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Changes in the Triglyceride-rich Lipoproteins and the Response to Dietary Cholesterol in man

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ABSTRACT The effect of a cholesterol-enriched diet was studied in nine healthy volunteers with special emphasis to the changes which occurred in the triglyceride-rich lipoproteins ($d < 1.019$ g/ml).

Compared to the habitual diet, a moderately increased intake of cholesterol (from 300–900 mg/day) resulted in changes of the lipid composition and in a decrease of the apoprotein bands apo B-100, C and E of the $d < 1.019$ g/ml fraction by 25–30%. On the average, the intensity of the apo B-48 band increased. The most consistent was a decrease of cholesterol and triglycerides in the $d < 1.019$ g/ml fraction binding to heparin-Sepharose considered to represent remnants of very low-density lipoproteins.

Low-density lipoprotein cholesterol increased by 0.2 mmol/l (ns). However, three subjects with relatively low concentrations of lipids and apoproteins in the $d < 1.019$ g/ml fraction showed a hyperresponse of LDL-cholesterol by more than 0.5 mmol/l. In these subjects the intensity of the B-48 band did not increase on the cholesterol-enriched diet.

The results suggest, that the rise in LDL-cholesterol caused by dietary cholesterol is mediated by an enhanced uptake of chylomicron- and VLDL-remnants by the liver, followed by a secondary down-regulation of the LDL-receptors.

INTRODUCTION

Dietary cholesterol elevates serum cholesterol and it does so more in some subjects (hyperresponders) than in others (hyporesponders) [1]. The mechanism by which dietary cholesterol affects lipoprotein concentrations and compositions, however, remains obscure. Animal experiments have shown, that in addition to low density lipoproteins (LDL), especially cholesterol and apolipoprotein-E enriched very-low density (VLDL) and high-density lipoproteins (HDL) accumulate in serum in response to cholesterol feeding [2, 3]. In man, similar observations have been reported [4, 5]. Nevertheless, till now attention was primarily focused on possible changes in the metabolism of LDL without realising that changes in the flux of VLDL and IDL (intermediate density lipoproteins) need not necessarily have to result in changes of their concentration [6–8]. However, a change in the flux of VLDL + IDL may have a large impact on LDL concentration, because the metabolism of these lipoproteins is closely linked. In an attempt to elucidate the basis of hyper-responsiveness to dietary cholesterol, we determined VLDL + IDL lipids and apolipoproteins (apo) in nine normolipidemic volunteers on a habitual diet and on a moderately cholesterol-enriched diet. Several of these subjects showed a hyperresponse to dietary cholesterol. Therefore, we related the changes in LDL-cholesterol with their initial and acquired concentration and composition of the VLDL + IDL fraction. Particular attention was paid to changes in the concentration of apo B-48 and apo B-100, which characterise exogenous and endogenous triglyceride-rich lipoproteins respectively and which presumably are removed by distinct hepatic receptors: the apo E or chylomicron-remnant receptor and the apo B/E or LDL receptor, respectively [9].

SUBJECTS, MATERIALS AND METHODS

Subjects and diets

All subjects, three women and six men, were highly motivated students or staff members (age: 35 ± 8 years), who had already participated in several other dietary trials. All were healthy, normolipidemics and had normal body weights. The cholesterol rich diet was composed of natural foodstuffs [1]. Nutrient composition (Table 1) was made similar to the habitually consumed diet, except for the cholesterol intake which was kept at about 900 mg/day compared to a habitual intake of about 300 mg/day. The polyunsaturated/saturated fatty acid ratio was in both diets about 0.4.

Table 1 Mean nutrient intakes on the habitual and cholesterol-rich diets

	Habitual diet	Cholesterol-enriched diet
Energy (MJ/day)	9.7 ± 2.5	11.2 ± 2.2
(Kcal/day)	2.3 ± 0.6	2.7 ± 0.5
Protein (% of energy)	15.5	14.4
Total fat (% of energy)	34.3	32.8
Saturated fatty acids (% of energy)	14.8	14.4
Polyunsaturated fatty acids (% of energy)	5.2	6.0
Carbohydrates % (of energy)	49.2	50.9
Alcohol (% of energy)	2.5	2.0
Cholesterol (mg/MJ)	29	82
(mg/day)	285 ± 111	917 ± 84

Results are given as mean (± SD) for nine subjects. The habitual diet was assessed by the dietary history method in October 1985. The composition of the cholesterol-rich diet is based on the chemical analysis of duplicate diets as supplied during the experiment, plus calculated contents of a small allowance of foods free from fat and cholesterol.

Table 2 Change in serum lipids and lipoproteins by dietary cholesterol

	Habitual diet	Cholesterol-enriched diet
Cholesterol	5.71 ± 0.89	5.81 ± 0.91
Triglycerides	0.96 ± 0.36	0.85 ± 0.36
VLDL + IDL-chol	0.54 ± 0.27	0.41 ± 0.29*
Heparin-unbound-chol	0.10 ± 0.08	0.09 ± 0.03
Heparin-bound-chol	0.45 ± 0.22	0.32 ± 0.14**
Ratio bound/unbound chol	3.8 ± 1.5	3.5 ± 1.6
VLDL + IDL-TG	0.60 ± 0.24	0.50 ± 0.29
Heparin-unbound TG	0.18 ± 0.18	0.14 ± 0.10
Heparin-bound TG	0.39 ± 0.18	0.26 ± 0.13**
Ratio bound/unbound TG	3.5 ± 3.2	2.4 ± 1.6
LDL-chol	3.80 ± 0.76	4.01 ± 0.84
HDL-chol	1.36 ± 0.40	1.40 ± 0.29

Concentration (mean ± SD) in mmol/l in nine volunteers.
*$0.05 < P < 0.1$ (Students' paired t-test)
**$P < 0.05$.

Blood sampling and analysis

Blood samples for biochemical analysis of the $d < 1.019$ g/ml fraction were obtained after an overnight fast of 16 h: on the habitual diet at the day prior to the start of the cholesterol-enriched diet and again 15 days later. To 10 ml of blood the following preservatives were added: 0.9 mg phenylmethylsulfonylchloride, 25 μg Naazide, 2 mg ethylenediaminetetraacetate, 1 mg, gentamycin and 0.1 mg aprotinin (6000 kallikrein inhibitors per mg no 236624, Boehringer, Mannheim, FRG). To reduce interference by biological variation serum lipids and HDL-cholesterol were also determined in one other blood sample taken while on the habitual diet and in blood samples taken while on the cholesterol-enriched diet during 19, 23 and 30 days. These values showed good agreement with those of the selected samples ($p > 0.2$; data not shown).

VLDL + IDL ($d < 1.019$ g/ml) were isolated by ultracentrifugation at 168.000 xg for 16 h [11]. The density $d < 1.019$ g/ml was reached by adding D_2O ($d = 1.10$ g/ml). The VLDL + IDL were fractionated further by heparin-Sepharose column chromatography using as eluens: 0.067 M phosphate buffer, pH 7.4, either with or without 1.0 M NaCl [11]. LDL ($1.019 < d < 1.063$ g/ml) was isolated by density gradient ultracentrifugation using whole serum as starting material [12]. Apoprotein electrophoresis of the VLDL + IDL was performed with 3%/4% sodium dodecylsulfate (SDS) discontinuous gradient gel electrophoresis [13]. The amount of VLDL + IDL loaded onto the gels was similar for all samples and corresponded to the amount present in 0.35 ml of serum. As described [13], the lipoproteins were mixed with SDS and reducing reagent and boiled. This resulted in a quantitative delipidation of apo B-48 and apo B-100 just at the interface between buffer and gel. Apoprotein concentrations were estimated on the basis of the colour intensities of the stained bands measured by densitometry. Due to excess background staining, the intensity of the apo B-48 band could not be measured accurately. Therefore, these bands were judged by visual inspection by two persons. It was assumed, that the binding of Coomassie Brilliant Blue to the various apoproteins is similar. To determine the apo E phenotype, VLDL + IDL were extracted with ethanol-aceton and ether, the apoproteins were separated by isoelectric focusing on 7.5% polyacrylamide gels containing 6.8 M urea and 2% ampholine (LKB, pH 4–6), as described [14].

Total cholesterol, and triglycerides were determined by enzymic methods (Boehringer, Mannheim, FRG cat. no 237574, and Sera PAK, Ames Italy, no 6639, respectively). HDL-cholesterol was determined with a precipitation method [15].

RESULTS

Changes in serum lipids and lipoproteins as induced by the cholesterol-enriched diet

Compared to the habitual diet, the cholesterol-enriched diet on the average did not result in significant changes of total serum cholesterol, triglycerides, VLDL + IDL-triglycerides, LDL-cholesterol and HDL-cholesterol (Table 2 and Fig. 1). However, the concentration of VLDL + IDL-cholesterol on the average tended to decrease by 24%. This was accompanied by a significant decrease of lipoprotein-associated cholesterol binding

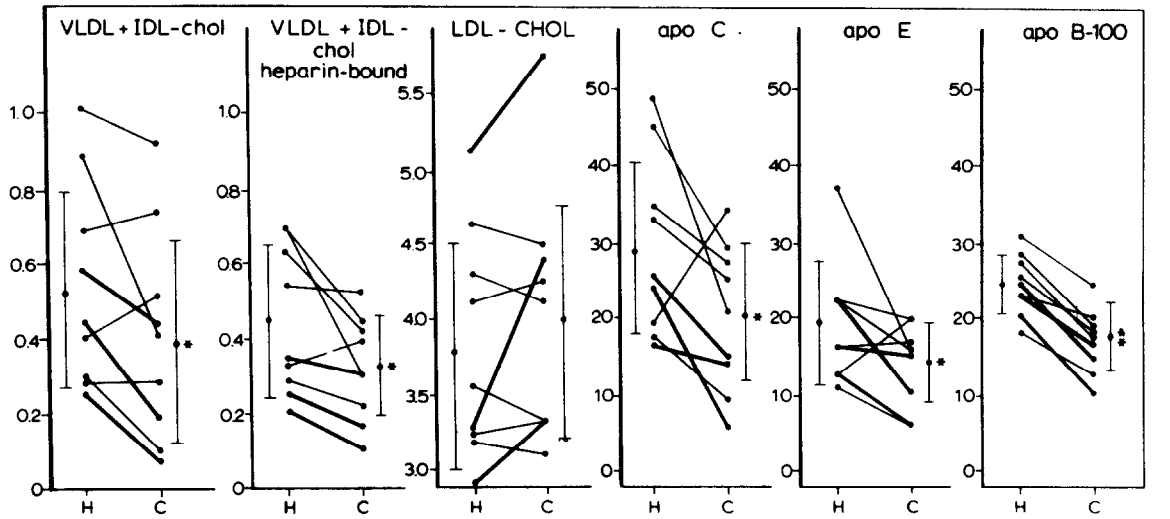

Fig. 1 Effect of the cholesterol enriched diet (C) on various lipoprotein lipids and on the apoproteins in the d < 1.019 g/ml fraction compared to the habitual diet (h). Lipoprotein lipids in mmol/l and apoprotein concentrations in absorbance per protein band after application and electrophoretic analysis of the VLDL + IDL from 0.35 ml of each serum.
The heavy lines represent the effect in the hyperresponders.
*$P < 0.05$ (Students' paired t-test)
**$P < 0.001$.

to heparin-Sepharose (−29%). The cholesterol content in the VLDL + IDL fraction not retained by heparin-Sepharose was low and did not change significantly. By the cholesterol enriched diet, the average concentration of triglycerides in the heparin-bound fraction decreased as well by 33%. On both diets the ratio heparin-Sepharose bound/unbound cholesterol and bound/unbound triglycerides were similar, indicating no significant changes in the binding properties of the d < 1.019 g/ml fraction to heparin-Sepharose. Hence, the number of particles in this fraction must have decreased.

Changes in VLDL + IDL apoproteins by the cholesterol-enriched diet

The intensity of the (VLDL + IDL)-apo C band decreased from 29 000 ± 12 000 (mean ± SD) to 20 300 ± 9600 absorbance units; a decrease of 32.2% ($P < 0.05$). The same was true for the intensities of the apo E and apo B-100 bands which decreased by 26.7 and 29.0% respectively ($P < 0.05$ and < 0.001 respectively) Figs 1 and 2). The ratio E/C which determines the binding of VLDL-remnant particles to heparin-Sepharose [16] and to lipoprotein receptors [17] was on the habitual diet (0.69 ± 0.13) and on the cholesterol-enriched diet (0.73 ± 0.20; 0.05p < 0.1); the ratio apo E + C/B100 and other ratios also did not change by the cholesterol-enriched diet. In five of the nine subjects studied, the intensity of the apo B-48 band increased clearly. In three subjects (numbers 1, 3 and 9) apo B-48 band was diffuse or the intensity of the B-48 band on the cholesterol-enriched diet was similar to that on the habitual diet (Fig. 2). In one subject (number 6), the intensity of the apo B-48 band decreased slightly. Thus, on the average, the intensity of the apo B-48 band increased, indicating an increased concentration of exogenous triglyceride rich lipoproteins.

Hyper-responsiveness to dietary cholesterol

Of the nine volunteers, three subjects (numbers 3, 6 and 9) showed a hyper-response to dietary cholesterol: total serum cholesterol and LDL-cholesterol increased considerably more than in the other subjects (Fig. 1). The increase of LDL-cholesterol amounted to 0.50, 0.62 and 1.19 mmol/l. In these subjects, the decrease on the cholesterol-enriched diet of VLDL + IDL-cholesterol, heparin-bound-cholesterol, apo E, apo C and apo B-100 was similar to the average decrease (Fig. 1). However, on the habitual diet but especially on the cholesterol-enriched diet, the concentrations of VLDL + IDL-cholesterol, heparin-bound-cholesterol and the apoproteins in the VLDL + IDL fraction tended to be lower in these subjects than in the subjects who showed no or only a slight response to dietary cholesterol. Hyper-responsiveness to dietary cholesterol was not related to the apo E phenotype (Fig. 2). In

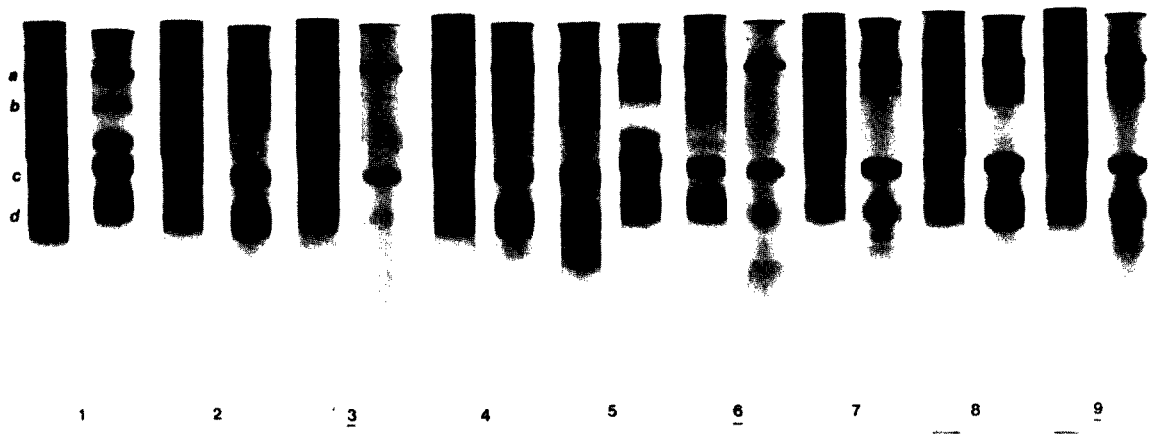

Fig. 2 VLDL + IDL apoprotein composition on the habitual diet and on the cholesterol-enriched diet (left and right of each pair of gels respectively.
a = apo B-100
b = apo B-48
c = apo E
d = apo C
On each gel VLDL + IDL from 0.35 ml of serum was applied, the numbers 3, 6 and 9 represent the hyper-responders.

the hyper-responders, the intensity of the apo B-48 band did not increase on the cholesterol-enriched diet.

DISCUSSION

In this study, we observed that lipids and apoprotein concentrations in the d < 1.019 g/ml fraction decreased by 25 to 30% on a moderately cholesterol-enriched diet (900 mg/day) with otherwise similar nutrient composition compared to the control diet. On the average, LDL-cholesterol remained similar. The concentration of particles binding to heparin-Sepharose decreased as well, indicating a decrease of VLDL-remnants. These data correspond with our earlier observations in which we observed a decrease of IDL-cholesterol by 16 and 5% in two studies on a high-cholesterol diet (1800 mg/day) [18].

The catabolism of VLDL is a stepwise process involving the formation of remnants which may then be either catabolised to form LDL or which are removed directly from the circulation without the formation of LDL [19]. In rats and rabbits, the major part of VLDL-apo B is degraded without conversion to LDL [19, 20]. It is known that in the rat hepatic receptors bind both chylomicron- and VLDL-remnants, generated through the action of lipoprotein lipase, more efficiently as they become poor in C-apoproteins [21]. There is evidence, that it is the chylomicron-remnant or apo E receptor that removes VLDL-remnants [22], rather than the LDL receptor which is thought to be responsible for VLDL remnant removal in rabbits [19] and dogs [23]. In man [24] the relative contribution of the hepatic chylomicron (apo E) receptor versus the LDL (apo B, E) receptor in the uptake of VLDL remnants is variable. Especially the apo receptor has been shown to have a rather broad specificity [24, 25]. There is general agreement, that cholesterol feeding reduces the activity of the apo B/E receptors [26]. At a normal or even an increased production rate as may be expected on a cholesterol-enriched diet VLDL-remnants increase rather than decrease [27, 28]. Therefore, the results suggest that an increased part of VLDL-remnants is cleared by the chylomicron-remnant receptor. This may result in the accumulation of chylomicron-remnants because of competition for binding to the receptor for both types of remnants. Beside upregulation of the apo E receptor, changes in the lipids and apoprotein composition may also increasingly direct the VLDL-remnants to the chylomicronremnant receptor, rather than to the LDL-receptor. It may be expected that particles with an increased affinity for the apo-E receptor are cleared rapidly from the circulation without causing significant changes in the composition of the fasting d < 1.019 g/ml fraction. At an increased production of these particles, the chylomicron remnant receptor-mediated removal of these particles may become saturated, resulting in changes of the composition of the d < 1.019 g/ml fraction due to accumulation of these apo-B-100 containing particles together with apo B-48 containing particles. This may explain the findings of Nestel *et al.* [4] in human volunteers on a high cholesterol diet (about 1700 mg/day).

The hypothesis formulated above is stressed by our observations in the hyper-responders. Those subjects with a rather low concentration of the d < 1.019 g/ml lipids showed a clear increase in the LDL-cholesterol concentration. The intensity of the apo B-48 band was

low and did not increase in these subjects in contrast to the other subjects. Apparently these subjects have a higher capacity for uptake of both types of remnants which ultimately may result in down-regulation of the LDL receptor.

In conclusion we suggest, that hyper- differ from hyporesponders to dietary cholesterol by a generally lower concentration of VLDL-remnants and, upon increased cholesterol intake, show an increased contribution of the apo B, E receptor, rather than the apo E receptor, in the removal of these remnants from the circulation.

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