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Cholesterol Level in Peripheral Blood Lymphocytes: Relationship with Ischemic Heart Disease in Patients with Various Forms of Hyperlipidemia

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Even though a correlation between the incidence of ischemic heart disease(IHD) and the plasma level of "atherogenic" cholesterol has been demostrated in a number of extensive population studies [1-3], the mechanism underlying this phenomenon remains unclear. It is generally accepted that a rise of plasma cholesterol (Ch) level above 6.5 mmol/liter increases the risk of atherosclerotic plaque formation [4]. Cholesterol accumulation in the vascular intima is due to the transformation of macrophages and smooth muscle cells into foam cells [5,6], which does not reflect the Ch content of other cells in the body. Cholesterol accumulation in tissues may occur without pronounced hypercholesterolemia. Yu. M. Lopukhin has described "cholesterosis" of red blood cells in patients with hypercholesterolemia [7]. However, these non-nucleated cells do not have their system for own controlling Ch balance, and the Ch concentration in their plasma membrane simply reflects the intensity of the concentration gradient transport. The mechanism responsible for the coupling and balance between the Ch content of the vessel wall and other tissues and circulating plasma lipids is unknown.

The lymphocytes circulating in human blood share a considerable number of parameters with vascular endothelium because these cells function at the blood-tissue interface and receive physiological stimuli from the blood and tissues. In addition, peripheral blood lymphocytes are a more convenient tool for biological investigations as compared with fibroblasts and endothelial cells.

This study is an attempt to compare the changes in the Ch content of peripheral blood lymphocytes with plasma concentrations of total Ch, triglycerides and Ch derived from low-density lipoprotein (LDL).

MATERIALS AND METHODS

Eighty-five patients (55 women and 30 men) aged 29-62 years were included in the study. Ischemic heart disease (IHD) with typical clinical signs and electrocardiological symptoms was identified in 66 patients; in 40 of these atherosclerotic lesions of the coronary arteries were documented angiographi-

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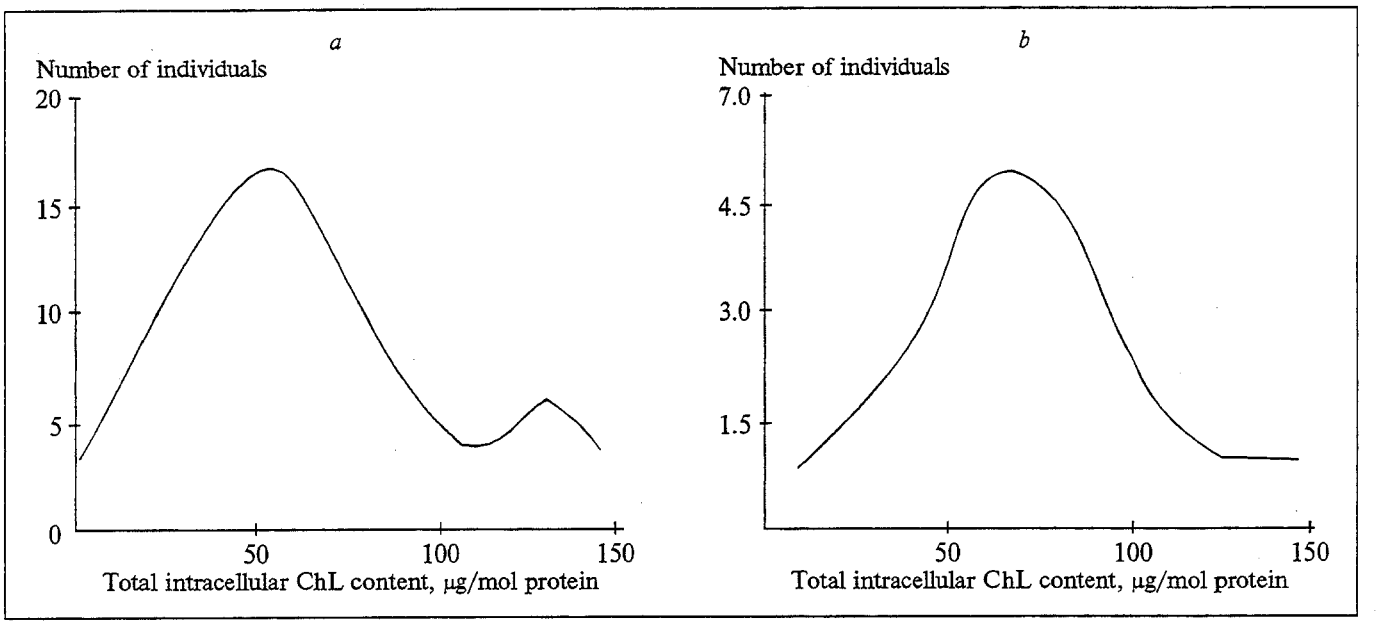

Fig. 1. Distribution of individuals by total intracellular Ch content. *a*) patients with various types of HPL; *b*) control subjects.

cally. The patients were divided into 3 groups according to the type of hyperlipidemia (HLP) defined by the WHO classification [8]. Forty-six patients had type IIa HLP, 23 patients had type IIb HLP, and 15 patients had type IV HLP (Table 1). Eighteen patients with HLP without clinical signs of IHD were enrolled as direct relatives of IHD patients, and in these, type IIa, IIb, and IV HLP was revealed in 9, 5, and 4 individuals, respectively. The control group consisted of 19 essentially healthy subjects aged 30-50 years with normal lipid levels and without clinical or electrocardiological symptoms of IHD. Blood was taken at the stage of remission 2 weeks after discontinuation of all drugs. Blood (25 ml) was collected from the cubital vein in the morning after a 12-hour fast in plastic vials with EDTA (Sigma, USA, final EDTA concentration 0.1 M). Plasma was separated by centrifugation (20 min, 1500 g). Mononuclear cells were isolated by the method of Boyum [9]. Lymphocytes were separated from monocytes by adhesion of the latter on plastic, after which the lymphocytes were lysed with 1% deoxycholate. Cell protein was measured after Lowry [10], total and free Ch, triglycerides, and LDL Ch were measured by spectrophotometry in a Titertek Multiskan device (Titertek, Finland) with the use of Boehringer Mannheim kits (Germany). Total and free intracellular Ch levels were calculated in μg/mg protein, plasma Ch concentration was calculated in mmol/liter. The amount of esterified Ch was calculated by subtracting free Ch from total Ch. The results were statistically processed.

RESULTS

The mean total Ch content of lymphocytes was 68.3±25.3 μg/mg protein and was not significantly

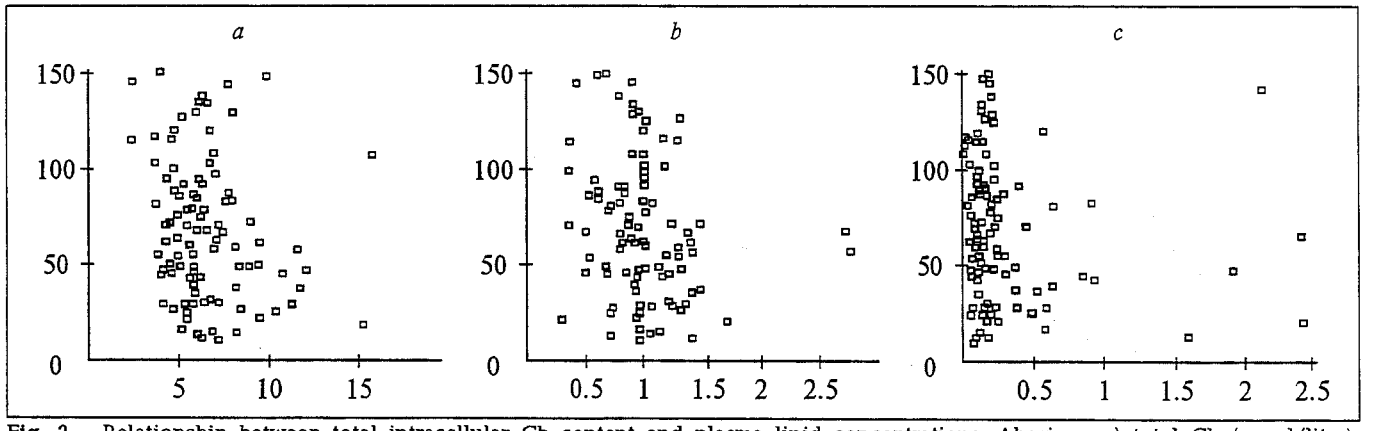

Fig. 2. Relationship between total intracellular Ch content and plasma lipid concentrations. Abscissa: *a*) total Ch (mmol/liter); *b*) LDL–Ch (mmol/liter); *c*) triglycerides (mmol/liter). Ordinate: total intracellular cholesterol content (μg/mg protein).

different in any of the groups examined, the values being 68.4±38.6, 63.4±32.2, 53.6±32.5, and 80.1±20.1 μg/mg protein for groups with type IIa, IIb, and IV HLP and the control group, respectively. Peripheral blood lymphocytes contained about 70% esterified Ch, which is in good agreement with reported data [11]. However, the percent ratio of total and esterified Ch varied considerably from patient to patient even within the same group, while the percentage of esterified Ch was almost the same in all groups: 67±4% (Table 1). Figure 1 shows the distribution of patients and controls by the total intracellular Ch content. The bimodal distribution may be attributed to the specific sample of patients, which does not reflect the general aggregate as a whole. There was no relationship between the total, free, and esterified Ch contents of peripheral blood lymphocytes and the plasma Ch concentration either for all the patients together or in each separate group. Moreover, in all patients these parameters were independent of the plasma triglyceride and LDL Ch levels (Fig. 2). A reverse correlation between the Ch:triglyceride ratio and the total Ch content of lymphocytes was established in patients with triglyceride levels above normal (Fig. 3). It should be mentioned that the presence of individuals without clinical signs and with age differences had no appreciable effect on the situation. A weak direct dependence between intracellular Ch and LDL Ch was revealed in males, while in females this relationship was inverse (Fig. 4). This finding should be further investigated, since the current study included male and female patients aged 29-62 years with normal or somewhat low levels of plasma LDL Ch (Table 1).

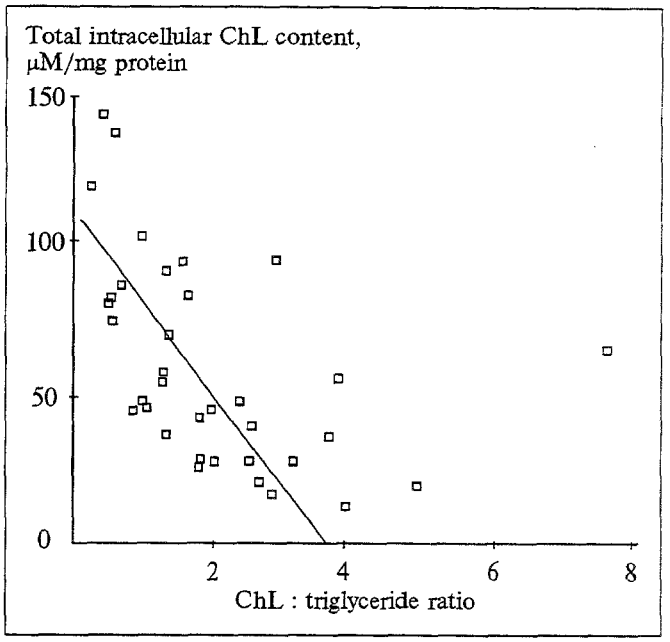

Fig. 3. Relationship between total intracellular Ch content and Ch:triglyceride ratio in hyperlipidemic patients.

Our results may indicate that the Ch content of nucleated cells is not directly related to the plasma Ch concentration. Therefore, the largest possible number of factors that influence lipid transport should be taken into account in the search for indirect relationships between these parameters. Our findings may be useful for individualizing antiatherosclerotic therapy.

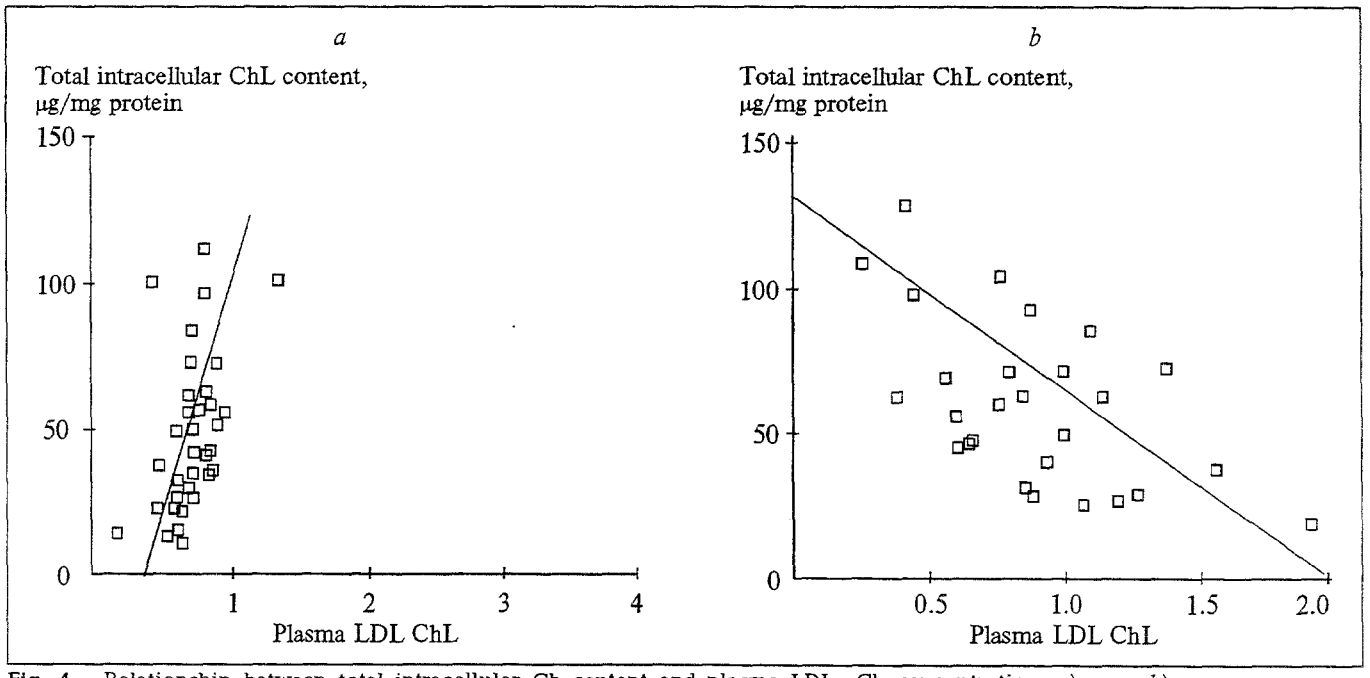

Fig. 4. Relationship between total intracellular Ch content and plasma LDL−Ch concentration. *a*) men; *b*) women.

TABLE 1. Plasma and Intracellular Lipid Contents in Patients with Different Types of Hyperlipidemia

Lipids	HPL type			
	Controls	IIa	IIb	IV
Plasma, mmol/liter				
Total ChL	4.8±0.6	6.9±1.9	4.7±1.2	7.3±1.6
Free ChL	1.6±0.2	2.07±0.3	2.1±0.3	2.8±0.6
Esterified ChL	3.5±0.4	4.8±0.9	2.6±0.9	4.4±1.4
Triglycerides	1.1±0.2	1.46±0.2	2.6±0.9	9.5±3.1
LDL ChL	1.1±0.2	1.05±0.15	1.1±0.15	1.1±0.2
Cells, μg/mg protein				
Total ChL	80.1±26.0	68.4±38.6	63.4±32.2	53.6±32.5
Esterified ChL	52.3±24.1	53.7±25.6	41.5±18.2	32.5±23.5
Total number of examines	19	46	23	15
Of these, number after myocardial infarction	—	27	6	2

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