

CYCLIC CHANGES IN BLOOD SERUM CHOLESTEROL AND PROTEIN CONCENTRATIONS IN THE COURSE OF HYPERCHOLESTEROLEMIA

G. Kh. Bozhko, V. M. Kulabukhov, and P. V. Voloshin

UDC 577.1:616.153.922:616.153.96

KEY WORDS: cholesterol; protein; hypercholesterolemia

Cholesterol is an essential component of biological structures, but its excessive accumulation in the animal body often leads to disturbances of the arteries (atherosclerosis) and to the development of other pathological states. To study the problem of the counteraction of atherogenesis, it is important to note that hypercholesterolemia develops irregularly, and that a relative fall of the cholesterol level is accompanied by temporary reduction of morphological disturbances in the vessels [5]. This phenomenon evidently reflects activation of hypocholesterolemic mechanisms [2]. Successful treatment, therefore, can be achieved if it is applied during periods corresponding to phases of relative hypocholesterolemia and hypocholesterolemia, so that the intrinsic adaptive metabolic processes of the body can be stimulated [3]. A central place in cholesterol metabolism is occupied by the liver cells, where the main mass of the blood serum proteins (BSP) also are synthesized. The risk of development of atherosclerotic changes is thus closely linked with disturbances of not only lipid, but also protein metabolism [8].

We investigated the cholesterol and BSP concentrations for 7 months in a model of hypercholesterolemia, formed by adding stearin to the animals' diet.

EXPERIMENTAL METHOD

The experimental animals were 24 male rabbits (3 kg) and 60 guinea pigs (0.4 kg). Half of the animals received cholesterol daily in the diet in a dose of 0.5g/kg body weight: rabbits for 210 days, guinea pigs for 119 days. The remaining animals were controls. Concentrations of cholesterol, total BSP, and the fraction isolated by acid treatment of the serum, were analyzed every week for 35 days, and again after 63, 91, 147, and 210 days in the control and experimental animals. No statistically significant differences were found in the parameters studied in the control animals before and during the experiment. Blood for obtaining serum was collected from the marginal vein of the rabbit's ear under local anesthesia, and from guinea pigs, from a forelimb vein, under slight negative pressure. Serum was obtained from the blood by centrifugation and the cholesterol concentration was recorded [6]. Concentrations of protein were determined by a modified Lowry's method after their precipitation by trichloroacetic acid [7]. Acid-soluble proteins were extracted from the residue with 0.2 N sulfuric acid (10:1). The components of this fraction migrated in an electric field during electrophoresis under acid conditions (pH 2.8) toward the cathode, and they were accordingly called cationic BSP [6]. The results were analyzed by traditional statistical methods.

EXPERIMENTAL RESULTS

The results given in Fig. 1 show that the curve describing changes in the blood serum cholesterol concentration of the rabbits over a period of 30 weeks can be divided into two distinct parts. In the initial period of hypercholesterolemia (up to and including 4 weeks) a sharp rise (by 4.7 times toward the end of the first week) and temporary stabilization of the cholesterol level during the next two weeks were observed. The cholesterol concentration then decreased compared with the

Laboratory of Biochemistry, Research Institute of Neurology and Psychiatry, Khar'kov. (Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Korzh.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 113, No. 3, pp. 259-261, March, 1992. Original article submitted July 22, 1981.

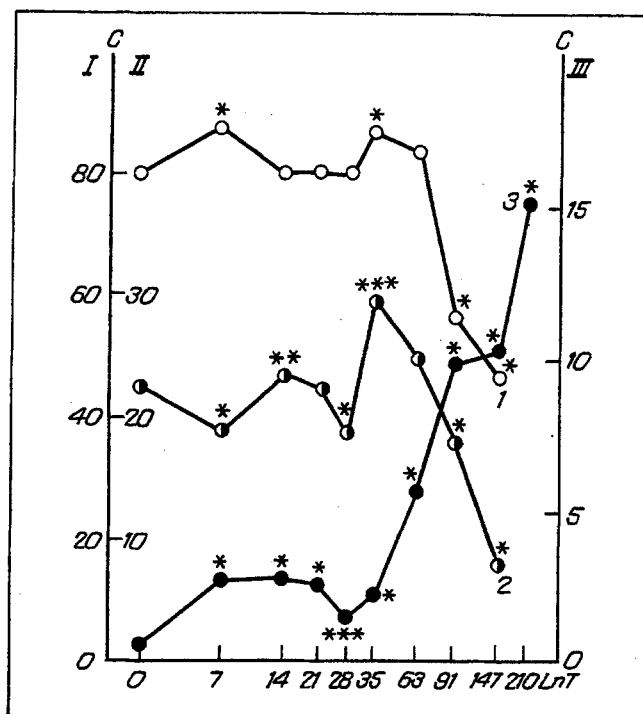


Fig. 1. Blood serum protein and cholesterol concentrations in rabbits in early and late stages of hypercholesterolemia. Abscissa, natural logarithm of time (days); ordinate, concentration (in mg/ml): III) cholesterol, I) total proteins, II) cationic proteins. 1) Total blood serum proteins, 2) cationic blood serum proteins, 3) cholesterol. *) Change significant compared with control, **) the same, compared with previous time of investigation.

previous time of investigation, although it still remained higher than in the control. The subsequent period of hypercholesterolemia (4-30 weeks) was characterized by a virtually linear increase in cholesterol concentration, excluding the period of time between the 13th and 21st weeks, when the rate of sterol accumulation was somewhat slower.

The BSP concentration rose after 7 days of hypercholesterolemia, but was quickly restored, and thereafter it remained unchanged for 2 weeks. After 5 weeks of the experiments the BSP concentration began to fall. This tendency was subsequently sharply intensified: by the 21st week of hypercholesterolemia the BSP concentration was only 61% of the control value.

Changes in the concentration of cationic BSP in the initial period of hypercholesterolemia were characterized by a more complex dependence on time. In the first week of hypercholesterolemia, by contrast with the total fraction, it showed a decrease. During the second week, compared with the previous period its value rose, and then again fell 4 weeks after the beginning of the experiments. During prolonged hypercholesterolemia, in the interval between the 4th and 21st weeks, dependence of changes in the cationic protein level on time resembled that of the total fraction. In the interval between the 4th and 5th weeks an increase in the protein concentration was observed, to be followed by a decrease in their concentration. The extremely low content of cationic proteins at the end of the experiments will be noted: only 37% compared with the control.

Thus differences in the time course of cholesterol are reflected in quantitative changes in BSP in the early and late stages of hypercholesterolemia. It can be concluded from the results given in Fig. 1 that after 5 weeks from the beginning of the experiment, the sharp increase in the cholesterol level correlated with an equally marked decrease in the content of total and cationic BSP. This correlation persists for a long time — for 16 weeks.

TABLE 1. Blood Serum Total Protein and Cholesterol Concentrations (mg/ml) in Guinea Pigs with Hypercholesterolemia

Substance	Time of investigation, days				
	control	35	63	91	119
Cholesterol	0.39±0.01	2.02±0.28*	2.09±0.32*	1.45±0.09*	0.69±0.06***
Protein	52.9±1.1	60.4±2.1*	53.9±1.7	53.5±1.0	57.8±1.1***

Legend. *) Change significant compared with control, **) the same, compared with previous periods of investigation.

The results suggest interaction between the metabolic systems of the animal which are involved in conversion of cholesterol and BSP. The mechanisms of this suggested interaction are unknown, and it is therefore difficult to judge their pause—effect relations. It is worth noting, however, that quantitative changes in BSP cannot be determined by the protein components of lipoproteins or lipid-carrier proteins, for their contribution to the total BSP is only a few per cent.

The data given in Table 1 show that in guinea pigs, as a result of the addition of cholesterol to the diet, just as in rabbits its blood serum level first rises. Later, during the next 8 weeks, no statistically significant deviations are observed. In this time interval, changes in the BSP concentration also are absent. By the 17th day of the experiment the cholesterol concentration is significantly reduced compared with its highest value and the value observed in the previous time interval. The time course of cholesterol in this period of investigation in guinea pigs thus resembles the development of the initial stages of hypercholesterolemia in rabbits. The BSP concentration at this time, on the other hand, rose compared with the control and with the preceding period of investigation. Similar changes in the concentration of cholesterol and BSP were evoked by discontinuation of cholesterol administration with the food, or its reduction to half the amount, after 3 months of hypercholesterolemia. Under these circumstances the cholesterol concentration fell by 48% in the course of a few days, whereas BSP rose by 38% [4]. In patients with atherosclerosis the opposite relationship is observed between the severity of the pathological process, correlating with the blood plasma cholesterol level and the content of cationic BSP [7]. Injection of exogenous proteins into the animals against the background of prolonged hypercholesterolemia is accompanied by a decrease in the cholesterol concentration and an increase in the relative BSP concentration [2].

The results are evidence of cyclic changes in the cholesterol and BSP concentrations in the course of hypercholesterolemia. The relative decrease in the cholesterol concentration in rabbits, and its stabilization in guinea pigs, in the initial period of hypercholesterolemia is accompanied by a transient rise of the BSP level. This suggests that the mechanisms preventing the development of hypercholesterolemia may include stimulation of synthesis of BSP.

LITERATURE CITED

1. G. Kh. Bozhko, P. V. Voloshin, V. M. Kulabukhov, et al., Zh. Éksp. Klin. Med., No. 5, 466 (1988).
2. G. Kh. Bozhko, T. P. Boiko, P. V. Voloshin, et al., Dokl. Akad. Nauk Ukr. SSSR, Ser. No. 5, 61.
3. G. Kh. Bozhko, T. P. Boiko, L. S. Kostyukovskaya, et al., Byull. Éksp. Biol. Med., No. 7, 40 (1990).
4. G. Kh. Bozhko, L. S. Kostyukovskaya, V. M. Kulabukhov, et al., Ukr. Biokhim. Zh., No. 5, 25 (1990).
5. V. V. Dolgov, T. A. Voino-Yasenetskaya, and V. S. Repin, Atherosclerosis in Man [in Russian], Moscow (1989), p. 55.
6. V. M. Kulabukhov, P. V. Voloshin, L. S. Kostyukovskaya, et al., Ukr. Biokhim. Zh., No. 2, 27 (1987).
7. G. L. Miller, Analyt. Chem., No. 5, 964 (1959).
8. L. Wendt, Raum und Zeit, No. 40, 12 (1989).