsaturated fatty acids, like oleic acid, leave the stomach at a slower rate than shorter chain fatty acids as myristic acid (25). Gastric emptying of MCFA has been reported to be even faster than glucose (26). However, compared with the oleic acid diet feelings of hunger did not change with the MCFA diet and were increased with the myristic acid diet.

The unchanged fasting concentrations of some proposed regulators of food intake -apoA-IV and leptin- with the myristic compared with the oleic acid diet may indicate that their concentrations change in a different time span (for example shortly after a meal) or that other regulatory components are involved. Cholecystokinin (CCK), which concentrations change shortly after a meal, might be one of these regulators. The intestinal release of CCK is stimulated by long chain fatty acids (27) and administration of CCK reduces short-term food intake (28). A recent study (29) showed that CCK release is fatty acid chain length dependent as MCFA did not, and lauric acid (C12:0) increased CCK release. Also oleic acid increased CCK release compared with saline (30). However, whether the oleic acid-induced release of CCK is higher than for myristic acid or other saturated fatty acids remains to be investigated in further postprandial experiments.

From our results, it is tempting to speculate that exchanging dietary saturated rich fats for fats rich in oleic acid decreases feelings of appetite. This may, on the longer term, help to maintain a proper weight. It is therefore interesting that an inverse association between the intake of vegetable fat, in which major fatty acids are oleic and linoleic acid, and body mass index has been observed (31). Stamler and Dolecek (32), in addition, demonstrated a greater weight loss at lower intakes of fats and oils rich in saturated fatty acids and with higher intakes of polyunsaturated fatty acids. It should, therefore, be also investigated whether exchanges of saturated fatty acids (especially the major fatty acid palmitic acid (C16:0) or mixtures of saturated fats) for polyunsaturated fatty acids reduces feelings of hunger and food intake.

Whereas MCFA did not change feelings of hunger compared with oleic acid and only slightly increased those compared with the myristic acid diet, a trend for increased leptin concentrations was demonstrated. So far, body mass is considered to be the most important regulator of leptin levels (16, 33). Also, in the present study a positive association between BMI and leptin levels was observed. In addition, leptin concentrations were higher in women than in men, as has been reported before (33). However, the fact that leptin concentrations tended to increase with the MCFA diet, without concomitant changes of body weight, indicates that leptin concentrations may also be finely tuned independent of changes in BMI. Recently this was also demonstrated in subjects, in whom fasting was associated with a decline of leptin concentrations (18, 34). This and the rise in leptin concentrations after a prolonged exposure to hyperglycemia and resultant hyperinsulinemia indicated that the leptin release is sensitive to changes in insulin and glucose levels (35-37). Since the time of fasting was on average similar for our subjects within the different diet groups, the hyperinsulinic effect of MCFA (10) compared with longer chain fatty acids, might be related to the elevated leptin levels with this diet.

To summarize, this study indicates that the diet rich in myristic acid increased feelings of hunger compared with the diet rich in oleic acid. The mechanism of regulation remains obscure. No changes in fasting apoA-IV or changes in leptin concentrations were observed for this comparison.

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9

General discussion

This thesis describes the effects of medium chain fatty acids (MCFA), lauric acid, myristic acid and palmitic acid on the cardiovascular risk profile in healthy women and men. So far, specific effects of these saturated fatty acids on serum lipid and lipoprotein concentrations have hardly been studied (MCFA and lauric acid) or results were conflicting (lauric, myristic and palmitic acids). In addition, the effects of individual saturated fatty acid on hemostatic factors have not been examined in sufficient detail, while mainly young and lean men were studied, whereas we now studied both women and men aged between 20 and 60 years.

Design of the studies

The first study (lauric and palmitic acid study) followed a cross-over and the second (MCFA and myristic acid study) a parallel design. The advantage of a cross-over design is that subjects receive each diet and, therefore, to achieve the same statistical power, less subjects are needed than in a parallel designed study. The disadvantage is that carry-over effects may occur, which, however, was not the case in our study. Also the long duration of a cross-over designed experiment together with the controlled nature of the study could have been one of the reasons that 11 of the 43 subjects that started the trial dropped out during the first study.

In the second study (MCFA and myristic acid study) a parallel design was applied. The test period was preceded by a run-in period of controlled diets. In this way between subject variation in the variables of interest was excluded as each subject was used as its own control. In addition, the variation in the results due to the different habitual diets was eliminated.

The diets contained 40 En% from fat, of which the major amount of fatty acids (70% in the first study and 63% in the second study) was supplied to the subjects with experimental margarines and products that were made of these margarines. The remaining fat-intake was left at the subjects' own decision, although controlled with a point system of "free-choice" items. To exclude some potential confounders, body weights were kept constant during the study by adjusting the level of energy intake when the weekly recorded body weight indicated changes. Subjects were asked to maintain the same activity level, the same smoking and drinking habits and not to change intakes of medicines such as oral contraceptives throughout the study.

The compliance to the protocols was checked in different ways. Subjects visited the university every week to receive a new supply of experimental products and were then interviewed by a registered dietitian to check their adherence to the study protocol and discuss possible difficulties with it. The self reported food intake records in each experimental period, records of the products chosen as "free-choice" items, body weight, and fatty acid analysis of pooled serum samples provided further evidence for good compliance to the protocol. The subjects did not receive any payment for their contributions, to avoid unmotivated subjects participating only for their financial benefit.

In both studies, values on a diet rich in oleic acid were used as references. In the parallel designed study, for the coagulation and fibrinolytic parameters it was noted that changes were still observed after three weeks of the run-in period in the oleic acid group. Whether this effect was caused by a drift in time, or because the run-in period was too short to achieve stable measurements of the different factors is not known from the present experiment.

Experimental fats

The lauric, palmitic and oleic acid rich experimental fats were composed of blends of natural fats. Edible consumption fats that are rich only in MCFA or myristic acid do not exist. Therefore, special MCFA and myristic acid rich synthetic fats were manufactured. To evaluate the extent to which the positional distribution of the fatty acids in these synthetic fats corresponded to that from natural fats (chapter 3), we determined the fatty acids at the *sn*-2 position. From these results, and the total fatty acid composition, the fatty acid distribution over the *sn*-1 or *sn*-3 position was calculated. As in dairy fat, MCFA from the MCFA experimental fat was attached mainly to the *sn*-1 and *sn*-3 position. In the myristic acid fat, myristic acid and the other saturates were equally distributed among the *sn*-2 and *sn*-1,3 positions. Myristic acid rich fat sources as palm kernel or coconut fat have myristic acid mainly at the *sn*-1,3 positions (1), whereas dairy fat carries half of the total proportion of myristic acid at the *sn*-2 position (2). So far, dietary experiments comparing fats rich in palmitic acid (3, 4) and stearic acid (5) suggest that the positional distribution of these saturates do not affect lipid and lipoprotein concentrations. Whether a different positional distribution of MCFA and myristic acid could have influenced their cholesterolemic effects or effects on hemostatic parameters is, however, not known.

When comparing the two studies carried out, it should be taken into account that the amounts of fatty acids exchanged, as confirmed by the food records, were lower in the first study (≈ 8 En% from lauric acid or from palmitic acid for oleic acid) than in the second study (10 En% from MCFA or from myristic acid for oleic acid). Because natural fats were consumed in the first study, the lauric acid diet contained somewhat more myristic acid (≈ 2 En%) than the other two experimental diets, which might have influenced the results. For proper comparisons, the values in the figures and in the tables presented in this chapter are therefore recalculated to effects that are expected when 10 En% of a particular saturated fatty acid would have been exchanged for oleic acid. In addition, the lipid and lipoprotein values on the lauric acid diet are corrected for the higher percent of energy myristic acid, using data of the study comparing the effects of myristic acid with those of oleic acid (Chapter 3).

INDIVIDUAL SATURATED FATTY ACIDS AND CARDIOVASCULAR RISK

The effects of MCFA and lauric, myristic and palmitic acids on lipid and hemostatic factors are summarized in table 1.

Table 1. Summary of the effects on fasting lipid and hemostatic factors of diets enriched in MCFA, lauric, myristic or palmitic acid compared with those of oleic acid.*

	MCFA	Lauric acid	Myristic acid	Palmitic acid	CVD risk
Lipid factors					
LDL cholesterol	+=	+	++	+	+
HDL cholesterol	=	+	+	=	-
Triacylglycerols	+=	=	=	=	+
Lipoprotein(a)	=	=	-	=	+
Apolipoprotein B	=	+	+=	+	+
Apolipoprotein A-I	=	+	+=	=	-
Hemostatic factors					
Whole blood aggregation	=	=	=	=	+
Coagulation					
Factor VIIam activity	=	+	+	+	+
Fibrinogen	=	+=	=	+=	+
Antithrombin activity	=	-	=	=	- or + ?
Fibrinolysis					
PAI activity	=	=	=	+	+

* See also table 2 in the general introduction for remarks on the relationships of these variables with CVD-risk. PAI: plasminogen activator inhibitor.

Lipid factors

Changes in LDL and HDL cholesterol concentrations and total to HDL cholesterol ratios, when 10 En% from oleic acid is exchanged for either MCFA or lauric, myristic or palmitic acids, are shown in figure 1.

Figure 1. Mean changes in LDL and HDL cholesterol concentrations and total to HDL cholesterol ratios when 10 percent of energy from oleic acid is exchanged for either MCFA or lauric acid or myristic acid or palmitic acid.

MCFA did not significantly change HDL and LDL cholesterol concentrations compared with the oleic acid or the myristic acid diet. MCFA, however, tended to increase LDL cholesterol concentrations compared with oleic acid. A recent controlled study (6), in which extremely high concentrations of MCFA (43 En%) were given, reported a more pronounced increase in LDL cholesterol compared with oleic acid. Recalculated to a 10 En% exchange from oleic acid, however, the LDL cholesterol-raising effect of MCFA was 0.13 mmol/L (6), which is smaller than the 0.23 mmol/L raise of LDL cholesterol concentrations found in the present study. The HDL cholesterol concentrations, in agreement with our results, did not change compared with the oleic acid diet (6). Therefore, referring to the effects of MCFA as neutral compared with carbohydrates (7, 8) does not seem justified, because carbohydrates do decrease HDL cholesterol levels compared with oleic acid (9). The effects on HDL cholesterol concentrations of MCFA compared with iso-energetic amounts of carbohydrates, of course, needs further investigation in a side-by-side comparison. In contrast to the other saturates, MCFA tended to raise triacylglycerol concentrations. Triacylglycerol-raising effects have been found in several other studies also (6, 10, 11). The less pronounced effects of the MCFA diets on the triacylglycerol concentrations in the present study was probably also related to the smaller doses of MCFA given in our diets.

Lauric and myristic acids demonstrated the strongest total cholesterol-raising effect compared with oleic acid. Lauric acid significantly raised total, LDL and HDL

cholesterol levels compared with oleic acid. The total cholesterol-raising effects of lauric acid compared with oleic acid were also found in an earlier experiment (12), but the total cholesterol-raising potency compared with other individual saturated fatty acids like palmitic acid is still not clear. Because of the presumed strong effects of myristic acid on lipids and lipoproteins concentrations, the values for lauric acid (see figure 1) were corrected for the 2 En% higher myristic acid in the lauric acid diet. Then, the expected rise in LDL and HDL cholesterol would be 0.34 mmol/L instead of 0.43 mmol/L for LDL cholesterol when 10 En% of oleic acid is exchanged for lauric acid, as 0.09 mmol/L was due to the higher myristic acid content of this diet relative to oleic acid. The expected change for HDL cholesterol is 0.16 mmol/L instead of 0.19 mmol/L, as 0.03 mmol/L is due to the higher myristic acid content. Combined results, therefore, suggest (figure 1), that the LDL cholesterol-raising effect of lauric acid is between that of palmitic acid and myristic acid. The major difference between lauric and palmitic acids is the more pronounced increasing effect of lauric acid on HDL cholesterol concentrations, which explains the stronger total cholesterol-raising effects of this saturated fatty acid compared with palmitic acid.

The myristic acid diet raised total, LDL and HDL cholesterol concentrations compared with the oleic acid diet. The presumed very potent effects of myristic acid on total (8) and LDL cholesterol levels (13), however, needs to be attenuated. Total cholesterol concentrations raised with 0.40 mmol/L relative to the oleic acid diet, which was less than expected from regression equations (8). Using Hegsted's regression equation, which includes myristic and palmitic acids, polyunsaturates and dietary cholesterol, total cholesterol concentrations are estimated to increase with 2.19 mmol/L when 10 energy percent of oleic acid is exchanged for myristic acid. Using the equation including all variables (MCFA, lauric, myristic and palmitic acids, monounsaturates, polyunsaturates and dietary cholesterol), the estimated change is 1.35 mmol/L (8). In agreement with our results, other controlled experiments found, smaller changes in total cholesterol when myristic acid was compared with oleic acid (14) or no change when it was compared with palmitic acid (15). Myristic acid does raise HDL cholesterol concentrations, which has been found earlier (14, 15). As for lauric acid, the unfavorable LDL cholesterol-raising effects of myristic acid, go with the postulated favorable effects on HDL cholesterol concentrations. Thus, the LDL and HDL cholesterol raising effects of palm kernel (16) or coconut fat (17-19), rich in both lauric and myristic acids, compared with for example palm or olive oils, are most likely due to the cholesterol-raising action of both lauric and myristic acid.

Relative to oleic acid, palmitic acid elevated total and LDL cholesterol and apoB concentrations, but not HDL cholesterol concentrations. This study, therefore, confirmed previous studies (12, 14, 20, 21) that palmitic acid is a cholesterol-raising fatty acid although the effects on LDL cholesterol concentrations were less than those of myristic acids (see figure 1). Hayes and colleagues have hypothesized that, under certain conditions, palmitic and oleic acids can be exchanged without affecting LDL cholesterol concentrations (13). Conditions, in which palmitic acid does not raise LDL cholesterol levels include those in which diets contain adequate linoleic acid and are low in cholesterol (17, 22). It has been postulated that, under these conditions,

linoleic acid increases LDL-receptor activity, and thereby counteracts the stimulating effects of palmitic acid on apoB production (13). As the linoleic acid content of the diets used was adequate and cholesterol intake was low, cholesterol-raising effects of palmitic acid presented in chapter 2 only fit in the proposed model (13) when a high total fat intake also moderates the LDL metabolism and consequently the cholesterol-raising effects of palmitic acid. In the present study dietary fat intake was around 40 En%, whereas in the few studies, which found that palmitic acid did not raise LDL cholesterol compared with oleic acid, total fat intake was around 30-34 En% (17, 22). With Western diets, containing on average 37 En% of fat (23), the major saturated fatty acid of this diet (palmitic acid) must be classified as a fatty acid that raises LDL cholesterol concentrations. HDL cholesterol levels remain stable after palmitic acid rich diets, relative to oleic acid rich diets. Whether this is not true at lower fat intakes needs more study.

Lipoprotein(a)

The myristic acid diet decreased Lp(a) concentrations compared with the oleic acid diet. Other studies indicate that mixtures of saturated fatty acids lower Lp(a) concentrations compared with oleic acid (*cis*C18:1(n-9)) (24), elaidic acid (*trans*C18:1(n-9)) (21, 24, 25) and stearic acid (C18:0) (26). Lp(a) concentrations on diets rich in linoleic acid did not differ from those on diets rich in oleic acid (24). Combining these results suggests that mixtures of saturated fatty acids lower Lp(a) concentrations compared with fatty acids containing 18 carbon atoms, irrespective of the number of double bonds. In the first study we did not observe Lp(a) lowering effects of the lauric or palmitic acid compared with oleic acid. The suggestion, however, that myristic acid in particular decreases Lp(a) is not supported by a recent study that reported similar Lp(a) concentrations after diets rich in myristic or palmitic acids (27). Since reduced Lp(a) concentrations on the myristic acid diet and mixtures of saturated fatty acids are accompanied by increased LDL cholesterol concentrations, both lipoprotein fractions are likely to be changed through mechanism that do not follow a similar pathway.

To summarize the effects on lipid factors: lauric, myristic and palmitic acids unfavorably raise LDL cholesterol concentrations relative to oleic acid, whereas the LDL cholesterol-raising effects of MCFA diets are less strong. The saturated fatty acids studied, sorted according to their LDL cholesterol-raising potency, are in ascending order: MCFA, palmitic acid, lauric acid and myristic acid. HDL cholesterol concentrations are favorably elevated by diets rich in lauric or myristic acids, but not by diets rich in MCFA or palmitic acid. Combined results on total to HDL cholesterol ratio (see figure 1), which has been proposed as a strong predictor of CVD-risk (28, 29), therefore, suggests higher ratios with the palmitic acid and the MCFA diets. The responses to the diets of lipid factors did not differ between women and men.

Hemostatic factors

Platelet aggregation

The saturated fatty acids studied did not induce changes in whole blood aggregation induced by either collagen (MCFA, lauric, myristic and palmitic acids), ADP (MCFA and myristic acid). Diets rich in lauric or palmitic acids did not change thromboxane B₂ production in collagen activated blood samples compared with the oleic acid diet (chapter 5). With the used method for measuring aggregation, we tried to mimic the *in vivo* aggregation situation as closely as possible. Hirudin was used as anticoagulant, because it binds directly to thrombin and does not lower the calcium concentrations as is the case in citrate-anticoagulated blood samples.

Partly due to the broad scale of platelet aggregation methods used by different laboratoria, the literature on the aggregatory effects of fatty acids is difficult to compare with each other and very confusing. In addition, saturated fatty acids have hardly been studied in detail and no studies have been carried out on whole blood aggregation with hirudinized anticoagulated blood samples. Using other methods, saturated fatty acids have been reported to increase PRP aggregation induced with thrombin (30), whereas others indicated lower PRP aggregation induced with ADP or collagen of a milkfat diet compared with diets enriched in sunflower or rapeseed oil (31). In this area of research, a validation and standardisation program for the methods to be used is needed, in addition to new approaches to assess the *in vivo* platelet activity.

Coagulation factors

Our studies demonstrated that diets enriched in lauric, myristic or palmitic acids significantly increased factor VIIam activity. Marked differences were noted in factor VIIam responses between women and men (figure 2). Changes in factor VIIam only reached statistical significance in women. Whether changes in factor VIIam were more pronounced in women using contraceptives or in postmenopausal women, could not be examined in the first study on lauric and palmitic acids because groups were too small to draw conclusions. The second study on MCFA and myristic acid, however, demonstrated an interaction effect between diet and factor VIIam activity after the run-in period, suggesting that dietary responses were more pronounced in women with higher factor VIIam activities. Because contraceptive users and postmenopausal women have higher factor VIIam activity or coagulant activity than non-users ((32) and results given in chapter 6 and 7), data from parallel designed studies must be corrected for the women's baseline values, or study groups need to be balanced according to menopausal status and contraceptive use.

As the gender differences were observed in both studies, this finding certainly deserves follow-up and the proposed explanations (in chapters 6 and 7), such as hormonal differences or differences in interactions between diet and genetic backgrounds need more research.

A recent population-based study sustains that the association between saturated fat intake and factor VIIc activity is stronger in elderly women than in elderly men (33).

Implications of a higher factor VII activity in women for CVD-risk are not known from prospective studies because these only included men (34, 35). Cross-sectional studies, however, do demonstrate, like in men, higher factor VIIc and factor VII-antigen in women with CVD compared with women without CVD (36).

Figure 2. Changes in factor VIIam activity for women and men when 10 percent of energy from oleic acid is exchanged for either MCFA or lauric acid or myristic acid or palmitic acid.

The factor VII elevating effects of myristic or palmitic acid rich fats were also demonstrated by Tholstrup et al (15, 16). It is, however, difficult to compare their (15, 16) and our studies. Firstly, we have compared the individual saturates with oleic acid, whereas Tholstrup et al (15, 16) compared diets rich in lauric plus myristic acid or palmitic acid with diets rich in stearic acid (16) or a diet enriched in myristic acid with a palmitic acid enriched diet (15). Secondly, the study populations differed. We investigated the effects of saturates in healthy women and men with a broad age distribution (20-60 years), while they included only young and lean male subjects (15, 16). The use of different factor VII assays (Tholstrup et al used a one-staged coagulant assay with human thromboplastin) was probably of less importance for the comparison of results, because in our and their assay a human thromboplastin was used and the correlation between factor VIIam activity measured with a chromogenic assay and factor VIIc activity is high (37). Tholstrup et al found, except for stearic acid, no differences in factor VIIc activity between the individual saturated fatty acids (for the lauric plus myristic acid diet compared with the palmitic acid diet (16)) or small differences (for the myristic relative to the palmitic acid diet (15)). Stearic acid

decreased factor VIIc activity compared with lauric plus myristic acids or palmitic acid (16).

Inhibitors of coagulation were affected differently by the individual saturated fatty acids. Antithrombin activity tended to be lower on the lauric acid diet and did not change on the MCFA, myristic or palmitic acid diets compared with the oleic acid diet. The myristic acid diet decreased the activity of TFPI, and the MCFA tended to decrease TFPI compared with oleic acid. TFPI activity on diets rich in lauric or palmitic acids, however, was not measured. The significance for CVD-risk of these findings is not clear. Higher activities of the coagulant inhibitors would be expected to be beneficial for CVD-risk. However, also elevated levels of antithrombin (38) and TFPI (39) have been found in CVD patients.

For a valid conclusion on the effects of saturated fatty acids on the overall thrombotic capacity of the plasma, it might be important to evaluate how much thrombin can be formed.

In the second study (MCFA and myristic acid) we measured overall thrombin generation capacity of plasma with a global coagulation assay: the thrombin potential assay. Our study showed that the tissue factor-induced thrombin potential increased on the myristic compared with the oleic acid diet, in women but not in men. This finding again underlined the gender-specific effects of dietary saturated fatty acid on the coagulant capacity of the blood.

Concentrations of the prothrombin fragment 1+2 (chapter 6), which is released when prothrombin is converted into thrombin and, therefore, is used as a marker for thrombin formation *in vivo*, did not change between the diets rich in lauric or palmitic acid compared with the oleic acid rich diet. This suggests an unaltered *in vivo* coagulant state of the blood after these diets. Why fragment 1+2 concentrations increased in another dietary experiment on the effects of diets rich in lauric plus myristic acids compared with a stearic acid rich diet (40) is not known.

The increased factor VIIam activity after lauric, myristic or palmitic acid rich diets relative to the oleic acid diet is of particular significance because results of epidemiological studies suggest that fatal coronary episodes are frequently preceded for months or years by increased plasma activity of this coagulation factor (34, 35). This then constitutes a hypercoagulable capacity of the blood implying an augmented risk on the longer term.

Prospective studies, in addition, suggest that both a high factor VIIc activity and high LDL cholesterol concentrations implicate the strongest risk for the development of CVD (34, 35, 41). When the effects of the individual saturated fatty acids on changes in LDL cholesterol concentrations and changes in factor VIIam activity are combined and compared with those of oleic acid (figure 3), they indicate that MCFA is less potent to increase these risk markers compared with the other saturated fatty acids. The action of myristic acid seems slightly less favorable than the action of lauric or palmitic acid, through the more potent LDL cholesterol-raising effects.

Fibrinolytic factors

Palmitic acid significantly increased PAI activity compared with oleic acid, which suggested a less favorable fibrinolytic state of the plasma. Differences in fibrinolytic capacity have not been reported before after diets enriched in specific saturated fatty acids (15, 16, 26), but a diet rich in butterfat decreased PAI activity compared with hydrogenated soybean oil (25). Besides differences in MCFA, lauric, myristic and especially palmitic acids contents, the diets compared in that study (25) also differed with respect to *trans* fatty acids, which prevent a valid comparison with our results.

Figure 3. Changes in factor VIIam and LDL cholesterol concentrations when 10 percent of energy from oleic acid is exchanged for either MCFA or lauric acid or myristic acid or palmitic acid.

Feelings of appetite and mood

In the second study we found significantly increased feelings of hunger with the myristic compared with the oleic acid diet (chapter 8), whereas mood and anxiety scores did not differ between the diets (chapter 4). The findings on feelings of hunger indicate that dietary fatty acid composition might be a determinant of food intake regulation. Further research, however, is needed to confirm these new findings.

Conclusions and recommendations

The present investigations show dissimilar effects on serum lipid and lipoprotein levels of the saturated fatty acids studied. Diets rich in myristic acid raise LDL cholesterol concentrations most pronounced, followed by lauric and then palmitic acid rich diets. High LDL cholesterol concentrations increase CVD-risk. The effects of MCFA are different from the other saturated fatty acids studied, because they increase LDL cholesterol concentrations only slightly. The raise of HDL cholesterol by lauric and myristic acid rich diets has potentially beneficial effects on CVD-risk, but the effects of diet-induced elevations of HDL cholesterol concentrations remain to be established.

Lauric, myristic and palmitic acid rich diets, to the same extent, unfavorably raise plasma coagulant activity, as measured with factor VIIam activity. Therefore, there appears little need to differentiate, with respect to this parameter, between individual saturated fatty acids, except for MCFA and probably stearic acid (16, 26). However, the differences in responses between women and men are evident and deserve further mechanistic study and CVD-risk assessment.

The increase of CVD-risk is most pronounced when elevated levels of LDL cholesterol go together with increased coagulant capacity (41). Therefore, it will be advisable to decrease the intake of fats rich in lauric, myristic acids and palmitic acids in favor of a higher intake of unsaturated fatty acids for example from oils rich in monounsaturated fatty acids (oleic acids) such as olive oil, high oleic sunflower oil or rapeseed oil. Additional positive effects on feelings of appetite might be expected with the exchange of saturated fatty acids for monounsaturated fatty acids, but need confirmation in future research.

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Samenvatting

INLEIDING

Door de gemiddelde Nederlander wordt per dag 37 procent van de totale op een dag opgenomen energie als vet geconsumeerd. Dit komt neer op gemiddeld 92 gram vet per dag. Deze hoeveelheid wordt niet alleen geconsumeerd in de vorm van zichtbare vetten zoals bijvoorbeeld margarines of bak- en braadvetten, maar ook via "onzichtbare" vetten in koekjes, brood, kaas, melk en dergelijke.

Vetten bestaan voor het grootste gedeelte uit vetzuren. Uitgaande van de chemische structuur bestaan er verschillende klassen van deze vetzuren: de verzadigde vetzuren, de enkelvoudig onverzadigde vetzuren en de meervoudig onverzadigde vetzuren. Elk vet bestaat steeds uit een combinatie van de vetzuren uit de verschillende groepen, waarbij één of twee vetzuren in meerderheid aanwezig zijn. De consumptie van verzadigde vetten bedraagt ongeveer 14% van de dagelijkse energiebehoefte van de mens en komt neer op ongeveer 35 gram per dag. In tabel 1 (*hoofdstuk 1*) worden de vetten met veel verzadigde vetten genoemd. De belangrijkste vetzuren in boter zijn bijvoorbeeld palmitinezuur (C16:0) en het enkelvoudige onverzadigde vetzuur oliezuur (C18:1), met daarnaast kleinere hoeveelheden myristinezuur (C14:0), korte- en middenlange keten vetzuren ($\leq$ C10:0) en stearinezuur (C18:0). Vetten met veel verzadigde vetzuren kan men herkennen doordat deze hard zijn in gekoelde staat, terwijl vetten met veel onverzadigde vetzuren vloeibaar zijn.

Binnen de groep verzadigde vetzuren zijn de volgende vetzuren met hun bijbehorende chemische structuur te onderscheiden: de korte- en de middenlange keten vetzuren ($\leq$ C10:0), laurinezuur (C12:0), myristinezuur (C14:0), palmitinezuur (C16:0) en stearinezuur (C18:0). In de westerse landen bestaat ongeveer de helft van de totale hoeveelheid geconsumeerde verzadigde vetzuren uit palmitinezuur. De andere helft bestaat uit een combinatie van stearinezuur, myristinezuur, laurinezuur en de korte- en middenlange keten vetzuren.

Mensen die veel vet rijk aan verzadigde vetzuren eten hebben een groter risico om hart- en vaatziekten te ontwikkelen. Al in de jaren zestig werd door Amerikaanse onderzoekers waargenomen, dat vetten met eenzelfde gehalte aan verzadigde vetzuren, maar met verzadigde vetzuren van een verschillende ketenlengte, een verschillend effect op het cholesterolgehalte van het bloed -één van de risicofactoren voor hart- en vaatziekten- hebben. Boter bijvoorbeeld verhoogt het totaal cholesterolgehalte ten opzichte van eenzelfde hoeveelheid cacaoet. Omdat resultaten van uitgevoerde studies voor sommige van de verzadigde vetzuren schaars of tegenstrijdig zijn, werden in onze studies de effecten van voedingen rijk aan middenlange keten vetzuren (MCFA), laurinezuur, myristinezuur of palmitinezuur onderzocht op het vetgehalte van het bloed (bloedlipiden, zoals onder andere cholesterol en triglyceriden) en op dat van de deeltjes die deze bloedvetten vervoeren (de lipoproteïnen). Processen betrokken bij de bloedstolling spelen waarschijnlijk ook een belangrijke rol bij het ontstaan van hart- en vaatziekten. De effecten van verschillende verzadigde vetzuren op de bloedstolling zijn tot nu toe nauwelijks onderzocht. Daarom werden in onze studies ook factoren gemeten die

betrokken zijn bij de bloedstolling, zoals de mate van samenklontering van de bloedplaatjes (bloedplaatjesaggregatie), en de activiteit van verschillende eiwitten betrokken bij de bloedstolling of de afbraak van stolsels (de fibrinolyse). Tevens hebben we de effecten van de voedingen onderzocht op de gemoedstoestand en de eetlust.

ONDERZOEKSMETHODEN

Er werden twee onderzoeken uitgevoerd. De effecten van de verschillende verzadigde vetzuren werden bestudeerd ten opzichte van een voeding rijk aan oliezuur.

Het eerste onderzoek bestudeerde de effecten van voedingen rijk aan laurinezuur of palmitinezuur ten opzichte van een voeding rijk aan oliezuur. Elke vrijwilliger in dit onderzoek consumeerde iedere voeding zes weken lang. Aan het einde van die periode werd tweemaal een nuchter bloedmonster afgenomen.

Het tweede onderzoek bestudeerde de effecten van voedingen rijk aan MCFA of myristinezuur vergeleken met een voeding rijk aan oliezuur. De groep vrijwilligers in deze studie consumeerde eerst gedurende drie weken de voeding rijk aan oliezuur (inlooperperiode) en vervolgens werd de groep in drieën opgedeeld voor de zesweekse experimentele periode; één groep kreeg de voeding rijk aan MCFA, één groep de voeding rijk aan myristinezuur en één groep bleef de oliezuurverrijkte voeding gebruiken. Aan het einde van de drieweekse inlooperperiode werd tweemaal een nuchter bloedmonster afgenomen evenals aan het einde van de experimentele periode.

Voedingen

Voor het bereiken van de juiste vetzuursamenstelling van de voedingen werden speciale vetten samengesteld. Voor het eerste onderzoek werden deze samengesteld uit bestaande vetten rijk aan verzadigde of enkelvoudig onverzadigde vetzuren (zie tabel 1, *hoofdstuk 1*). Het vet rijk aan laurinezuur bestond bijvoorbeeld uit een mengsel van palmpitvet en een zonnebloemolie met veel oliezuur; het palmitinevet uit een mengsel van palmstearine (dit wordt gemaakt uit palmolie), botervet en een deel zonnebloemolie.

Voor het tweede onderzoek was het minder gemakkelijk de juiste vetzuursamenstelling te bereiken door het gebruik van "natuurlijke" vetten en daarom werden er speciale vetten met een bepaalde vetzuursamenstelling gemaakt met behulp van een chemisch proces genaamd interesterificeren.

De experimentele vetten werden in beide onderzoeken in producten verwerkt zoals margarine, brood, koekjes, vlaai, cake, kaas en desserts.

Alle deelnemers schreven voorafgaande aan de studies gedurende drie dagen op wat ze aten en dronken. Aan de hand van deze gegevens werd berekend hoeveel energie zij per dag gebruikten en vervolgens hoeveel van onze experimentele producten zij dan zouden moeten gebruiken. De berekende voedingen bevatten in

totaal 40 energie-procenten vet, waarvan 25 (studie 1) of 28 energieprocent (studie 2) geleverd werd door de experimentele vetten. Het overige deel van de vetconsumptie kon worden ingevuld met behulp van een systeem van vrije punten voor de verschillende vet bevattende levensmiddelen. De voedingen rijk aan de verschillende verzadigde vetzuren werden zo samengesteld dat ze 8,5 (studie 1) of 10 (studie 2) energieprocenten meer van het te onderzoeken vetzuur bevatten vergeleken met de voeding rijk aan oliezuur.

Vrijwilligers

De vrijwilligers voor de eerste studie waren 18 vrouwen en 14 mannen. In de eerste studie was de leeftijd gemiddeld 41 jaar. De vrouwen wogen gemiddeld 69 kg en hadden een lengte van 166 cm. De mannen wogen gemiddeld 81 kg en waren 179 cm lang. Aan de tweede studie namen 37 vrouwen en 23 mannen deel. De leeftijd van de vrijwilligers was gemiddeld 40 jaar. De vrouwen wogen gemiddeld 67 kg en waren 167 cm lang terwijl de mannen 78 kg wogen en 178 cm lang waren. De karakteristieken van de vrijwilligers wat betreft leeftijd, lengte en gewicht in beide studies waren dus vergelijkbaar.

Compliance

Op verschillende manieren werd getracht na te gaan of de deelnemers zich aan de voorgeschreven voedingen hielden en de producten in de juiste hoeveelheden en volgens de richtlijnen consumeerden (compliance). De vrijwilligers kwamen elke week naar de Universiteit en kregen daar een nieuwe portie van de producten voor de komende week. Tevens werden dan met een diëtist de eventuele problemen met het studie-protocol besproken. Tijdens dit wekelijkse bezoek werd verder het gewicht gemeten. Als bleek dat de deelnemer in gewicht toenam of juist afviel werden de richtlijnen aangepast. In de praktijk kwam het er op neer dat er soms in het begin van het experiment inschattingfouten waren gemaakt, maar dat daarna nauwelijks meer aanpassingen van de richtlijnen voor consumptie van de producten nodig waren.

Tijdens de studie schreef de vrijwilliger gedurende vier (studie 1) of drie (studie 2) dagen op wat er gegeten en gedronken was en ook dit werd doorgesproken met de diëtist. De gegevens gaven aan dat de voedingen gemiddeld als voorgeschreven gebruikt werden. Tevens verzamelden de vrijwilligers in de eerste studie gedurende twee dagen een duplicaat portie van het eten en drinken. Deze porties werden gemengd, het vet eruit gehaald en de vetzuursamenstelling van dit vet bepaald. De verkregen gegevens kwamen overeen met de getallen berekend uit de gegevens van de opschrijfdagen.

Tenslotte hebben we de vetzuursamenstelling van het bloed gemeten. Als er veel van een bepaald vetzuur gegeten wordt, zouden verhoogde nivo's van dat vetzuur in het bloed aangetroffen kunnen worden. Dit was ook het geval.

METINGEN EN RESULTATEN

Bloedlipiden en lipoproteïnen (*hoofdstukken 2 en 3*)

Cholesterol wordt in het bloed getransporteerd door kleine vetbolletjes, lipoproteïnen genaamd. In het bloed komen diverse lipoproteïnen voor. De lipoproteïnen verschillen van elkaar in samenstelling en in dichtheid. Ongeveer 30% van het cholesterol wordt getransporteerd door Hoge Dichtheids Lipoproteïnen (HDL) en ongeveer 65% in de vorm van Lage Dichtheids Lipoproteïnen (LDL). Te hoge concentraties van de cholesterolrijke LDL-deeltjes kunnen indirect hart- en vaatziekten veroorzaken. HDL-deeltjes hebben dit effect niet en lijken juist gunstig om het risico voor hart- en vaatziekten te verminderen.

Zoals verwacht zijn er duidelijke verschillen tussen de verzadigde vetzuren op lipiden en lipoproteïnegehalten. Myristinezuur verhoogt het ongunstige LDL cholesterolgehalte het meest, gevolgd door laurine- en daarna palmitinezuur (zie ook figuur 1, *hoofdstuk 9*). Het mogelijk gunstige HDL cholesterolgehalte wordt alleen verhoogd door laurine- en myristinezuurrijke voedingen, maar niet door palmitinezuurrijkevoedingen. Het is echter nog de vraag of deze door de voeding veroorzaakte verhoging van het HDL cholesterolgehalte ook het risico op hart- en vaatziekten daadwerkelijk verlaagt. De effecten van MCFA zijn verschillend van de overige bestudeerde verzadigde vetzuren. MCFA verhogen het totaal en LDL cholesterolgehalte nauwelijks en het HDL cholesterolgehalte niet ten opzichte van oliezuur.

Vanwege de effecten op het LDL cholesterolgehalte zal een vervanging van vetten rijk aan laurine- en myristinezuur (zoals bijvoorbeeld cocosvet of boter) of palmitinezuur (zoals palmolie, boter, runder- of varkensvet) door oliezuurrijke vetten (zoals olijf-, zonnebloem- of raapzaadolie) gunstige effecten opleveren voor het risico op hart- en vaatziekten.

Gemoedstoestand (*hoofdstuk 4*)

Uit de langdurige epidemiologische onderzoeken blijkt in de categorie mensen met een laag totaal cholesterolgehalte, (gunstig voor het risico op hart- en vaatziekten), de sterfte door oorzaken die niet ziekte gerelateerd zijn hoger te zijn dan bij mensen met een hoger cholesterolgehalte. Niet ziekte gerelateerde sterfte zijn sterften als gevolg van ongevallen, zelfdoding of geweld. Uit onderzoeken blijkt daarnaast dat verlaging van het totaal cholesterolgehalte door medicijnen of dieet, ook stijgingen van de niet-ziekte gerelateerde sterfte teweeg brengt. Een lager cholesterolgehalte zou mogelijk samengaan met meer gevoelens van depressiviteit. Omdat wij in onze studies veranderingen van het cholesterolgehalte verwachtten, hebben we ook verschillende items, met betrekking tot gemoedstoestand, door de deelnemers laten scoren. Wij zagen evenwel geen effect van de verschillende voedingen -en de daarbij horende veranderingen in cholesterolgehalten- op de gemoedstoestand. Dus een richtlijn voor mensen met een normaal cholesterolgehalte om verzadigde vetzuren te vervangen door enkelvoudig onverzadigde vetzuren zal geen negatieve gevolgen hebben voor het gemoed.

Bloedplaatjesaggregatie (hoofdstuk 5)

Bloedplaatjes spelen een grote rol bij de ontwikkeling van een bloedstolsel. Wij hebben geprobeerd met behulp van speciale apparatuur de samenklontering van bloedplaatjes na te bootsen, vlak na de bloedafname. De snelheid van samenklontering van de bloedplaatjes (bloedplaatjesaggregatie) werd daarbij gemeten. Ten opzichte van oliezuur, had geen van de bestudeerde verzadigde vetzuren effect op de snelheid van de bloedplaatjesaggregatie in een bloedmonster.

Factoren betrokken bij de bloedstolling en afbraak van stolsels (hoofdstukken 6 en 7)

In het bloed zijn verschillende eiwitten aanwezig die betrokken zijn bij de bloedstolling. Deze eiwitten zijn in een normale situatie niet actief, en dat is maar goed ook want het bloed moet vloeibaar blijven. De stollingsfactoren komen pas in actie als er bijvoorbeeld een beschadiging in de vaatwand ontstaat. Een teveel aan actie kan echter problemen veroorzaken, een bloedstolsel kan bijvoorbeeld in kleine aderen vast blijven zitten en zo de bloedtoevoer van een gedeelte van het hart afsluiten, met een hartinfarct als gevolg. In het afgenomen bloed bepaalden we de activiteit van verschillende stollingsfactoren, bijvoorbeeld de factor VII activiteit, en hiermee de potentie van het bloed om te stollen. Een verhoogde activiteit van stollingsfactor VII is geassocieerd met een verhoogd risico op hart- en vaatziekten, en met name de ziekten met fatale afloop.

Voedingen rijk aan laurine, myristine en palmitinezuur verhoogden de factor VII activiteit van het bloed in ongeveer dezelfde mate ten opzichte van de oliezuurvoeding. Opmerkelijk daarbij was, dat de stollingsactiviteit van factor VII sterker werd beïnvloed bij de groep vrouwen dan bij de groep mannen (zie figuur 2, hoofdstuk 9). Een verklaring daarvoor zou de verschillende hormonale of genetische achtergrond van vrouwen kunnen zijn of een verschillend samenspel van de verzadigde vetzuren en de genetische of hormonale achtergrond. Hierover is helaas nog weinig bekend. De voedingen rijk aan laurinezuur of myristinezuur hadden ook effect op verschillende remmers voor de bloedstolling. De myristinezuurvoeding verlaagde het TFPI ten opzichte van de oliezuurvoeding en de laurinezuurvoeding verlaagde het antithrombine. De betekenis van deze remming voor het risico op hart- en vaatziekten is ook nog onvoldoende bestudeerd.

De voeding rijk aan palmitinezuur verhoogde de activiteit van plasminogeen activator inhibitor (PAI). Dit is een remmer van het proces van bloedstolsel-afbraak. Een hogere PAI activiteit is geassocieerd met een toegenomen risico op hart- en vaatziekten. Misschien betekent onze bevinding dat de palmitinezuur voeding een verlaagde activiteit oplevert om stolsels weer te kunnen oplossen, maar dit zal nog moeten worden bevestigd met andere metingen van de fibrinolyse. De voeding rijk aan MCFA gaf nauwelijks effecten op de verschillende factoren betrokken bij de stolling en de fibrinolyse.

Eetlust (hoofdstuk 8)

In de tweede studie, die MCFA en myristinezuur onderzocht, hebben we ook de effecten van de verschillende vetten op gevoelens van honger bestudeerd. De deelnemers gaven vlak voor het naar bed gaan op lijstjes aan of ze die dag heel veel of juist heel weinig honger hadden gehad. De hongerscores werden op hetzelfde moment gemeten als de scores voor de gemoedstoestand. Het bleek dat vrijwilligers, die de myristinezuurvoeding consumeerden, gemiddeld meer gevoelens van honger hadden dan de vrijwilligers die de oliezuurvoedingen consumeerden. Dit zou kunnen betekenen dat de mensen die een myristinezuurrijke voeding eten in "normale situatie zonder verdere richtlijnen" misschien meer hadden gegeten vergeleken met mensen die een oliezuurrijke voeding consumeren met wellicht gevolgen voor het gewicht.

CONCLUSIE

Vergeleken met oliezuur hebben de onderzochte individuele verzadigde vetzuren een verschillend effect op het lipidenprofiel van het bloed. Myristinezuur verhoogt het ongunstige LDL cholesterolgehalte het meest, gevolgd door laurinezuur en daarna palmitinezuur. Het potentieel gunstige HDL cholesterolgehalte wordt alleen verhoogd door laurinezuur- en myristinezuurrijke voedingen, maar het is nog de vraag of deze door de voeding veroorzaakte verhoging ook daadwerkelijk het risico op hart- en vaatziekten verlaagt. De effecten van MCFA zijn verschillend van de overige bestudeerde verzadigde vetzuren; ze verhogen het LDL cholesterolgehalte nauwelijks en het HDL cholesterolgehalte niet.

Laurine-, myristine- en palmitinezuur hebben vergelijkbare effecten op de stollingscapaciteit van het bloed (gemeten als factor VII activiteit), alhoewel er kleine nuances zijn in de effecten op andere stollingsfactoren of stollingsremmers. Het lijkt dus nauwelijks zin te hebben, wat de effecten op stollingsactiviteit betreft, onderscheid te maken tussen de verschillende verzadigde vetzuren, behalve voor MCFA en wellicht het niet onderzochte stearinezuur. Opmerkelijk is dat de effecten van de voedingen op de stollingscapaciteit van het bloed sterker zijn bij vrouwen dan bij mannen. Deze bevinding zal verder ondersteund moeten worden met mechanistisch onderzoek en risicoschattingen voor hart- en vaatziekten in vrouwen.

Het risico op hart- en vaatziekten is het hoogst bij mensen met zowel een hoog LDL cholesterolgehalte als een hoge stollingsactiviteit van het bloed. Het verdient daarom aanbeveling vooral het eten van vetten rijk aan laurine en myristinezuur (o.a. cocosvet en boter) en palmitinezuur (o.a. palmolie, boter, runder- of varkensvet) te verminderen. Deze vetten kunnen dan vervangen worden door oliën rijk aan onverzadigde vetzuren, zoals bijvoorbeeld oliën rijk aan oliezuur (o.a. olijf- of raapzaadolie of oliezuurrijke zonnebloemolie). De vervanging zal mogelijk ook gunstige effecten hebben op de eetlust.

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Curriculum vitae

Elisabeth Helena Maria Temme, geboren 10 november 1966 te Heemskerk (Noord Holland), volgde de middelbare school opleiding aan het St. Michael College te Zaandam. Van 1985 tot en met 1991 studeerde zij aan de Landbouwuniversiteit te Wageningen, Voeding van de Mens. Zij verrichtte afstudeeronderzoeken op het gebied van Fysiologie (Vakgroep Fysiologie van Mens en Dier te Wageningen), Voeding (Afdeling Interne Geneeskunde van het Radboudziekenhuis te Nijmegen) en Toxicologie (Vakgroep Toxicologie te Wageningen). Als onderdeel van haar studie deed ze een stage binnen de "Commonwealth, Scientific and Industrial Research Organization" (CSIRO) divisie Humane Voeding. Vanaf april 1992 was zij werkzaam als assistent in opleiding (AIO) op de Vakgroep Humane Biologie van de Faculteit Gezondheidswetenschappen van de Universiteit Maastricht. Zij verrichtte onderzoek in de gezonde mens naar de effecten van vetzuren op risicoindicatoren voor hart- en vaatziekten. Ze presenteerde haar onderzoeksresultaten op internationale congressen te Lugano (Zwitserland), Adelaide (Australië) en Maastricht (Nederland). Zij heeft in 1995 enkele zomercursussen gevolgd van "The New England Epidemiology Institute" te Boston USA. Sinds oktober 1997 is zij tijdelijk medewerkster van het Instituut Voeding (afdeling Epidemiologie) van de Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (TNO) te Zeist.

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