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METABOLIC ASPECTS OF HUMAN ADAPTATION IN THE NORTH

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THE CORRELATION BETWEEN COLLAGEN AND LIPID METABOLISMS IN RESIDENTS OF THE ARCTIC ZONE OF THE RUSSIAN FEDERATION

ABSTRACT

The purpose of the study is to examine the relationship between collagen metabolism and lipid profile and the risk of cardiovascular pathology in middle-aged men living in the Arctic.

Material and methods. The study based on written consent was included apparently healthy middle-aged overweighted men, living and working in the European North (n=28). Anthropometric survey was conducted by measuring body height and weight, waist and hip circumference (WC, HC). Assessment of cardiovascular risk (CVR) was carried out on a scale of SCORE. The comparison group consisted of men, residents of Western Siberia (n=6). Blood and urine sampling was carried out in the morning. In the urine, assessed were the contents of total hydroxyproline (tGOP) and its forms: free (fGOP), peptid-bound (peGOP), protein-bound (prGOP). The serum contents of cholesterol and triglycerides, HDL, LDL, apolipoprotein A1 and apolipoprotein B (Apo A1, Apo B), matrix metalloproteinases - MMP-1, MMP-2, MMP-3, MMP-9, tissue inhibitors of matrix metalloproteinases - TIMP-1 and TIMP-2, TIMP-4 were measured. The results were process using Statistica package applications.

Results of the study. The northerners had 2 times increase in the content of tGOP mainly due to peGOP and prGOP. The enhanced fibrosis involves local activation of the regulation system, which is manifested as an increase in the content of MMP-1 and MMP-9 and TIMP-1 and TIMP-4. Contents of LDL, HDL, Apo A1 and Apo B were in line with the reference values. Atherogenic coefficient in northerners was higher than 3, but less than that in the comparison group, but the cardiovascular risk was higher. Correlations between the contents of the GOP and cardiovascular risk factors, MMP and TIMP were established.

Conclusion. Increased cardiovascular risk scale SCORE, a relationship between the content of hydroxyproline and cardiovascular risk factors among northerners are evidences of the involvement of altered collagen metabolism in the pathogenesis of atherosclerosis.

Keywords: hydroxyproline, lipids, MMP, TIMP, cardiovascular risk, Arctic zone.

INTRODUCTION

The relevance of the study of collagen metabolism in the Arctic region is connected with the problem of fibrosis and the development of structural and functional changes in tissues and organs, which manifest in varying degrees of functional impairment. Clinical and morphological features of fibrosis in lungs and heart of the northerners have been described [3]. It has been found that the magnitude of changes in respiratory function depends on the state of organism adaptation, seasonality, physical activity and duration of exposure to extreme environmental factors (low temperature, dust, etc.) [8]. Revealed changes of external respiration, oxygen transport function of blood, the oxygen permeability of capillaries and the oxygen balance of blood, which are associated with polar experience and photoperiod, showed the involvement of these systems in the development of northern tissue hypoxia [1]. Regardless of the pathogenesis of hypoxia, it is a powerful inducer of collagen synthesis [9]. Type I collagen is particularly sensitive to hypoxia: organ deposits of type I collagen increase sharply in hypoxia [10]. Since the main function of type I collagen is to prevent tissue distension [7], its accumulation can be expected to decrease the elasticity of tissues and vessels, which is especially important. However, data on the content of this extracellular matrix component in northerners are not available, since there were no direct investigations in the Arctic region.

It is known that men aged 40-59 years are more susceptible to atherosclerosis [4]. This is partially associated with hormonal status changes induced, among other factors, by the function of sex hormones. Male northerners aged 30-39 years have been shown to have 1.4 and 1.2 times lower levels of testosterone and dehydroepiandrosterone sulfate levels, respectively, than men under 29 [5]. There is evidence that Finnish men (24-45 years old) with low testosterone had higher levels of triglycerides and LDL [13]. The relationship between testosterone and risk of cardiovascular disease is widely discussed [11, 14]. Thus, the combination of factors such as northern tissue hypoxia, male gender, reproductive age, and low temperatures can increase the risk of pathology associated with impaired lipid and collagen metabolism.

The purpose of the study is to examine the relationship between collagen metabolism and lipid profile and the risk of cardiovascular pathology in middle-aged men living in the Arctic.

MATERIALS AND METHODS

Apparently healthy men (n = 28, average age 47.1 ± 2.3 years) working at mining facility in Murmansk region (67° N) were enrolled in the study on the basis of a written informed consent. Calculated average life expectancy in the North was 22.6 ± 2.1 years. The study was conducted in the period of polar night (November-December). **Comparison group** (Novosibirsk citizens, n = 6) and northern group had no differences in gender, age, body mass index, marital status and chronotype.

The study was approved by RIECM Bioethical Committee and performed in compliance with the “Ethical principles for medical research involving human subjects” and in accordance with the “Rules of clinical practice in the Russian Federation”.

Anthropometric examination, including measurement of height (cm), body weight (kg), waist circumference (WC, cm) and hips (HC, cm) was performed. Body mass index (BMI - kg / m2) was determined, and WC/HC relationship was evaluated. Cardiovascular risk (CVR) was assessed in accordance with National guidelines on cardiovascular prevention (2011).

Biological material (blood plasma and urine) was collected in the morning after an overnight fast, and was deep-frozen at -70ºS after pre-treatment. Lipid profile was assessed using an automatic biochemical analyzer 480 AU Beckman Coulter (USA). Thermo Fisher Scientific sets (USA) were used to determine the levels of cholesterol (cholesterol, mmol / L) and triglyceride (TG, mmol / l). Levels of HDL (mg / dL), LDL (mmol / L), Apolipoprotein A1 (apo A1 mg/dl), and apolipoprotein B (Apo B mg/dL) were determined using DiaSyS sets (Germany).

The level of the following components was determined in blood plasma using ELISA kits according to instructions: matrix metalloproteinases MMP-1, MMP-2, MMP-9 (Sigma-Aldrich Co. LLC, USA), MMP-3 (AESKU DIAGNOSTICS GmbH & Co. KG, Germany), tissue inhibitors of matrix metalloproteinases TIMP-1 and TIMP-2 (Sigma-Aldrich Co. LLC, USA), TIMP-4 (R & D Systems Inc., USA). The results were read using a microplate reader Stat Fax-2100 (Awarenes Technology Inc., USA).

Collagen content was evaluated by the level of total hydroxyproline (tGOP) and its forms in the urine: free (fGOP), peptide-bound GOP (peGOP), protein-bound GOP (prGOP) [6]. The calibration curve was obtained for GOP standard dilutions (MM - 131,13, «Sigma»). Measurement of the optical density of the analyte was performed using a spectrophotometer PD-303S («Apel», Japan) at a wavelength of 560 nm.

The study was performed using the equipment of RIECM Shared Equipment Center “Modern optical systems”.

Statistical processing of the results was carried out using Statistica v. 10 (Stat Soft Inc., USA). To compare the two groups, Mann–Whitney U test was used. Relationships between parameters were established using Spearman’s rank correlation. The results were presented as M ± m. Differences were considered statistically significant at p <0.050.

Table 1

The content of hydroxyproline in urine and the content of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in serum of men in the European North

Index	European North	Western Siberia	p
tGOP, mcg/ml	25,13±1,94	12,15±1,51	0,001
fGOP, mcg/ml	5,25±0,66	3,17±0,36	0,010
peGOP, mcg/ml	17,71±1,56	7,87±1,24	0,002
prGOP, mcg/ml	2,17±0,22	1,12±0,38	0,033
Index of fibrosis, SU	5,60±0,91	2,85±0,24	0,010
MMP-1, ng/ml	0,90±0,09	0,73±0,15	0,020
MMP-2, ng/ml	67,95±6,76	52,08±10,26	
MMP-3, ng/ml	31,90±2,73	35,17±5,20	
MMP-9, ng/ml	380,29±28,69	239,00±41,64	0,033
TIMP-1, ng/ml	713,05±79,29	310,42±62,92	0,015
TIMP-2, ng/ml	206,52±24,45	271,50±92,16	
tGOP, mcg/ml	1,62±0,14	1,17±0,11	0,020

Table 2

Anthropometric indices, lipid profile and the degree of cardiovascular risk in men in the European North

Index	European North	Western Siberia	p
BMI, kg/m2	26,74±0,73	28,06±1,61	
WC, см	93,68±2,05	100,17±4,50	
HC, см	100,82±1,36	106,20±2,52	
WC/ HC	0,93±0,01	0,91±0,01	
Cholesterol, mmol/l	5,01±0,23	5,08±0,31	
TG, mmol/l	1,37±0,19	2,03±0,35	
HDL, mmol/l	1,21±0,09	0,77±0,06	0,009
LDL mmol/l	2,70±0,14	2,95±0,21	
Apo A1, mg/dl	148,40±3,89	121,62±3,17	0,001
Apo B, mg/dl	50,64±3,48	91,38±10,20	0,001
Atherogenic coefficient, SU	3,53±0,35	5,88±0,90	0,018
Risk SCORE, %	3,89±0,83	0,96±0,56	0,044

RESULTS AND DISCUSSION

tGOP urine concentration in northerners was 2 times higher than in men from comparison group (Table 1). The increased concentration was caused by the increased level of all GOP forms, especially prGOP, which had 2 times higher level than in the comparison group. The results of a special pilot study involving young Swedish men showed that increased excretion of tGOP and fGOP in the urine is caused by low temperatures [12].

There is an opinion that peGOP reflects the rate of biological collagen turnover (both synthesis and degradation), fGOP reflects the degradation of collagen and prGOP reflects synthesis of a young, immature collagen [6]. If so, the index of fibrosis can be calculated by obtaining the fGOP concentration value from tGOP concentration value and subsequent assignment to fGOP. This index reflects the synthesis of collagen (Table 1). In northerners, this index was 2 times higher than in the comparison group.

There is a hypothesis that serum prGOP is a component of the C1q complement, which is also related to acute-phase proteins [16]. However, high level of prGOP was shown to promote atherosclerosis in healthy males aged 40-59 [4].

Local regulation of extracellular matrix metabolism based on MMP/TIMP system seems to play a key role in increased content of GOP and the index of fibrosis. Northerners had an increased levels of MMP-1 and MMP-9, whereas the level other enzymes (MMP-2, MMP-3) did not differ from the control group (Table. 1). The levels of TIMP-1 and TIMP-4 in northerners were increased, while TIMP-2 level was similar to those of the comparison group.

Notably, the TIMP-4 level in blood plasma of miners in the European North did not differ from those of Finnish middle-aged men, who had no symptoms of cardiovascular disease [15]. The authors noted the direct association of TIMP-4 concentration with age, LDL, thickness of the carotid artery intima-media, and systolic blood pressure, which show the effect of TIMP-4 on the process of atherogenesis.

Thus, fGOP level increase can be attributed to increased levels of MMP-1 and MMP-9, and a significantly increased level of another GOP forms, peGOP, can be attributed to the high values of TIMP-1 and TIMP-4. However, TIMP-1 and TIMP-4 inhibitory effect on MMP-9 was insufficient.

Anthropometric indices northerners did not differ from those of comparison group (Table. 2). There were no differences in the content of cholesterol and triglycerides. Contents of LDL, HDL, Apo A1 and Apo B corresponded to the reference values, but varied between the groups. Atherogenic coefficient exceeded the limit in both groups, but the risk of cardiovascular disease on a scale of SCORE was significant only in the group of northerners (Table. 2). It is important that the results of men in the European North were similar to the results of men (40.4 ± 0.6 years) working at the mine in Mirny (North Asia). For example, total cholesterol content in blood serum in a group of overweight men (25.0-29.9 kg / m2), was 5.25 ± 0.07 mmol / l, HDL was 1.21 ± 0.02 mmol / l, TG was 1.30 ± 0.05 mmol / l, and atherogenic coefficient was 3.66 ± 0.10 SU [2].

The relationship between collagen metabolism and lipid metabolism in northerners is presented in Table 3. There was a reverse medium strength correlation between anthropometric indicators (BMI, WC, HC) and all forms of GOP. There was a significant inverse relationship of medium strength between triglyceride and tGOP, fGOP, prGOP; as well as between cholesterol, LDL, Apo B, and fGOP; between atherogenic coefficient and tGOP, fGOP, peGOP. We established a direct correlation between MMP-1 and TIMP-4 with peGOP reflecting the dependence of this form of GOP on regulatory system.

Table 3

Correlation between the content of individual forms of hydroxyproline and lipid, anthropometric indices in men in the European North (r; p)

Index	tGOP	fGOP	peGOP	prGOP
BMI	-0,56; 0,002	-0,41; 0,032	-0,47; 0,011	-0,48; 0,010
WC	-0,55; 0,002	-0,46; 0,014	-0,42; 0,024	-0,58; 0,001
HC	-0,57; 0,001	-0,40; 0,034	-0,47; 0,011	-0,57; 0,002
WC/HC	-0,39; 0,038	-0,44; 0,019	–	-0,43; 0,024
TG	-0,47; 0,018	-0,61; 0,001	–	-0,47; 0,016
cholesterol	–	-0,45; 0,022	–	–
LDL	–	-0,45; 0,025	–	–
Apo B	–	-0,47; 0,017	–	–
Atherogenic coefficient	-0,46; 0,025	-0,41; 0,045	-0,42; 0,042	–
MMP-1	–	–	0,55; 0,018	–
TIMP-4	–	–	0,48; 0,040	–
TIMP-4, ng/ml	1,62±0,14	1,17±0,11	0,020	

References

1.Kim L.B. Transport kisloroda pri adaptacii cheloveka k usloviyam arktiki i kardiorespiratornoj patologii [Oxygen transport in human adaptation to the Arctic conditions and cardiorespiratory diseases]. Novosibirsk: Nauka, 2015, 216 pp.

2.Ginsar E.A. [et al.] Rasprostranennost metabolicheskogo sindroma i ego struktura v zavisimosti ot massy tela u rabotayushchih muzhchin g. Mirnogo [The prevalence of metabolic syndrome and its pattern in relation to body weight in working males of the town of Mirny, Republic of Sakha (Yakutia)] .Prof. med., 2010, Vol. 13, № 1, P. 37–41.

3.A.P. Avtsyn [et al.]. Patologiya cheloveka na severe [Human pathology in the North]. Moscow: Meditsina, 1985, 416 pp.

4.E.A. Bazanov [et al.]. Pokazateli obmena kollagena soderzhanie lipidov ciklicheskogo 3´,5´-AMF i nekotoryh gormonov v plazme krovi muzhchin 40–59 let [Collagen metabolism indices, and plasma concentrations of lipids, cyclic 3',5'-AMP, and certain hormones in 40–59 year old men]. Vopr. Med. Khim., 1980, Vol. 26, № 4, P. 464–470.

5.L.B. Kim [et al.]. Polovye gormony i kardiovaskulyarnyj risk u muzhchin gornorabochih v usloviyah Evropejskogo Severa [Sex hormones and cardiovascular risk in male mining workers living in the European North]. Fiziologiya Cheloveka. 2016, Vol. 42, № 2, P. 92–99.

6.P.N. Sharaev [et al.]; ed. prof. R.R. Kildiyarovoj. Soedinitelnaya tkan v detskom vozraste [Connective tissue during childhood]. Izhevsk, 2005, 152 p.

7.Faller D.M. Shields D. Molekulyarnaya biologiya kletki [Molecular biology of the cell]. Moscow: BINOM Publ., 2003, 272 pp.

8.Shishkin G.S. Ustyuzhaninova N.V. Funkcionalnye sostoyaniya vneshnego dyhaniya zdorovogo cheloveka [Functional state of respiration in healthy human]. Novosibirsk: Publ. SB RAS, 2012, 329 pp.

9.Falanga V. Low oxygen tension stimulates collagen synthesis and col1A1 transcription through the action of TGFβ1 / V. Falanga, L. Zhou, T. Yufit // J. Cell Physiol. – 2002. – Vol. 191, Is. 1. – P. 42–50.

10.Hypoxia-induced increase in the production of extracellular matrix proteins in systemic sclerosis / J.H. Distler [et al.] // Arthritis Rheum. – 2007. – Vol. 56, № 12. – P. 4203–4215.

11.Kelly D.M. Testosterone and cardiovascular risk in men / D.M. Kelly, T.H. Jones // Front. Horm. Res. – 2014. – Vol. 43. – P. 1–20.

12.Lennquist S. Urinary excretion of hydroxyprolines in man under the influence of cold / S. Lennquist // Scand.

J. Clin. Lab. Invest. – 1975. – Vol. 35, Is. 2. – P. 103–107.

13.Relation of total and free testosterone and sex hormone-binding globulin with cardiovascular risk factors in men aged 24–45 years. The Cardiovascular Risk in Young Finns Study / S. Firtser [et al.] // Atherosclerosis. – 2012. – Vol. 222, Is. 1. – P. 257–262.

14.Tambo A. Testosterone and Cardiovascular Disease / A. Tambo, M.H.K. Roshan, N.P. Pace // Open Cardiovasc. Med. J. – 2016. – Vol. 10. – P. 1–10.

15.Tissue inhibitor of matrix metalloproteinase 4 (TIMP-4) in a population of young adults relations to cardiovascular risk markers and carotid artery intima-media thickness. The Cardiovascular Risk in Young Finns Study / M. Oikonen [et al.] // Scand. J. Clin. Lab. Invest. – 2012. – Vol. 72, Is. 7. – P. 540–546.

16.Varghese Z. Plasma hydroxyproline fractions in patients with dialysis osteodystrophy / Z. Varghese, J.F. Moorhead, M.R. Wills // Clin. Chim. Acta. – 1981. – Vol. 110, Is. 1. – P. 105–111.

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COMPARATIVE ANALYSIS OF BIOCHEMICAL PARAMETERS OF BLOOD IN THE MEN, LIVING IN YAKUTIA RURAL AND URBAN AREAS

ABSTRACT

We surveyed the men of Yakutia indigenous population (n = 150) living in rural and urban areas. The shift of lipid metabolism in the direction of dyslipidemia was observed in men in Central Yakutia, while among urban residents the signs of disadaptation were more marked. The men who lived in the north of Yakutia, there was no deviation from the norm. We revealed the correlation of lipid metabolism index (atherogenic index) with metabolic equilibrium index (De Ritis Ratio) that allows recommending the use of De Ritis Ratio for the formation of groups at risk of cardiovascular pathology and timely implementation of preventive measures.

Keywords: indigenous population of Yakutia, the activity of enzymes, lipids, metabolic equilibrium, De Ritis Ratio.

Recent studies show that in today's socio-economic environment of genetically enshrined mechanisms of energy metabolism in the restructuring of the indigenous population is not enough [2, 4, 8, 9, 11, 14]. Increased metabolism of lipids needed for adaptation to the climate and geographical conditions of the North, with a small amount of replenishment of reserves of the body can lead to pre-pathological changes in the body. Currently functional exhaustion manifested in the increase of cardiovascular diseases among the indigenous population of Yakutia [1, 5, 6]. The role of lipid metabolism in the development of atherosclerotic vascular disease which is a risk factor for cardiovascular diseases, early detection of changes in biochemical parameters involved energy metabolism is important.

Purpose of the study. Detection of maladjustment biochemical parameters of blood in indigenous men depending on place of residence.

MATERIAL AND METHODS

In total we studied 150 indigenous men of Yakutia aged 22 to 70 years (mean age 43.23 ± 1.23 yrs). People living in the northern regions were 55, in Central Yakutia: 68 living in urban area, in rural area - 27. Criteria of exclusion were: exacerbation of chronic diseases, the presence of cancer, infections and viral diseases. In addition, people with coronary artery disease who underwent heart attack and stroke were excluded.

To assess the objective state during survey questionnaire was conducted, developed in FBGNU "Yakult Science Center of complex medical problems"; we obtained informed consent of respondents to be studied, blood test. Blood for biochemical studies was taken from the cubital vein on an empty stomach in the morning, 12 hours after a meal.

Determination of biochemical parameters was carried out by enzymatic method on an automatic biochemical analyzer «Cobas Mira Plus» company «La Roche» (Switzerland) using reagents "Biocon" (Germany). Calculation of LDL cholesterol (low density lipoprotein) and LDL (cholesterol VLDL) was performed according to the formula Friedewald et al. (1972). Atherogenic coefficient was calculated according to the formula proposed by A.N. Klimov (1977): Ca = (cholesterol - HDL cholesterol) / HDL-C.

The study was approved by the decision of the local Ethics Committee of the Yakult Science Center of complex medical problems.

RESULTS AND DISCUSSION

Blood biochemical parameters in men living in rural areas in the north of Yakutia were varied in the range of normal values (Table). The relatively high level of albumin in this group compared with the Central Yakutia residents can be attributed to the increased energy metabolism, as one of the important functions of albumin is him participation in the transport of fatty acids. In addition, albumin is the low molecular anti-