

## Comparison of the Rates of Changes in the Lipid Spectrum of Human Blood Serum at Moderate Altitudes

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**Abstract**—Antiatherogenic changes in the lipid spectrum of blood serum under various conditions, such as mountain and climate therapy in Caucasian resorts at moderate altitudes, interval hypobaric therapy, and trekking at moderate altitude, combined with the regular application of cold and the performance of special exercises increasing cold resistance, based on Tibetan Tummo Yoga, have been studied. Using an exponential model, the rates of changes in the total cholesterol, high- and low-density lipoproteins (HDL and LDL, respectively), very low-density lipoproteins (VLDL), and triglycerides in blood serum were compared. Application of a new calculation algorithm allowed demonstrating a higher rate of antiatherogenic changes in the lipid spectrum of the blood, such as a decrease in total cholesterol and LDL and an increase in HDL contents after the use of a combination of altitude hypoxia, moderate physical training, and special exercises to raise resistance to cold temperatures (Tibetan Tummo Yoga).

**Keywords:** lipid spectrum, atherogenicity index, altitude hypoxia, cold resistance

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In the present study, we extend the results of our previous experiments [1, 2], in which we studied the lipoprotein spectrum of blood serum under high-altitude conditions, combined with the regular performance of special exercises to increase the body's resistance to cold temperatures, analogous to those included in Tibetan Tummo Yoga [3].

It has been shown in several studies [4–16] that climbing a mountain changes the lipid spectrum of the human blood. Researchers focused their studies on antiatherogenic changes in the lipid spectrum, including a decrease in the total cholesterol (TC) content and an increase in the level of high-density lipoproteins (HDL) in persons living under moderate altitude conditions [4, 5], because this allows using the climate in the mountains and altitude hypoxia as tools for treating dyslipoproteinemia and prophylaxis, as well as sanatorium/resort therapy of atherosclerotic alterations of arteries [7, 12–16]. Unfortunately, the antiatherogenic effects of a mountainous climate and altitudinal hypoxia on the blood serum lipid spectrum are not supported by control case studies. Austrian researchers did not find antiatherogenic changes in the lipid spectrum in 22 men after a three-week staying at an altitude of 1500–2500 m in the Austrian Alps [9]. Conversely, the data are accumulating, showing an increase in the risk of atherosclerosis during living under altitude conditions due to the growth of the cholesterol content of the blood. In the study [10], it was reported that acclimation to the altitude of 3105 m was associated with an increase in the total cholesterol

content from 177 to 190 mg/dl ( $p < 0.002$ ) and a substantial growth of the risk of atherosclerotic pathology. A recent multicenter study that was performed in Switzerland on a large group of subjects ( $n = 19272$ ) and published in 2008 did not demonstrate any significant changes in the cholesterol content in blood serum during living under high-altitude conditions [11].

Let us state a contradiction. On the one hand, staying at high altitude is applied as a form of mountain-climate therapy for the treatment of atherosclerosis and nonmedication prophylaxis of atherogenic dyslipoproteinemia [1–4, 7–9, 12–18]. On the other hand, the antiatherogenic effects of moderate altitude hypoxia on the lipid composition of blood are not confirmed by control studies on large samples [6, 10, 11].

We suppose that this contradiction represents different grades of hypoxic influence on the human body. The combination of altitude hypoxia related to lower barometric pressure and working hypoxia related to controlled physical training is a feature of conditions under which antiatherogenic changes in the lipid spectrum, i.e., a general decrease in cholesterol and increase in HDL, were observed in subjects. This refers to different types of sport and sanatorium modes of staying at moderate and high altitudes [1–4, 7–9, 12–18]. On the contrary, living at, or elevation to, a high altitude without an accompanying physical load may result in the opposite changes in the lipid spectrum of blood serum in healthy and diseased individuals [5, 6, 10, 11].

**Table 1.** Content of total cholesterol in the blood in the course of therapy ( $X \pm m$ )

Age group	Frequency of beneficial effect (number of patients)	Content of total cholesterol in the blood, mmol/l	
		in the beginning of the course	at the end of the course
1	2	3	4
41–50 years	17 from 18	7.77 ± 0.163	6.68 ± 0.173
51–60 years	21 from 28	7.69 ± 0.158	6.63 ± 0.224
Older than 60	13 from 19	7.64 ± 0.198	6.93 ± 0.186

**Table 2.** Contents of lipids in blood serum of patients who suffered from macrofocal heart attacks prior to and after their stay at a moderate altitude for 30 days ( $X \pm m$ )

Index	Prior to therapy	Immediately after therapy
Total cholesterol (TC), mg/dl	241 ± 5.05	228 ± 6.51
HDL cholesterol, mg/dl	37.5 ± 0.95	42.9 ± 1.01*
Triglycerides (TG), mg/dl	215.3 ± 10.3	170.8 ± 11.2*
LDL cholesterol, mg/dl	161.0 ± 6.78	155.9 ± 6.51
LDL cholesterol, mg/dl	44.4 ± 2.03	35.0 ± 2.51*
Index of atherogenicity (IA), arb. un	5.42 ± 0.43	4.32 ± 0.54

**Table 3.** Effects of adaptation to interval hypoxia in an altitude chamber on the lipid level in the blood of patients suffering from ischemic heart disease ( $X \pm m$ )

Index	Before adaptation, $n = 46$	After adaptation, $n = 46$	$p_{1-2}$
TC, mmol/l	5.29 ± 0.14	4.96 ± 0.10	<0.002
TC, mg/dl	204.2 ± 5.4	191.6 ± 3.86	
HDL, mmol/l	1.28 ± 0.05	1.36 ± 0.04	<0.02
HDL, mg/dl	49.4 ± 1.93	52.5 ± 1.54	
VLDL, mmol/l	0.30 ± 0.018	0.26 ± 0.016	<0.01
VLDL, mg/dl	11.58 ± 0.7	10 ± 0.6	
LDL, mmol/l	3.70 ± 0.14	3.33 ± 0.11	<0.0004
LDL, mg/dl	142.8 ± 5.4	128.5 ± 4.25	
TG, mmol/l	1.49 ± 0.09	1.29 ± 0.08	<0.007
TG, mg/dl	131.9 ± 7.97	114.2 ± 7.1	
IA, arb. un	3.56 ± 0.27	2.83 ± 0.17	<0.0001

Notes: Abbreviations are explained in the text.

We assumed that the conditions determining the antiatherogenic modification of the lipid spectrum, including decreases in the total cholesterol and low-density lipoprotein (LDL) contents and an increase in the level of HDL during mountain-climate therapy of dyslipoproteinemia, are altitude and working hypoxia in combination with regular cold effects (mountain-

climate therapy). Therefore, we compared the rates of changes in the lipid spectra under different protocols of isolated and combined hypoxic and climate treatment.

## METHODS

We compared the results of the measurement of the lipid spectrum, in which changes in the contents of lipid fractions were observed after staying in mountains for no more than 30 days. We chose studies performed in Russia because mountain-climate correction of atherogenic dyslipoproteinemia in cardiology has been widely used in our sanatoria located in resorts at moderate altitudes [12–16].

From study [13], we extracted the numerical data on changes in total cholesterol that were observed during therapy in resort at Pyatigorsk (Table 1).

*Note.* For convenience of analytical comparisons of the rate of change in the lipid content, the numerical data of the first two age groups were pooled into one sample, for which point estimations of ordinary and central moments were summarized for both age groups. According to the properties of the sum of the expected values and variances and independence of division in accordance with the patient's age, these calculations can be considered correct ([19], pp. 81, 91). Newly calculated weighted average values that took into account the total number of patients in each of two groups (Table 1, column 2) for the total cholesterol content in the combined sample were expressed as mg/dl ( $X \pm m$ ). They were 297 ± 11.6 mg/dl before therapy and 260 ± 13.1 mg/dl after therapy.

From study [14], we took the numerical data on changes in the lipid spectrum that are presented in Table 2.

*Note.* Changes in some indices were nonsignificant (\*, significant differences taking into account the number of subjects  $n = 225$  [14]). For the next comparisons of the rates of lipid changes, we shall use only the mean values of these parameters ( $X$ ).

From study [16], we took numerical data on the changes in the lipid spectrum after adaptation to interval hypoxia in an altitude chamber for 22 days (Table 3).

*Note.* For convenience of the subsequent comparisons of the rates of changes in the lipid content, we presented the values of indices as mg/dl in the second lines of cells in Table 3. In order to recalculate the values from mmol/l to mg/dl, we used coefficients 38.6 and 88.55 for the contents of cholesterol and its fractions, and triglycerides, respectively.

Our own experiments were performed at moderate altitude in the Himalayas in the spring of 2008. We studied the lipoprotein spectrum of blood serum during the combined influence of the conditions that were similar to mountain-climate therapy used in the resorts of the former Soviet Union [12–14]. These conditions included altitude hypoxia, regular physical

load in the form of trekking, which we additionally combined with exercises adapted from Tibetan Tummo Yoga [3].

Sixteen subjects, including eight men and eight women aged 19–50 years participated in the study. From April 27 to May 8, 2009, they stayed at an altitude of 2000–3000 m (the Kullu Valleys, the Himalayas) and daily performed moderate physical training, such as trekking at moderate altitude. They were additionally subjected to cold tests combined with exercises aimed at enhancing heat production based on Tibetan Tummo Yoga. Samples of blood were obtained from the cubital vein in the morning on an empty stomach. Sampling was performed on day 1 and day 10 of staying at a moderate altitude of 1500 m in the Himalayas in the laboratory in the town of Patlikul (in Himachal Pradesh, India) and on day 5, at an altitude of 3000 m the Dundi khud in the same state. In the laboratory of Patlikul town, the blood samples were centrifuged and plasma was used for assessment of the lipid spectrum using an Erba Chem-5 Plus v. 2 semiautomatic closed-type biochemical analyzer. Lipid content was assayed spectrophotometrically.

The significance of changes in the lipid spectrum was estimated using the nonparametric Wilcoxon's test for matched pairs, because the distribution laws were not known for random samples [20]. Then, at a significance level of  $\alpha = 0.05$  (the probability of the first-type error), we confirmed the following significant differences of changes in the lipid spectrum between days 1 and 10 of staying under conditions prevalent at a moderate altitude and under regular physical training: (1) a decrease in the total cholesterol level; (2) an increase in the content of cholesterol in the high-density lipoprotein fraction; (3) a decrease in the content of cholesterol in the low-density lipoprotein fraction; (4) an absence of changes in the level of cholesterol in the very low-density lipoprotein fraction; (5) stability of the triglyceride (TG) content; (6) a decrease in the index of atherogenicity (IA).

In order to make a presentation of statistical estimations consistent for the next comparison of the rates of changes in the lipid spectrum, we have to use, in our case, point estimations of the arithmetical mean of samples and the standard deviation, taking into account a priori that our numerical data could be described by parameters of the normal or Gaussian distribution ( $X \pm s(m)$ ). Moreover, for our sample ( $n = 16$ ), it is impossible to find an appropriate law of distribution of random values [20, 21].

*Note.* In Tables 1, 2, 3, and 4, numerical data on the blood lipid spectrum are presented as ( $X \pm s(m)$ ), where  $X$  is the arithmetical mean;  $s$  is the standard deviation; and  $m = \frac{s}{\sqrt{n}}$  is the standard error of the

mean. However, for comparison of the rates of changes in the lipid spectrum, we need only data on the mean values  $X$ . Therefore, we will not use the values of the

standard deviation  $s$  and the standard error of the mean in the subsequent calculations.

## RESULTS AND DISCUSSION

**Mathematical model of lipid changes in blood serum.** We reported [1, 2, 17] antiatherogenic changes in blood serum lipoproteins in healthy subjects at the altitude of 4000 m. We have demonstrated that changes in the lipid content in the blood during a limited time interval from several hours to several weeks can be described using a known equation, based on a simple one compartment model of changes in blood lipid content with first-order kinetics [22–24]:

$$X(t) = Ce^{kt}, \quad (1)$$

where  $X$  is lipid content;  $t$  is time; and  $k$  and  $C$  are coefficients the values of which can be calculated using the least square method (LSM). This model was considered to be evidence of the validity of Trincer's results on the thermogenic lung function, for which lipids are used as a substrate [2, 17]. Accordingly to Trincer [18], a hypoxemic signal, which the blood transmits to the lungs, activates chemical heat production in the lungs independently of the factors that have caused hypoxemic changes in the blood.

**Comparisons of the rates of lipid changes in blood serum.** Using the LSM, we can calculate the  $k$  and  $C$  coefficients for exponential model (1), which will describe lipid changes in the blood during a limited time interval of up to 30 days.

Then, taking into account time intervals of 10 to 30 days, the numerical data on the decreases in the total cholesterol contents presented in Tables 1, 2, 3, and 4 will be correctly approximated by analytical equations:

$$X_1^{chol}(t) = 297.1e^{-0.004t}, \quad (2)$$

$$X_2^{chol}(t) = 241.1e^{-0.002t}, \quad (3)$$

$$X_3^{chol}(t) = 204.2e^{-0.003t}, \quad (4)$$

$$X_4^{chol}(t) = 190.8e^{-0.01t}, \quad (5)$$

where  $X_{1,2,3,4}^{chol}$  is the total cholesterol content of the blood (the low index indicates the number of the table containing numerical data on cholesterol level); and  $t$  is time.

In order to compare the rates of changes, let us present equations for the first-order time derivatives:

$$\frac{dX_1^{chol}}{dt} = (297.1e^{-0.004t})' = -1.32e^{-0.004t},$$

$$\frac{dX_2^{chol}}{dt} = (241.1e^{-0.002t})' = -0.43e^{-0.002t},$$

**Table 4.** Changes in the lipid spectrum of apparently healthy subjects ( $n = 16$ ) on days 1, 5, and 10 of their stay at a moderate altitude in the Himalayas under conditions of moderate physical training and cold treatment (Tibetan Tummoo Yoga)

Index	Lipid content in the blood		
	Day 1	Day 5	Day 10
Total cholesterol (TC), mg/dl	192.1 ± 27.7(6.9)	177.4 ± 20.3(7.17)	173.6 ± 18.7(4.7)
HDL cholesterol, mg/dl	34.2 ± 5.6(1.4)	38.25 ± 3.2 (1.15)	40.8 ± 5.0(1.45)
LDL cholesterol, mg/dl	132.4 ± 32.4(8.1)	120.1 ± 12.9(4.55)	114.4 ± 14.3(3.6)
VLDL cholesterol, mg/dl	20.6 ± 7.7(1.9)	19 ± 7.6(2.7)	18.4 ± 5.4(1.35)
Triglycerides (TG), mg/dl	101.1 ± 40.2(10)	96.25 ± 37.2(13.2)	92.4 ± 26.9(6.7)
Index of atherogenicity (IA), arb. un	4.7 ± 1.1(0.26)	3.65 ± 0.41(0.14)	3.37 ± 0.62(0.15)

$$\frac{dX_3^{chol}}{dt} = (204.2e^{-0.003t})' = -0.6e^{-0.003t},$$

$$\frac{dX_4^{chol}}{dt} = (190.8e^{-0.01t})' = -2.2e^{-0.01t}.$$

Because we have to make comparisons using equal time intervals, we have to choose the maximum duration of mountain-climate treatment in the sample under study. This is 30 days. Then, after definite integration of the first-order derivatives of approximated functions (2)–(5) with integration limits ranging from 0 to 29 and taking the square of these results, we solve the task of comparing the rates of changes in the approximation exponential functions in the space  $L_2$  (in the square of integrable functions) [25]. After integrating and calculation, we receive the following values:

$$\int_0^{29} (-1.32e^{-0.004t}) dt = -35.8,$$

$$(-35.8)^2 \approx 1285 \text{ for } X_1^{chol},$$

$$\int_0^{29} (-0.43e^{-0.002t}) dt = -12.1, \quad (-12.1)^2 \approx 146 \text{ for } X_2^{chol}, \quad (6)$$

$$\int_0^{29} (-0.6e^{-0.003t}) dt = -16.6, \quad (-16.6)^2 \approx 275 \text{ for } X_3^{chol},$$

$$\int_0^{29} (-2.2e^{-0.01t}) dt = -53.9, \quad (-53.9)^2 \approx 2902 \text{ for } X_4^{chol}.$$

Equations (6) demonstrate that the combination of altitude hypoxia, moderate physical training, and regular cold treatment (Tibetan Tummoo Yoga applied to increase resistance to cold temperatures, ( $X_4^{chol}$ ) result in the maximum decrease in the level of total cholesterol, which is substantially higher as compared to the decreases in the total cholesterol contents after altitude hypoxia alone ( $X_2^{chol}$ ) or adaptation to hypobaric

hypoxia ( $X_3^{chol}$ ). The combination of altitude hypoxia and moderate physical training in the course of sanatorium/resort therapy ( $X_1^{chol}$ ) significantly elevates the rate of decrease in the content of total cholesterol, which becomes important when the subjects perform specific Tibetan Tummoo Yoga exercises to enhance cold resistance  $X_4^{chol}$ .

A similar calculation procedure was applied to compare the rates of the increase in the HDL and LDL levels using the numerical data that are presented in Tables 2–4. The approximating exponential equations obtained by the LSM for numerical data on the lipid spectrum presented in Tables 1–4 and the squares of definite integrals of their first-order derivatives are presented in the Table 5.

*Note.* We evaluated the rates of changes in the anti-atherogenic (HDL) and atherogenic (total cholesterol and LDL) fractions of the lipid spectrum of blood serum alone. In our experiments, changes in VLDL and triglycerides were nonsignificant; therefore, we did not compare these fractions of the lipid spectrum.

Table 5 demonstrates that a combination of altitude hypoxia, moderate physical training, and regular cold treatment, such as Tibetan Tummoo Yoga for elevation of cold resistance (row 4), was more efficient for a decrease in the content of total cholesterol, an increase in the HDL level, and a decrease in the LDL content as compared to the effects of the isolated application of altitude hypoxia (row 2) and adaptation to hypobaric hypoxia (row 3).

Atherogenic shifts of the blood lipid spectrum and lipid spots on the internal wall of the arteries are known to appear long before the clinical manifestation of ischemia, namely, beginning from the age of 10 years. Therefore, prophylaxis of dyslipoproteinemia is an important part of efficient therapy and rehabilitation of atherosclerosis [26]. Non-medication prevention of acquired dyslipoproteinemia, which is the main risk factor of the atherosclerotic modification of the arterial intima, will be the subject of future studies on our method of combined hyperoxytherapy for further increasing the efficacy of mountain-climate treatment

**Table 5.** Summary of exponential models of  $Ce^{kt}$  type for changes in total cholesterol, HDL, and LDL levels (columns 2, 4, and 6) and the values of squares of definite integrals of their first-order time derivatives with integration limits from 0–to 29 (days)

Table no.	Total cholesterol		Cholesterol HDL		Cholesterol LDL	
	2	3	4	5	6	7
1	$297.1e^{-0.004t}$	1285				
2	$241.1e^{-0.002t}$	146	$37.5e^{0.0045t}$	27.1	$161e^{-0.001t}$	24.3
2	$204.2e^{-0.003t}$	275	$49.4e^{0.003t}$	17	$142.8e^{-0.005t}$	344
4	$190.8e^{-0.01t}$	<b>2902</b>	$34.3e^{0.02t}$	<b>702</b>	$131.8e^{-0.016t}$	<b>2480</b>

of cardiological patients in terms of restorative medicine.

### CONCLUSIONS

(1) We found a new application of the known calculation algorithm that allows us to compare the rates of changes in the lipid fractions of the blood in healthy and sick subjects under different conditions.

(2) Application of a new calculation algorithm demonstrated the maximum rate of antiatherogenic changes in the lipid spectrum of the blood, including a decrease in the cholesterol and LDL levels and an increase in the HDL content, under combined conditions of altitude hypoxia, moderate physical training, and the performance of specific exercises to increase resistance against cold conditions based on Tibetan Tummo Yoga.

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