Increased removal of remnants of triglyceride-rich lipoproteins on a diet rich in polyunsaturated fatty acids

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Abstract. We studied the effect of two diets, one rich in polyunsaturated and the other in saturated fatty acids, on the postprandial processing of exogenous and endogenous triglyceride-rich lipoproteins (chylomicrons, very-low-density lipoproteins, and their remnants).

For this purpose, 12 normolipidaemic young volunteers were fed, in a cross-over design of 9 days on each diet, either a diet rich in saturated fat (21% of their daily energy intake from saturated fat, 12% from monounsaturated fat, and 3% from polyunsaturated fat) or a diet rich in polyunsaturated fat (10% saturated fat, 9% monounsaturated fat, and 18% polyunsaturated fat) (P/S ratios 0·14 and 1·8, respectively). On the last day of each dietary period blood samples were drawn six times over a 24-h period for determination, by densitometric scanning of SDS gels, of the diurnal pattern of apoprotein B-48 and B-100 in the $d < 1·019$ g ml^{-1} fractions, as estimates for the processing of chylomicrons and very-low-density lipoproteins.

In addition to the usual decrease in the fasting and diurnal concentrations of total serum cholesterol and of cholesterol in the low-density lipoprotein fractions (between 15 and 21%), the diet rich in polyunsaturated fat resulted in 43% lower daily concentrations of chylomicrons and their remnants. This was due to differences in the clearance rate of chylomicrons and their remnants, rather than to differences in the absorption rate of exogenous fat. In addition, the concentrations of very low density lipoproteins and their remnants during the day were 20% lower on the diet rich in polyunsaturated fat.

As remnants, especially those from intestinal origin, are considered to be atherogenic, our observations provide an additional explanation why atherosclerosis is reduced on diets with a relatively high ratio of polyunsaturated to saturated fat.

Introduction

Studies on the influence of dietary fat composition on the concentration and metabolism of lipoproteins have usually been limited to measurements in the fasting state [1–3]. This approach can be criticized because it ignores the existence of chylomicron remnants, which are usually absent from fasting blood. Chylomicron remnants are formed in the process of catabolism of dietary fat by the action of lipoprotein lipase which hydrolyses the triglycerides in the particles [4–6]. In people eating high-fat diets, chylomicron remnants probably circulate in the plasma for most of the day. The concentration of chylomicrons may influence the clearance of very-low-density lipoproteins (VLDL) and their remnants, because both chylomicrons and VLDL share the same removal mechanism. Animal and cellular experiments indicate that remnants, especially those of exogenous origin, are very atherogenic [7]. This explains why subjects with familial dysbetalipoproteinaemia, characterised by increased remnant concentrations, have premature atherosclerosis despite their low normal concentrations of the atherogenic low-density lipoproteins (LDL) [8]. It has been suggested, that high diurnal levels of chylomicron remnants are responsible for the high prevalence of premature coronary atherosclerotic disease in populations consuming high-fat diets [9]. However, this hypothesis of atherogenesis as a postprandial phenomenon appears to be contradicted by the observation that coronary heart disease is reduced on diets that are low in saturated but high in total fat; the fat consisting largely of (n-6) polyunsaturates [10,11]. A diet rich in polyunsaturated fat has consistently been shown to result in a decrease of the fasting concentrations of cholesterol in LDL [1–3] and less consistently, in VLDL [1–3,12], but effects on chylomicron and VLDL remnants in man have not been reported as yet. This prompted us to delineate the effect of a diet rich in polyunsaturated fat on remnant concentration and

metabolism. The major problem here is to distinguish remnants of chylomicrons made in the intestine from VLDL remnants from endogenous origin. They share an overlapping density range. Therefore, they cannot be separated by ultracentrifugation. However, they can in principle be distinguished by their apoproteins: both chylomicron and VLDL remnants contain a high-molecular-weight protein, called apolipoprotein B (apo B). Apo B from hepatic origin is approximately twice as large as apo B from intestinal origin, which was the reason to call these proteins apo B-100 and apo B-48, respectively [13-15].

In the present study we have measured apo B-48 and apo B-100 by densitometric scanning of sodium dodecyl sulphate–polyacrylamide gels. By this method the effect of a meal on apo B-48 and apo B-100 concentrations in the fraction containing the triglyceride-rich lipoproteins and their remnants from both origins could be followed well; also the influence of a change in the fatty acid composition in the diet on the fate of these particles in the circulation could be established.

Subjects and methods

Subjects

The 12 subjects studied (six males and six females), were students or employees of the University of Nijmegen. They were apparently healthy, as indicated by a medical questionnaire. None had anaemia, glycosuria, proteinuria, or hypertension. Their ages ranged from 21 to 26 years (mean 23 ± 3 years). Mean height was 1.76 ± 0.05 m, body weight 68.7 ± 5.6 kg and body mass index 22.2 ± 1.1 kg m^{-2}. The volunteers had a fixed pattern of daily activities. They did not smoke, and all lived within 2 km of the out-patient clinic in which the experiments were performed. The subjects were not taking medication known to affect plasma

lipids, except for oral contraceptives which were use by four of the women. Their fasting plasma trigl; cerides, plasma cholesterol and lipoprotein concentr; tions were in the usual range for this age grou (Table 1). After the purpose of the study had bee explained to the subjects, they were selected on th basis of their willingness to co-operate and on the apoprotein E phenotype: seven subjects had the apc protein E phenotype E$_{3/3}$, one had E$_{3/2}$ and four had E 3. All subjects gave their informed consent.

Design and diets

The subjects were divided into two groups with simil; initial distributions of serum lipoproteins, apoprotei E phenotypes, energy intake and frequency of use (oral contraceptives and energy intake. Each grou consisted of three males and three females. One grou received the saturated fat diet first for 9 days, and th other group received the polyunsaturated fat diet firs After a 4-week wash-out period with the habitual di the reverse treatments were applied, again for 9 day A period of 4 weeks was chosen to control for the effe of the menstrual cycle in the fertile women studied. I addition, in this way the dietary influence of the h meal could be reproduced because exactly the sam menu was served. In the saturated-fat periods, th basal diet was enriched with butter fat, in the polyur saturated-fat period the basal diet was enriched wit linoleic acid-rich margarine. The composition of th diets consumed by the volunteers in a cross-over desig is shown in Table 2. The volunteers were instructed t prepare their own sandwiches for breakfast and evei ing meals at home using sandwich dressings low in f; plus the fat ingredients supplied by us (butter (margarine, and coffee-creamer containing either satu rated or polyunsaturated fats with the same f; percentages). Each day the volunteers were inte viewed about their adherence to the diet prescription

Table 1. Fasting concentrations of serum lipids, lipoproteins and apoproteins on the final day of the saturated and polyunsaturated fat diets in 12 subjects

	Saturated	Polyunsaturated	Difference (%)
Serum cholesterol	5.03 ± 0.16*	4.29 ± 0.23‡	17.2
triglycerides	1.00 ± 0.09	0.89 ± 0.07	12.3
LDL cholesterol	3.04 ± 0.16	2.52 ± 0.22‡	20.6
HDL cholesterol	1.56 ± 0.09	1.47 ± 0.08	6.1
$d < 1.019$ g ml^{-1} fraction†			
cholesterol	0.41 ± 0.04	0.30 ± 0.03‡	36.7
triglycerides	0.61 ± 0.09	0.53 ± 0.07	15.1
apo B-48	0.37 ± 0.09	0.34 ± 0.07	8.8
apo B-100	4.08 ± 0.37	3.20 ± 0.37‡	27.5

* Mean $\pm$ SD in mmol l^{-1} (except apoprotein B-48 and B-100 in absorbance units $\times 10^3$ and 10^6, respectively).
† Including chylomicrons.
‡ $P < 0.01$ by Wilcoxon's paired test.

Table 2. Mean daily intake of energy and nutrients of subjects on t saturated fat or the polyunsaturated fat diets

Energy/nutrient	Diet	
	Saturated	Polyunsaturate
Energy		
mJ day^{-1}	9.8	9.8
kcal day^{-1}	2400	2400
Protein (%)	15	15
Fat (%)	36	37
saturated fatty acids	21	10
C12:0+C14:0+C16:0		
monounsaturated fatty acids	12	9
polyunsaturated fatty acids	3	18
polyunsaturated/saturated ratio	0.14	1.8
Carbohydrates (%)	48	47
Alcohol (%)	1	1
Cholesterol (mg mJ^{-1})	30	30

They appeared to be highly co-operative. Lunch as a hot meal was prepared by the hospital kitchen, and was based on the lunch served to hospital in-patients on low fat diets. To this meal 30 g of either saturated or polyunsaturated fat was added in the form of a gravy. The daily lunch contained 2800 kJ for each participant and had to be consumed completely. The number of sandwiches allowed was adjusted to meet each participant's energy need. The use of additional food or snacks was strictly limited to two at most per day, to be chosen from a restricted list of foods which did not influence the composition of the diet. Food intake was measured by dietary recall and was converted into nutrients using the National Dutch Nutrient data bank NEVO [16]. The distribution of fat over the day on average was 16% at breakfast, 41% at lunch and 43% at dinner. The meals had to be consumed at fixed time intervals; breakfast between 07.30 and 08.30 hours, lunch at 12.30 hours, and the evening meal between 17.30 and 18.30 hours. The volunteers were instructed to maintain their usual pattern of physical activity, especially on the day preceeding the day of blood sampling. On day 9, breakfast, lunch and dinner were supplied by us and were consumed in the hospital. In order to compare intestinal absorption of dietary fat on both diets 100 000 IU retinyl palmitate (Davitamon A, Organon, Oss, The Netherlands) were added to the lunch and retinyl palmitate was measured in the $d < 1.019$ g ml^{-1} fraction obtained at 15.00 hours. Other blood was sampled at 09.00, 20.00 and 23.00 hours, and at 03.00 and 09.00 hours on the next day. From 20.00 until 03.00 hours the subjects remained in a room under supervision where they passed the time talking and watching video films. Between 03.00 and 08.30 hours on day 10 the subjects slept at their homes; then they returned to the hospital for the final blood sampling. During both dietary periods body weights were recorded daily. Energy intake was adjusted when necessary in order to maintain bodyweight. During both dietary periods, changes in body weight were not significantly different from zero.

Blood sampling, ultracentrifugation, and determination of lipoproteins

Blood was sampled in Vacutainer (Corvac) tubes and was allowed to clot at room temperature for 1 h followed by centrifugation for 15 min at 1200 g. The following preservatives were added to 10 ml of serum: 0.9 mg phenylmethylsulphonylfluoride, 25 μg sodium azide, 2 mg ethylenediamine tetra-acetate, 1 mg gentamycin and 40 μl Trasylol (Bayer, FRG; 10 000 kallikrein units per ml). The serum was stored at 4°C until analysis which was performed within 36 h. In order to recover all triglyceride-rich lipoproteins and their remnants, serum was ultracentrifuged at $d = 1.019$ g ml^{-1} (VLDL + IDL fraction). To reach this, 2.9 ml of serum was mixed with 0.45 ml of an NaCL solution of density $d = 1.10$ g ml^{-1} and the $d < 1.019$ g ml^{-1} was isolated after ultracentrifugation for 16 h at 160 000 g

at 14°C in a Sorvall TFT 45.6 rotor in a Beckman L7-55 ultracentrifuge [17]. This fraction was aspirated in a volume of 1.0 to 1.3 ml. All fractions were subsequently diluted to 1.5 ml with sodium chloride solution, 150 mmol l^{-1}, in order to load similar equivalents of triglyceride-rich lipoproteins from serum onto the gels. HDL cholesterol was determined in whole serum after precipitation of the apoprotein B containing lipoproteins with polyethylene glycol 6000 [18]. LDL cholesterol was calculated by subtraction.

Determination of apo B-48 and apo B-100

Exactly 0.1 ml of the $d < 1.019$ g ml^{-1} fraction, corresponding to the material present in 0.4 ml of serum, was heated for 3 min in boiling water in a solution with the final concentrations of 4% (weight volume) sodium dodecylsulphate, 4% saccharose and 0.8% dithiothreitol. After cooling these samples were loaded onto 3%/4% discontinuous SDS disc gels [19,20]. Electrophoresis was performed at 2 mA per gel for 16 h at room temperature. Protein bands were stained with 0.2% (weight/volume) Coomassie bril-
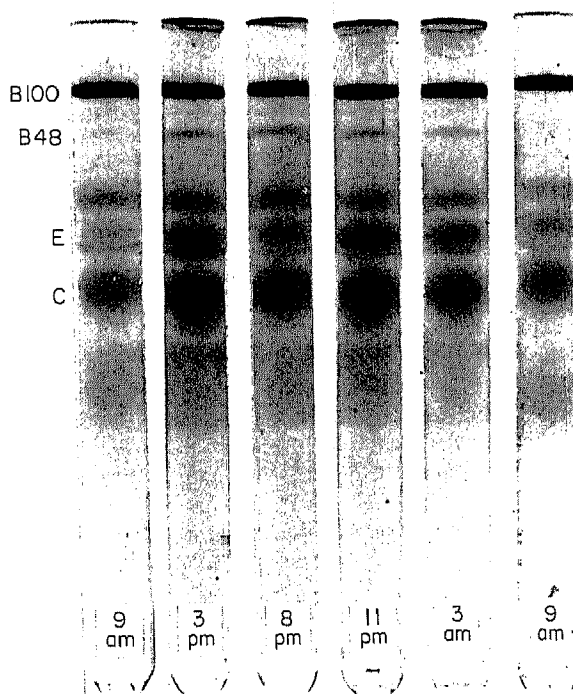

Figure 1. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of apoproteins in the VLDL + IDL fractions ($d < 1.019$ g ml^{-1}) obtained at the indicated times from a volunteer on the last day of the saturated fat period. From top to bottom the gels display proteins with a decrease in molecular weight. Next to apoprotein B-100 and B-48 the apoproteins E and C are also indicated.

liant blue R in methanol/water/acetic acid 36/36/8 (vol/vol/vol) at 60°C. Destaining was performed in the same solution without Coomassie brilliant blue twice for 48 h at 60°C. Gels were scanned with the LKB 2202 Ultroscan laser densitometer. Each gel was measured twice and the mean value was calculated as an expression of absorbance units. The method was tested by loading onto the gels between 2 and 70 μg of a VLDL + IDL ($d < 1\cdot019$ g ml^{-1}) fraction from a normal donor bled 3 h after an oral fat load. The absorbance areas obtained increased steadily with the applied μg of VLDL + IDL protein for apo B-48 from 2 to 70 μg and for apo B-100 from 2 to 50 μg with correlation coefficients of 0·93 and 0·97, respectively ($n = 18$). In view of these results, 30 μg of the $d < 1\cdot019$ g ml^{-1} was loaded onto the gel. A representative series of gels obtained for one subject is shown in fig. 1. All $d < 1\cdot019$ g ml^{-1} fractions of one subject on the saturated fat and one subject on the polyunsaturated fat diet were always analysed within one series. The coefficient of variation of the determination of apo B-100 in this way was 3·1% ($n = 8$) and 6·2% for apo B-48.

Analytical methods

Cholesterol and triglycerides were determined by enzymatic methods (CHOD-PAP reagent no. 237574, Boehringer, Mannheim, FRG; Sera-Pak triglycerides Miles, Milan, Italy) using a centrifugal analyser (Multistat III). The within-run coefficient of variation for control sera was 1·2% for cholesterol and 1·4% for triglycerides. Retinyl palmitate in the $d < 1\cdot019$ g ml^{-1} fraction was determined by means of high-pressure liquid chromatography [21].

One-dimensional isoelectric focusing of VLDL apoproteins and determination of the apo E phenotypes was carried out as previously described [22].

Statistical methods

Differences in the fasting concentrations within subjects were analysed by Wilcoxon's paired test. Diurnal concentrations on both diets were calculated as the area below the curve, and were compared using Wilcoxon's paired test. Differences in results were also
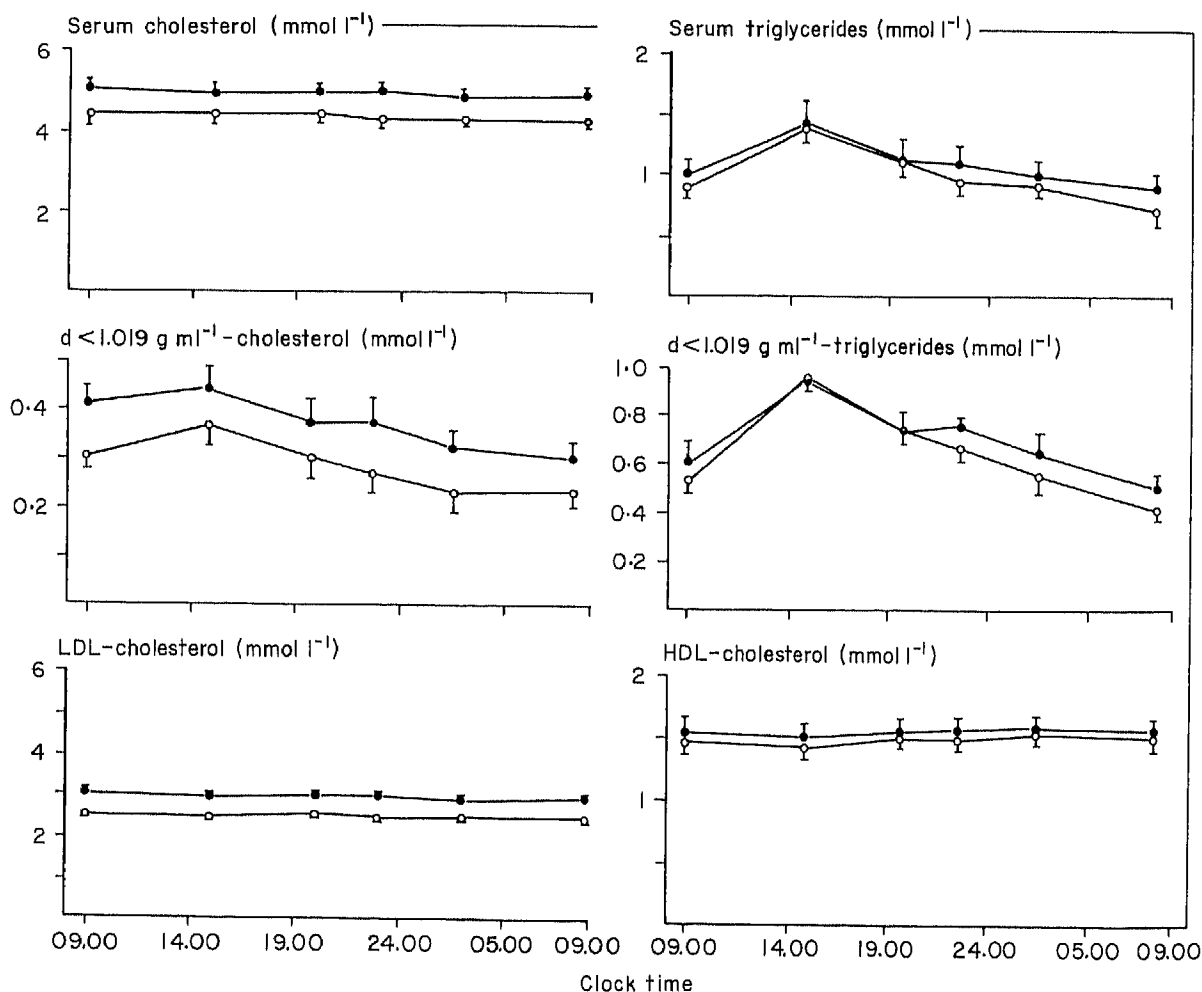

Figure 2. Diurnal variation and the effect of dietary fatty acid composition on the concentrations of serum cholesterol and triglycerides, cholesterol and triglycerides in the $d < 1\cdot019$ g ml^{-1} fractions, and cholesterol in the LDL and HDL fractions. All concentrations (mean ± SEM) were expressed in mmol l^{-1}. o———o Polyunsaturated fat diet; ●———● saturated fat diet.

examined by three-way analysis of variance using the Statistical Analysis System (SAS) General Linear Methods (SAS Institute Incorporated, Cary, NC, USA).

Results

Diet-induced changes in the fasting concentrations of serum lipids, lipoproteins and apoproteins B-48 and B-100

As expected, the diets resulted in significant differences in the fasting concentrations of total cholesterol, of cholesterol and apo B-100 in the $d < 1.019$ g ml^{-1} fraction, and of LDL cholesterol (Table 1). Other fasting values did not differ significantly between diets.

Diurnal variations in the values of serum lipids, lipoproteins and apoproteins B-48 and B-100

Except for serum cholesterol and LDL cholesterol, all serum lipid, lipoprotein and apoprotein levels measured showed a significant diurnal variation as judged by analysis of variance. On both diets triglycerides in serum and in the $d < 1.019$ g ml^{-1} fraction were highest at 14.00 hours (Fig. 2). The rise in triglycerides followed the intake of dietary fat. The rise in the concentration of apo B-48 in the $d < 1.019$ fraction, a marker for chylomicrons and their remnants, continued up until 18.00 hours (Fig. 3). Thereafter, apo B-
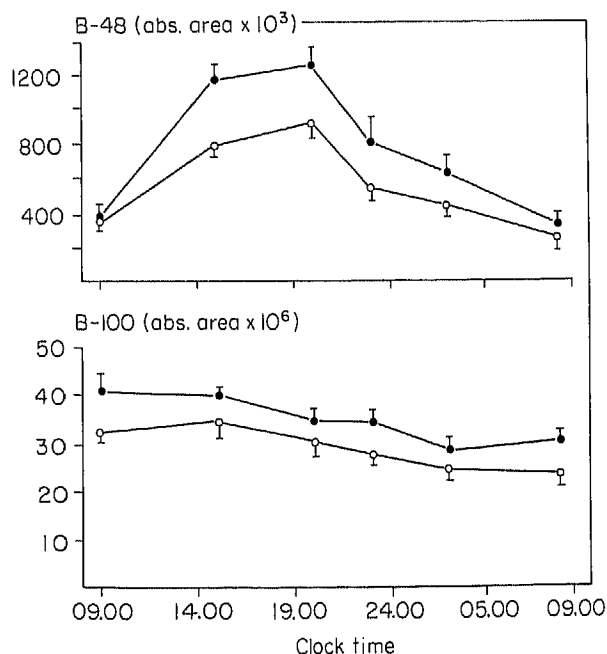
Table 3. Integrated 24-h concentrations of serum lipids, lipoproteins and apoproteins on the final day of the saturated and polyunsaturated fat diets in 12 subjects

| | Area below the curve | | |
| | Difference | | |
	Saturated	Polyunsaturated	(%)
Serum cholesterol	117·9±13·8*	102·3±16·0†	15·2
triglycerides	26·8±9·2	24·5±8·0	9·4
LDL cholesterol	71·2±12·3	59·4±15·5†	19·9
HDL cholesterol	37·8±7·4	36·1±6·8	4·7
$d < 1.019$ g ml^{-1} fraction			
cholesterol	8·9±3·5	6·9±2·6†	29·0
triglycerides	17·3±8·5	16·1±7·8	7·5
apo B-48	2·0±1·1	1·4±0·7*	42·9
apo B-100	82·6±24·7	69·0±22·2*	19·7

* $P < 0.05$, mean±SD in mmol l^{-1} (except for apo B-48 and apo B-100 in area 10^{-7} h^{-1}).
† $P < 0.01$ by Wilcoxon's paired test.

48 returned to basal values. Probably because of a deviation of the normal daily activity between 23.00 and 03.00 hours on the day blood was sampled, concentrations of cholesterol and of apo B-100 in the $d < 1.019$ g ml^{-1} fraction tended to decrease on both diets, especially from 24.00 hours onwards.

Diet-induced differences in the postprandial concentrations of serum lipids, lipoproteins and apoproteins

The integrated 24-h concentrations of apo B-48 were 43% lower on the polyunsaturated fat diet than on the saturated diet, indicating a lower chronic exposure of the arterial wall to chylomicron remnants (Fig. 3). Furthermore, compared with the saturated fat diet, the diet rich in polyunsaturated fat resulted in significant differences in the integrated 24 h concentrations of various other parameters (Table 3). However, most concentrations that changed significantly were already significantly different in the fasting state, as cholesterol in total serum; in the $d < 1.019$ g ml^{-1} fraction and in the LDL fraction, and apo B-100 in the $d < 1.019$ g ml^{-1} fraction.

As apoprotein E₂ has a lower affinity for the apo E receptor than either apoproteins E₃ or E₄ it is relevant to know that the results of the subject with the apoprotein E phenotype E$_{3/2}$ did not deviate from the mean values. The results obtained for this subject were, therefore, included in the statistical analysis.

Comparison of the rate for intestinal absorption of dietary fat using retinyl palmitate as a tracer

Diet-induced differences in apo B-48 could be due to differences either in the rate of intestinal absorption or in the clearance of apo B-48 containing particles from

Figure 3. Diurnal variation and the effect of dietary fatty acid composition on the concentrations of apoprotein B-48 and apoprotein B-100. Values as integrated absorbance area, in thousands or millions respectively, are expressed as means±SEM. ○——○ Polyunsaturated fat diet; ●——● saturated fat diet.

the circulation. This was studied by comparing the influx rates of retinyl palmitate into the circulation on the two diets. From previous experiments it was known that retinyl palmitate concentrations in serum peak 5 h after a fat load [21]. The retinyl palmitate concentrations measured 2 h after consumption of 100000 IU of retinyl palmitate can be considered to reflect largely the rate of absorption rather than the decay rate. The retinyl palmitate concentrations measured at 15.00 h, 2 h after consumption of the fat were $2 \cdot 2 \pm 1 \cdot 5 \, \mu mol \, l^{-1}$ on polyunsaturated fat and $1 \cdot 9 \pm 0 \cdot 8 \, \mu mol \, l^{-1}$ on saturated fat ($P > 0 \cdot 05$). Thus the differences in the 24-h time curve of apo B-48 were most probably due to differences in the clearance rate of chylomicrons and their remnants.

Discussion

In the present study we found that the integrated diurnal concentration of apo B-48 on the polyunsaturated fat diet was considerably lower than on the saturated fat diet. These differences are not likely to be due to differences in the gastrointestinal handling of exogenous lipids, because the absorption of retinyl palmitate, used as a measure for the absorption of dietary fat, was similar on both diets. This is supported by previous findings in man and rat that the absorption of linoleic acid is higher or at least similar to that of other fatty acids [23–26]. Apparently, the clearance of chylomicrons and their remnants is accelerated on a diet rich in polyunsaturated fat compared with a diet rich in saturated fat.

Chylomicron metabolism is considered to occur in two stages. Initially, the particles interact with lipoprotein lipase in extrahepatic tissues [4,5] resulting in triglyceride hydrolysis and delivery of the free fatty acids to the adipose tissues and for approximately 20% of the free fatty acids to the liver [27,28]. After most triglycerides have been hydrolysed remnant particles are formed that are removed from the circulation by hepatocyte receptors that recognize apo E [29–31]. The diet-induced changes in the processing of chylomicrons mentioned above are supported by experiments in animals. Chylomicrons produced on a diet rich in saturated fat appeared to be a poorer substrate for lipoprotein lipase *in vitro* [23,32–34] and were catabolized slower *in vivo* than chylomicrons produced on a diet rich in unsaturated fat [23].

The postprandial rise in triglycerides is thought to be largely from exogenous triglycerides marked by apo B-48 [21]. As the postprandial lipidaemia is inversely related to the HDL concentration [35,36], the diet-induced differences in the clearance of chylomicrons which we observed in our relatively young volunteers may even be larger in a middle-aged population which usually has lower HDL concentrations.

During the postprandial phase the processing of chylomicrons may also influence the metabolism of VLDL, because both particles are hydrolysed by lipoprotein lipase and are internalized in the liver by means of the apoprotein E as a ligand [29,31,37]. If not internalized, VLDL remnants give rise to the production of LDL [38]. The slower clearance of apo B-48-containing particles may result in a delay in the processing of VLDL and their remnants and consequently in an increased fraction converted to LDL. By this mechanism the changes in cholesterol and apo B-100 in the $d < 1 \cdot 019 \, g \, ml^{-1}$ fraction and of cholesterol in the LDL fraction, as observed in our study, can be explained.

The approximately 20% lower LDL cholesterol concentration on the polyunsaturated fat diet observed in this relatively short-lasting study is comparable with values in other studies with a duration of several weeks performed in larger groups of subjects and with a greater variation in age [3,39]. Thus, after 9 days of dieting which, equals approximately five times the half-life of LDL [40], LDL metabolism has probably reached a stationary phase. Therefore, it may be expected that the metabolism of chylomicrons and VLDL with a half life of minutes [41] or several hours [42] has been stabilized as well.

In summary, we found that chylomicron remnants were 43% more rapidly removed on a diet rich in polyunsaturated fat compared with saturated fat. The more rapid processing of chylomicron remnants may also explain the usual observed decreased concentrations of VLDL and of LDL on the polyunsaturated fat diet. Given the fact that chylomicron and VLDL remnants are atherogenic [7,9], and that LDL only becomes atherogenic after chemical or biological modification [43], these findings provide a new explanation as to why coronary heart disease is reduced on diets rich in polyunsaturated fat [10,11,44,45].

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