QUANTITY OF CHOLESTEROL EXTRACTED FROM THE HUMAN SKIN SURFACE: A POSSIBLE DISCRIMINANT OF ATHEROSCLEROSIS?

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Despite progress in the study of atherosclerosis, much attention is being paid, as before, to the search for its diagnostic screening. The most widely used approach so far has been to characterize the lipoprotein (LP) system of the blood plasma, including lipid and/or protein parameters [1]. However, these data, revealing the principal risk factor of atherosclerosis, namely dyslipoproteinemia (DLP), give information more relevant to predicting the development of atherosclerosis than to the degree of its formation by the time of testing. For that reason a number of workers have tried to obtain indirect information about the state of the aorta, proceeding in a different manner, namely by studying the cholesterol (Chs) concentration and, in some cases, the apoprotein B concentration also, in biopsy material from the skin. This course was preceded by a series of morphological and biochemical investigations, which revealed a parallel between damage to the aorta and Chs and apo-B accumulation in human skin. It has been shown that whereas healthy human skin contains from 1.4 to 7.2 μg Chs/mg dry weight of tissue [3, 7-10], in patients with atherosclerosis, as a rule the Chs content is on average 50% higher [7-10, 13]. According to one opinion [7] the diagnostic informativeness of this parameter is higher than that of traditional blood plasma levels [1]. An obstacle to the widespread use of this approach still remains the traumatic nature of biopsy. This can be avoided in a quantitative colorimetric diagnostic test recently developed, which uses the color reaction of specific reagents, sensitive to the Chs concentration, applied to the skin surface [2]. Under these circumstances, different stains of a reagent on the skin of healthy human subjects and patients with atherosclerosis [2] show that differences in the Chs level of skin biopsy specimens [7-10, 13] are a feature also of the superficial layer of the epidermis. However, there are no direct quantitative data in the literature on the Chs content in the surface layers of the skin, accessible by a noninvasive procedure, either in healthy individuals or in patients with atherosclerosis. Meanwhile, an answer to this question could not only give information on the distribution of cutaneous Chs, but could also provide a basis for the development of quantitative diagnostic tests.

In the investigation described below the quantity of cholesterol extractable from the upper layers of the skin, calculated per square centimeter, was compared after superficial extraction with a mixture of organic solvents. Optimal conditions of extraction were found and differences discovered between the level of extractable Chs in healthy individuals and patients with atherosclerosis. The results indicate that determination of the quantity of Chs extractable from the skin can be used as an informative, simple, and painlessly determined discriminant of atherosclerosis, for which purpose a comparison was drawn with the traditional lipid parameters of the blood plasma.

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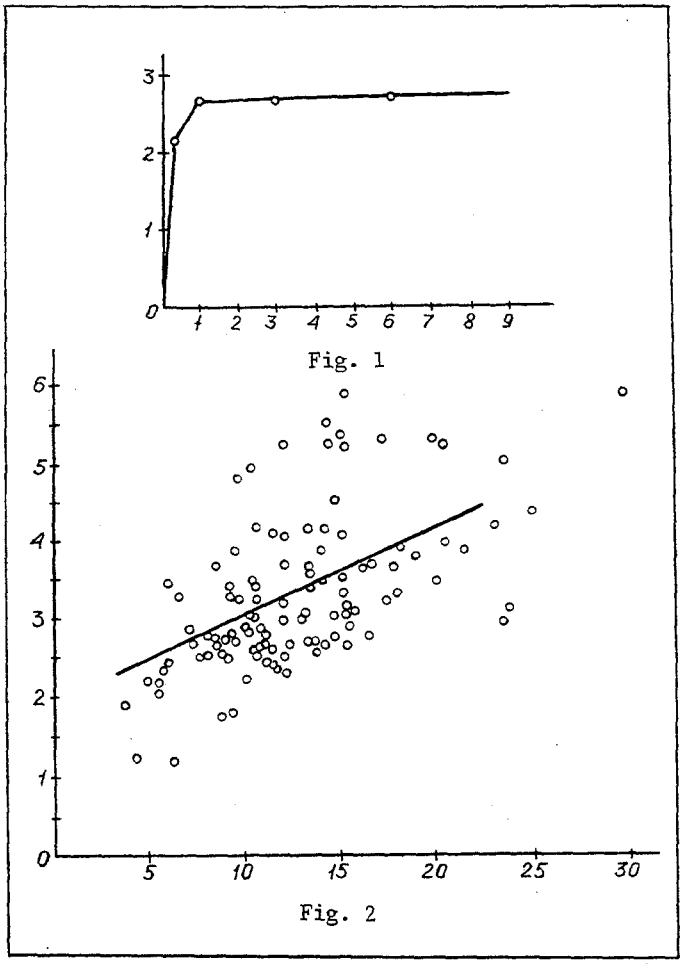

Fig. 1. Quantity of surface-extractable cutaneous cholesterol as a function of extraction time. Abscissa, time (in min); ordinate, quantity of extractable cholesterol (in $\mu g/cm^2$ skin surface).

Fig. 2. Cholesterol content of skin surface ("coefficient of dyslipoproteinemia" – Chs + TG)/α-Chs. Abscissa, coefficient (Chs + TG)/α-Chs (in relative units); ordinate, quantity of extractable cholesterol (in $\mu g/cm^2$ skin surface).

EXPERIMENTAL METHOD

Altogether 20 healthy individuals and 92 patients with atherosclerosis were investigated. The diagnosis was based on the results of an electrocardiographic study, loading tests, and a history of previous myocardial infarction. The levels of Chs, triglycerides, and α-cholesterol in the blood plasma were determined by enzyme kits from "Boehringer Mannheim" and on a Centrifichem-400 automatic analyzer. The control for lipid analysis was carried out in the lipid investigation standardization laboratory of the All-Union Research Center for Public Health, Academy of Medical Sciences of the USSR. Lipids were extracted from the skin with a mixture of ethyl alcohol and diethyl ether, in the ratio of 3:1 by volume. For this purpose, a tube with 2 ml of the above mixture was held firmly pressed against the palm for 0.5-9 min. The quantity of cholesterol in the extracts was determined by a modified method in [5]. The results were subjected to statistical analysis by Student's test and by regression analysis.

TABLE 1. Quantity of Cholesterol Extracted from Skin of Normal Individuals and Patients with Atherosclerosis, during External Treatment

Subjects	Number of cases	Quantity of cholesterol, $\mu g/cm^2$	p
Normal individuals	20	2,16±0,17	—
Patients with atherosclerosis	92	3,04±0,19	<0,05

TABLE 2. Quantity of Choelsterol Extractable from Skin Surface of Persons with Different Values of the Coefficient (Chs−α-Chs)/α-Chs

	Values of (XC − α-XC)/α-XC				
	3,5	3,5—5	5—7	7—10	>10
Number of cases	10	5	28	10	8
Skin cholesterol ($\mu g/cm^2$)	2,02 ±0,09	2,94 ±0,12	3,01 ±0,14	3,12 ±0,12	4,10 ±0,15

EXPERIMENTAL RESULTS

Comparison of the quantity of Chs extractable from the skin surface depending on the length of exposure (Fig. 1) showed that extraction reaches a maximum after only 2-3 min. Consequently, the optimal time for analysis is 3 min. In different people, extraction of cholesterol ranged from 1.5 to 5 $\mu g/cm^2$.

Data on the cutaneous Chs level in the literature relate only to biopsy material: its content was calculated only per mass of tissue [7-10], making comparison with the results of the present study difficult. Conversion, however, taking into account information on the area of the biopsy specimens taken (17-18 mg dry mass/cm^2) [10]) gives values of 24-32.5 $\mu g/cm^2$ [13, 15], or 60-82 $\mu g/cm^2$ [6-10, 12, 14], i.e., 10 times higher than values obtained in the present study. Hence it can be suggested that under our conditions of extraction, only lipids from the surface layer of the epidermis, perhaps the stratum granulosum, were extracted [15].

This is confirmed by calculation on the basis of the results in [11] of lipids extracted by skin surface treatment with hexane. For the skin in different parts of the body the amount of lipids varied from 19 to 59 $\mu g/cm^2$, including 4-17% of free and esterified Chs, i.e., the quantity of Chs was between 1.3 and 6.6 $\mu g/cm^2$, close to the value which we ourselves obtained.

The absence of an increase in the quantity of cholesterol with an increase in the extraction time indicates the constancy of this parameter. This is confirmed by the low value of the coefficient of variation (5%) during three-fourfold repetition of the analysis. Sensitivity depends on the method used to analyze the quantity of cholesterol in the extract, and in this case it was 0.1 μg, whereas the accuracy of the value determined was 1.6-5%. These characteristics may be improved by the use of an enzymic micromethod or of gas−liquid chromatography.

Table 1 gives the results of determination of Chs extractable from the skin of normal individuals and patients with IHD.

As Table 1 shows, the mean value of this parameter in patients with atherosclerosis (3.04 ± 0.1 $\mu g/cm^2$) was higher than in healthy individuals (2.16 ± 0.05 $\mu g/cm^2$). Consequently, accumulation of Chs is observed in atherosclerosis in the surface layer of skin, accessible for external extraction, just as also in biopsy specimens [7-10].

Possible correlation between the Chs level in the skin surface and parameters of the plasma lipoprotein system was analyzed. Under these circumstances (just as for Chs in biopsy specimens [7]), no correlation was found either with the Chs level, triglycerides (TG), or α-Chs, No correlation likewise was found with the cholesterol coefficient of atherogenicity (Chs-α-Chs)/α-Chs [1], by contrast with the results for Chs in the erythrocytes [4]. However, subdivision of the subjects into five groups corresponding with the values of this coefficient (Table 2) showed no difference in the skin cholesterol level over a wide range of its values: it varied from 2.96 to 3.12 $\mu g/cm^2$. Subjects with values of (Chs-α-Chs)]/α-Chs < 3.5 (the lower limit of normal), from whom only 2.02 ± 0.9 $\mu g/cm^2$ was extracted, and subjects with values of the coefficient of atherogenicity above 10, i.e., with the most severe DLP, whose cutaneous Chs level was 4.1 ± 0.15 $\mu g/cm^2$, constituted the exceptions.

We also analyzed the effect of another plasma lipoprotein coefficient, in which the two "atherogenic" parameters (Chs + TG) form the numerator and α-cholesterol forms the denominator, namely (Chs + TG)/α-Chs, on the cutaneous Chs level (Fig. 2).

Under these circumstances positive correlation ($r = 0.54$, $p < 0.001$) was found between the surface cutaneous Chs level and the suggested coefficient within the range of values of the latter up to 10, and weaker, but still significant correlation ($r = 0.48$, $p < 0.001$) up to the value of 17. At higher values of this coefficient, correlation was not present. Regression analysis in order to discover dependence of cutaneous Chs (y) on the coefficient (Chs + TG)/α-Chs (x) yielded a regression line with an angle of slope ($b = 0.12$), significantly different from zero ($p < 0.001$). Dependence of the surface cutaneous Chs content on the selected coefficient (which can be conventionally called the "coefficient of dyslipoproteinemia") evidently reflects the additive effect of the atherogenic DLP, leading to rapid accumulation of Chs in peripheral tissues. The absence of any further increase in Chs is probably due to saturation of the skin tissue, arising as a result of its specific properties.

A difference in the content of Chs in the surface skin of patients with IHD and normal individuals was thus demonstrated: the only lipoprotein parameter of the plasma which correlates with this value was the ratio (Chs + TG)/α-Chs. The results can be interpreted from the standpoint of future development of a new diagnostic screening test.

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