BIOPHYSICS AND BIOCHEMISTRY

Plasma Content of Cholesterol and Glycerol Alcohols Depends on the Number of Fatty Acid Double Bonds in Lipoprotein Lipid Pool

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The number of the fatty acid double bonds (unsaturation degree) in the plasma lipid pool was evaluated by automated ozone titration. A positive relationship between the content of double bonds, cholesterol, and glycerol was detected. The higher was plasma cholesterol level, the more double bonds contained fatty acids. At cholesterol level of 0.8-18.9 mmol/liter the double bond/cholesterol ratio approached 4.0 and double bond/glycerol ratio was 1.3-1.5. The maximum content of double bonds was detected in LDL. It was hypothesized that the greater part of plasma cholesterol is esterified with arachidonic acid possessing 4 double bonds; three fatty acids are esterified with glycerol, which altogether have 1-3 double bonds. It seems that plasma cholesterol level indirectly but significantly reflects the level of essential polyenic fatty acids, while glycerol reflects the levels of saturated and unsaturated fatty acids. The role of cholesterol in the plasma consists in the participation in transfer of essential fatty acid in the nonpolar form and their absorption by cells. LDL are the main transmitters of essential polyenic fatty acids in the form of cholesterol esters.

Key Words: *cholesterol; glycerol; fatty acids; double bonds; lipoproteins*

Measurement of plasma cholesterol (CH) alcohol is a diagnostic test; its presence in the plasma is the main factor in atherosclerosis development [1] under conditions when CH transfer with low density lipoproteins (LP) from the liver to cells *in vivo* and LP absorption by the cells through receptors is impaired [10]. Cholesterol is synthesized *de novo* from acetate by all live cells *in situ* [8]. All plasma LP contain CH, but it is present in different chemical forms: nonesterified (polar) CH predominates in VLDL, a great part of alcohol is esterified with essential polyenic fatty acids (FA) in the form of nonpolar CH polyesters in LDL [13], while in HDL the greater part of CH is esterified with monoenic endogenous oleic FA in the form of nonpolar CH monoesters and less so in the form of CH polyesters [6].

After hydrolysis of alimentary lipids, enterocytes absorb all FA in the form of nonesterified FA, 2-monoacylglycerides, glycerol alcohol, and only partially cholesterol. Alcohol absorption little depends on its level in the food. Enterocytes resynthesize essential FA into phospholipid composition, while saturated and unsaturated FA are resynthe-

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sized into triglycerides (TG), from which apoA-I then forms HDL and apoB-48 chylomicrons. Enterocytes absorb CH in quantities needed for the formation of polar lipid monolayer (phosphatidylcholine and nonesterified CH) on the chylomicron surface. Enterocytes do not synthesize CH esters and they are present in LP in just trace amounts [5]. Presumably, the main function of CH in LP consists in FA transfer and absorption by cells.

More than 150 FA with different structure, stereochemistry, and physicochemical characteristics in the LP transporting system are the substrate which should be delivered to cells. All classes of plasma lipids are transport forms for FA. All LP classes are lipid-transporting protein macromolecules. Lipid-transporting macromolecules apoA-I, apoB-148, and apoB-100 in association with different numbers of nonpolar lipids have different conformation and expose the ligand domains on LP surface. Cells binding the ligands absorb separately saturated and unsaturated FA and essential polyenic FA [5].

The aim of this study was to clear out the functional role of CH in plasma LP.

MATERIALS AND METHODS

A total of 167 patients with hyperlipidemia and coronary heart disease, familial heterozygotic hypercholesterolemia, with breast cancer and diabetes, and healthy children aged 6-12 years were examined. Summary levels of esterified and nonesterified CH and glycerol - TG, the content of choline base (phosphatidylcholine), and the concentrations of nonesterified CH and glycerol (separately) were evaluated by clinical biochemical methods on a Hitachi-912 autoanalyzer. The level of double bonds in plasma lipid pool after extraction by Folch' method was evaluated by O_3 titration on an ADS-5 double bond analyzer (Russia) [3] (the results were expressed in mmol ozone required for titration). VLDL, LDL, and HDL were isolated by successive ultracentrifugation on an L-50 Beckman centrifuge (40.2 rotor). Individual lipid classes were isolated by thin layer chromatography on plates coated with silica gel (Merck); double bonds were titrated with ozone after spot scraping and extraction after Folch.

Plasma CH level in patients varied from 0.6 to 18.9 mmol/liter, being maximum in familial hypercholesterolemia (12.6±1.6 mmol/liter) and minimum in cancer (1.21±0.13 mmol/liter). The level of TG was the highest in diabetes (2.26±0.20 mmol/liter); choline level in hypertriglyceridemia was 3.13±0.16; the concentration of nonesterified CH was 1.42±0.11 mmol/liter at a total level of 4.49±0.19 mmol/liter. The content of nonesterified glycerol did not surpass 3% of TG level. When estimating the double bonds/CH and double bonds/glycerol (TG) ratios we took into consideration the only double bond of CH alcohol and the esterification of IG with oleic FA with one double bond or of dienic linoleic FA with two double bonds in the second position of TG. Oleic or linoleic FA is esterified in the second position of glycerol (similarly as in TG) in phosphatidylcholine. The higher the content of double bonds (DB) in lipids, the higher is CH level (Fig. 1). The DB to CH ratio was estimated by the formula:

$$DB/CH = \frac{DB-(CS_{total}+TG+cholin)}{CS_{total}-CS_{nonester.}}$$

The double bond to CH ratio was thus estimated as 3.81±0.19. The double bond/CH ratio in volunteers with normal CH and TG levels was 4.46±0.11, in coronary patients it was 5.05±0.12, in females with breast cancer and hypercholesterolemia 4.22±0.16, and in patients with familial hypercholesterolemia 4.42±0.14.

The next step was estimation of the double bonds/glycerol (TG) ratio:

$$DB/glycerol = \frac{DB-(CS_{total}-CS_{ester}\times 3.81+choline)}{TG}$$

The 3.81 value is taken from our estimations. The double bonds/glycerol (TG) ratio was 1.13±0.07 in the total group of patients. In individual groups of patients the double bond to glycerol (TG) ratio increased to 1.49±0.17 in hypertriglyceridemia and was lower in diabetics. On the other hand, we failed to estimate double bonds in the plasma fraction of nonesterified FA.

After isolation of separate LP classes the content of double bonds in VLDL lipids was 32.1±3.6% of their total content in plasma lipids. LDL contained 50.5±3.9% and HDL contained 17.4±1.8% of total content of double bonds. In patients with familial hypercholesterolemia LDL CH contained 66.3±1.47% double bonds, the maximum level being 72% (Fig. 2). The double bond/LDL CH ratio in hypercholesterolemia (16.2 mmol/liter) in these patients was 4.12 (virtually the same as in plasma lipids). The percentage of double bonds in VLDL lipids increased from 32.1±3.6 to 53% in normal plasma TG level, the double bonds/glycerol (TG) ratio remaining unchanged. Isolation of individual lipid classes in volunteers showed that half

of double bonds (49.3±1.9%) were contained in the CH ester fraction, 16.7±2.1% in nonesterified FA, the levels of double bonds in TG and phospholipids being 14.1±1.9 and 19.2±2.1%, respectively. Hence, by using the priority physicochemical methods we evaluated the degree of FA unsaturation, FA distribution in separate lipids, and the lipoprotein lipid ratio in the form of polar and nonpolar lipids.

RESULTS

Our data indicate that plasma CH and glycerol levels are mainly determined by the content and physicochemical properties of FA (number of double bonds, unsaturation degree), which should be delivered to cells. Plasma content of CH and glycerol (TG) is in fact the content of "package material" needed for transformation of saturated and unsaturated FA into nonpolar transporter form of TG and of essential polyenic FA into nonpolar form of CH esters. The HDL then deliver essential FA in the form of phospholipids to cells, VLDL transport saturated and unsaturated FA in the form of TG, while LDL serve as the main transporters of essential polyenic FA in the form of CH esters.

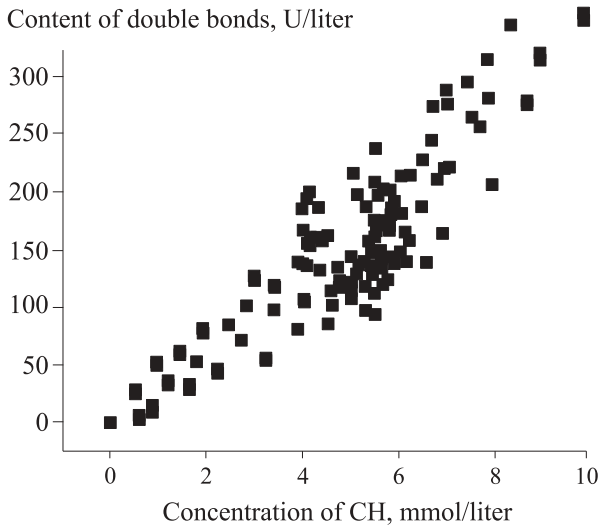

Fig. 1. Relationship between plasma levels of double bonds and CH.

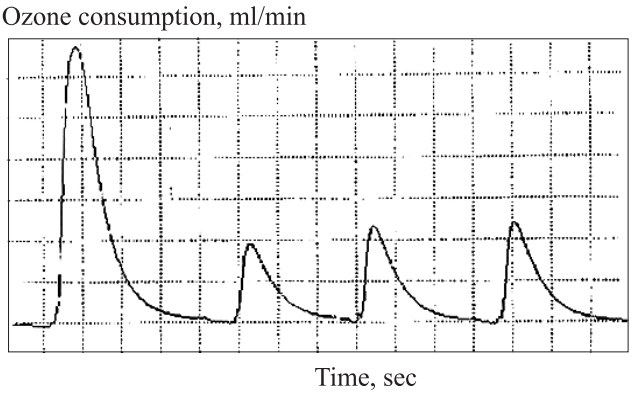

Fig. 2. Kinetic curves of double bond titration in FA of lipids in individual LP classes.

The apoB-100 zero receptor mutation in homozygotic familial hypercholesterolemia blocks cell absorption of essential polyenic FA, esterified by CH into nonpolar esters (but not CH absorption). ApoA-I in HDL binds extracellular CH, esterifies it with endogenous oleic FA into nonpolar transport form of cholesterol oleate. Hence, CH polyesters are a nonpolar transpor form of essential polyenic FA, while CH monoesters (cholesterol oleate) are a nonpolar transport form of CH.

Stable double bond/CH ratio close to 4.0 *in vivo* at all CH concentrations (0.8-16.4 mmol/liter) is due to the transport function of CH in the blood plasma, because cells can actively absorb essential FA by apoB-100 receptor endocytosis only in the form of nonpolar CH esters. In humans C 20:4 arachidonic acid is the main essential FA, which determines the double bond/CH ratio close to 4.0. CH in LDL is completely esterified with essential FA, including C 20:5 eicosapentaenoic and C 22:6 docosahexaenoic FA [11].

Plasma content of CH indirectly reflects the level of essential FA ($p<0.05$). The higher CH level in LDL, the more essential FA cannot be absorbed by cells and the more probable is the development of essential FA deficiency in cells and compensatory intensification of surrogate endogenous FA synthesis. For this reason all clinical manifestations of atherosclerosis are in significant correlation with plasma CH level.

Plasma TG level indirectly reflects the levels of saturated and unsaturated FA ($p<0.05$): the more saturated FA in TG (primarily C 16:0 palmitic FA), the lower the double bond/glycerol ratio and the higher plasma TG level and CH level in VLDL and the lower CH content in HDL. VLDL transport saturated and unsaturated FA to cells. Accumulation of TG in the plasma indicates impairment of absorption of saturated and unsaturated FA with VLDL by cells and possible development of energy substrate deficiency in cells, which would be compensated for at the expense of more intense passive absorption of FA in the form of nonesterified FA from associations with albumin.

Impaired transport of poly-FA with LP and its active absorption by cells through apoB-100 receptor endocytosis underlies the pathogenesis of atherosclerosis [7]. Atherosclerosis is a syndrome of essential polyenic FA deficiency in cells and compensatory production of surrogate dihomo-γ-linoleic FA, from which the cells synthesize pro-

stacyclins, thromboxanes, and leukotrienes. LDL which failed to form the apoB-100 ligand are absorbed by resident macrophages, including those in the arterial intima, forming atheromatosis. The level of CH significantly reflects disorders in active receptor absorption of essential polyenic FA by cells. Fatty acids, lipids, and LP in pathological processes should be regarded as a universal biological transporting system *in vivo*. The double bond/CH ratio close to 4.0 is observed not only in human plasma lipids, but also in mice, rats, dogs, and is presumably a biological constant.

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