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ИЗМЕНЕНИЕ СОСТАВА НЕНАСЫЩЕННЫХ ЖИРНЫХ КИСЛОТ ПЛАЗМЫ КРОВИ У МУЖЧИН, ПРОЖИВАЮЩИХ В СЕЛЬСКИХ РАЙОНАХ НОВОСИБИРСКОЙ ОБЛАСТИ, В ЗАВИСИМОСТИ ОТ УПОТРЕБЛЕНИЯ АЛКОГОЛЯ

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Changes In the Composition of Unsaturated Fatty Acids in The Blood Plasma In Men Living in Rural Areas of the Novosibirsk Region, Depending on Alcohol Consumption

Резюме

Цель исследования — изучить различия в содержании ненасыщенных жирных кислот (ННЖК) в плазме крови у мужчин, проживающих в сельских районах Новосибирской области, в зависимости от их алкогольного статуса. **Материалы и методы**: в рамках одномоментного эпидемиологического исследования по Новосибирской области были обследованы жители сельских районов (мужчины) в возрасте 60,04±10,55 (от 35 до 74 лет). Алкогольный статус участников определяли с помощью анкетирования. Количество разных алкогольных напитков было пересчитано в дозы чистого алкоголя. Все участники исследования были разделены на три группы по употреблению доз алкоголя в неделю: 1 группа — малое потребление алкоголя (МП); 2 группа — умеренное потребление алкоголя (УП); 3 группа — высокое потребление алкоголя (ВП). Методом высокоэффективной жидкостной хроматографии в плазме крови определяли уровни омега-3, -6 и -9 ННЖК. **Результаты**. Установлено, что в группе мужчин с ВП алкоголя более высокие уровни альфа-линоленовой омега-3 (p=0,041) и дигомо-гамма-линоленовой (p=0,002), докозатетраеновой (p=0,017), докозапентаеновой (p=0,023) омега-6 ННЖК в крови, по сравнению с группой мужчин с МП алкоголя. **Выводы**. Получены статистически значимые различия концентраций в крови альфа-линоленовой, дигомо-гамма-линоленовой, докозатетраеновой, докозапентаеновой ненасыщенных жирных кислот у мужчин 35-74 лет, проживающих в сельских районах Новосибирской области, в зависимости от употребления алкоголя.

Ключевые слова: *жирные кислоты, кровь, алкоголь, факторы риска*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

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Abstract

The aim of the study was to investigate differences in the content of unsaturated fatty acids (UFA) in blood plasma in men living of rural areas of the Novosibirsk region, depending on their alcohol status. **Materials and methods:** as part of a single-stage epidemiological study in the Novosibirsk region, rural residents (men) aged 60.04±10.55 (from 35 to 74 years) were examined. The alcohol status of the participants was determined by means of a questionnaire. The number of different alcoholic beverages was recalculated in doses of pure alcohol. All study participants were divided into three groups based on alcohol consumption per week: group 1 — low alcohol consumption (LC); group 2 — moderate alcohol consumption (MC); group 3 — high alcohol consumption (HC). The levels of omega-3, -6, and -9 UFA in blood plasma were determined by high-performance liquid chromatography. **Results**: It was found that the group of men with HC had higher concentrations of omega-3 alpha-linolenic acid (p=0,041) and omega-6 digomo-gamma-linolenic (p=0,002), docosatetraenoic (p=0,017), docosapentaenoic (p=0,023) UFA in blood, compared with group of men with LC. **Conclusions**: In the study, we found statistically significant differences in blood concentrations of alpha-linolenic acid, digomo-gamma-linolenic acid, docosathetraenoic acid, and docosapentaenoic acid unsaturated fatty acids were obtained in men aged 35-74 years living in rural areas of the Novosibirsk region, depending on alcohol consumption.

Key words: *fatty acids, blood, alcohol, risk factors*

Conflict of interests

The authors declare no conflict of interests

Sources of funding

The work was carried out within the framework of the budget topic under State Assignment No. FWNR-2024-0004

Conformity with the principles of ethics

The study was approved by the local ethics committee of the Research Institute of Therapeutic Microbiology and Microbiology — branch of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences (protocol No. 69, dated September 29, 2020). Each participant signed an informed consent

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IHD — ischaemic heart disease, MI — myocardial infarction, CCF — chronic cardiac failure, CVD — cardiovascular diseases, TC — total cholesterol, LDL cholesterol — low-density lipoprotein cholesterol, HDL cholesterol — high-density lipoprotein cholesterol, TG — triglycerides, NAFLD — non-alcoholic fatty liver disease, FA — fatty acids, UFA — unsaturated fatty acids, ALA — α-linolenic fatty acid, EPA — eicosapentaenoic fatty acid, DHA — docosahexaenoic fatty acid, LA — linoleic fatty acid, GLA — gamma-linolenic fatty acid, DHGLA — dihomo-gamma-linolenic fatty acid, AA — arachidonic fatty acid, DTA — docosatetraenoic fatty acid, DPA — docosapentaenoic fatty acid, GEX — hexadecenoic fatty acid, OL — oleinic fatty acid, MID — mead fatty acid, SEL — selacholeic fatty acid

Introduction

Globally, three million people (5.3% of all deaths) die of alcohol abuse annually. The share of men is 7.7%, while women account for 2.6% of all alcohol-related deaths. Among people aged 20 to 39 years old, alcohol-related mortality is approximately 13.5% [1].

Alcohol abuse and problem drinking are associated with a higher risk of cardiovascular diseases (CVD). However, the dose/reaction correlation between the amount of alcohol consumed and the risk of ischaemic heart disease (IHD) varies a lot between those regularly consuming alcohol and those drinking alcohol from time to time [2]. An increase in the average daily ethanol consumption positively correlates with the risk of arterial hypertension, hypercholesterolemia, including higher levels of low-density lipoprotein cholesterol (LDL cholesterol), smoking, physical inactivity [3]. However, studies of the correlation between alcohol consumption and the risk of CVD show contradicting results [4, 5]. A combination of various effects leads to U-shape or J-shape dose-dependent correlation between alcohol and IHD [2, 6, 7]. The Northern European and Northern American population consume beers and spirits, usually on weekends, while episodic consumption of spirits is typical for the Eastern European population [8].

The study of fatty acids (FA), including unsaturated fatty acids (UFA), is gaining more attention, with the emphasis on the amount and type of consumed acids. However, FAs possess various physiological functions even within one class [9]. The association between omega-3 UFA and alcohol consumption [10] has been actively discussed. A number of studies established that the prognostic value in CVD belongs to lower concentrations of blood omega-3 (eicosapentanoic,

docosahexaenic) and increased levels of omega-6 (linolic, gamma-linolenic, dihomo-gamma-linolenic, arachidonic, docosatetraenoic, docosapentaenoic) UFAs. A study of the omega-3/omega-6 UFA ratio showed higher blood concentrations of omega-6 UFA in decompensated chronic cardiac insufficiency. However, after fluid retention was managed, this ratio changed, and omega-3 UFA levels rose [11]. LURIC study demonstrated direct associations between docosatetraenoic and docosatetraenoic omega-6 UFA concentrations and inflammation markers (C-reactive protein, interleukin-6, fibrinogen and VCAM-1), as well as association with a higher risk of CVDs [9].

Alcohol abuse is an established risk factor of CVD [1-3]. As compared to non-drinkers, alcohol abuse is associated with a high risk of hypercholesterolemia (OR 1.76; CI: 1.12–2.75), hypertriglyceridemia (OR 2.69; CI: 1.52–4.77), overweight (OR 1.68; CI: 1.04–2.71), smoking (OR 2.24; CI: 1.48–3.41). An increase in the average daily dose of ethanol is related to the risk of arterial hypertension (OR 1.04; CI: 1.11–2.75) [3].

The objective of this study was to evaluate the differences in plasma omega-3, omega-6 and omega-9 UFA concentrations in male subjects living in rural area of the Novosibirsk Region, depending on their drinking status.

Materials and Methods

Subjects were examined during the cross-sectional, epidemiological study titled Epidemiology of Cardiovascular Diseases and Their Risk Factors in the Novosibirsk Region, in 2022–2023. Within the scope of this study, physicians at the Scientific Research Institute of the Therapy and Preventive Medicine, a branch of the Federal State Budgetary Scientific Institution Federal Research Centre, Cytology and Genetics Institute of the Siberian Section of the Russian Academy of Science examined 300 male subjects living in rural area of the Novosibirsk Region, aged 60.04±10.55 (35 to 74 years of age). Key inclusion criteria: correctly completed questionnaire, including data on alcohol consumption. Exclusion criteria for all subjects: incomplete questionnaire or missing data on alcohol consumption in the questionnaire. The study was approved by the Local Ethics Committee at the Scientific Research Institute of the Therapy and Preventive Medicine, a branch of the Federal State Budgetary Scientific Institution Federal Research Centre, Cytology and Genetics Institute of the Siberian Section of the Russian Academy of Science (Minutes No. 69 dated September 29, 2020). Each subject signed the informed consent form.

According to the WHO data, a standard alcohol unit is an amount of an alcoholic beverage, containing ethyl alcohol, the amount of which equals to 10 g of absolute alcohol (12.7 mL). One unit is: for beer (5%) — 250 mL, red strong wine (18%) — 70 mL, dry wine/champagne (13%) — 100 mL, vodka (40%) — 30 mL [1].

All subjects underwent an analysis of their alcohol consumption status: amount of alcohol consumed (daily/weekly/monthly/yearly alcohol consumption; amount of alcohol consumed at a time); type of alcoholic beverages (beer, dry wine/champagne, strong wine, homemade strong tinctures, vodka, cognac, and other spirits). Alcohol consumption at a time (a short period of time, e.g., one evening) was evaluated: strong spirits ≥ 200 mL, strong wine ≥ 500 mL, dry wine ≥ 700 mL, beer ≥ 2 litres. The study questionnaire was adopted from the ESSE-RF project questionnaire [12].

In order to analyse alcohol data, all data from the questionnaire were converted to absolute alcohol units (various alcoholic beverages) for each subject. Units were added together, and all subjects were divided into three groups, depending on weekly alcohol consumption (Table 1). Group 1 (mild alcohol consumption (MC)): < 8 units for men; group 2 (moderate alcohol consumption (ModC)): ≥ 8 units to < 16 units for men; group 3 (high alcohol consumption (HC)): ≥ 16 units for men [1, 13]. The groups were comparable in terms of their clinical-biochemical attributes.

All subjects underwent plasma UFA spectrum examination. Blood was drawn from the ulnar vein following 12 hours of fasting. Laboratory tests included measurement of plasma levels of the following FAs: α-linolenic (C 18:3, omega-3, ALA), eicosapentanoic (C 20:5, omega-3, EPA), docosahexaenic (C 22:6, omega-3, DHA), linolic(C 18:2, omega-6, LA), gamma-linolenic (C 18:3, omega-6, GLA), dihomo-gamma-linolenic (C 20:3, omega-6, DHGLA), arachidonic (C 20:4, omega-6, AA), docosatetraenoic (C 22:4, omega-6, DTA), docosapentaenoic (C 22:5, omega-6, DPA), hexadecenoic (C 16:1, omega-9, GEX), oleinic (C 18:1, omega-9, OL), mead (C 20:3, omega-9, MID), selacholeic (C 24:1, omega-9, SEL) using high-performance liquid chromatography. Serum lipid profile (total cholesterol (TC), high-density lipoprotein cholesterol (HDL cholesterol), triglycerides (TG)) was measured using Konelab Prime 30i analyser (Thermo Fisher Scientific, Finland) and DiaSys kit (Germany). Friedewald formula was used to calculate LDL cholesterol levels.

For statistical processing of the results, SPSS (version 23.0) (Statistical Package for the Social Sciences, USA) was used. The normality of parameter distribution was assessed using Kolmogorov-Smirnov test. The data are presented as a median value or percentiles [25%; 75%]. The Kruskal-Wallis non-parametric test was used to compare several groups, while independent groups were compared with the help of the Mann-Whitney test. Differences in quality attributes were identified using Pearson's test (χ^2). Presence of the correlation between FA and alcohol units was determined using correlation analysis (Spearman's rank correlation (r)). Differences were statistically significant at $p < 0.05$.

Results

The questionnaire completed by the subjects allowed obtaining information on the eating habits, chronic disease status (Table 1). There were no statistically significant differences in dietary preferences, such as consumption of fish, meat, seafood, butter and vegetable oil, vegetables, fruit, dairy products among subjects. In terms of serum lipid profile, groups differed in TC and HDL cholesterol levels (Table 1).

We established statistically significant differences between high alcohol consumption and mild alcohol consumption groups in α-linolenic, dihomo-gamma-linolenic, docosatetraenoic and docosapentaenoic FA (Table 2).

A correlation analysis was performed in order to identify possible correlations between FAs and alcohol units. The following correlations were found: gamma-linolenic FA (r=0.293; p=0.006), docosapentaenoic FA (r=0.308; p=0.004) and beer consumption. Also, there is moderate correlation between arachidonic FA (r=0.401; p=0.034) and wine consumption, as well as weak correlation between dihomo-gamma-linolenic FA (r=0.206; p=0.016) and vodka consumption.

Table1. *Clinical and biochemical characteristics of the study participants, Me [25%; 75%]*

Parameters	Group 1 (MC) n=260	Group 2 (ModC) n=25	Group 3 (HC) n=15	P*
Age, years	63,0 [55,0; 68,0]	56,0 [45,0; 62,0]	50,0 [42,0; 65,0]	0,001
BMI, kg/m^2	29,3 [26,2; 33,6]	29,4 [22,4; 33,8]	27,7 [22,7; 35,9]	0,692
SAD, mmHg	146,5 [135,5; 164,5]	151,5 [134,0; 166,25]	148,0 [135,0; 165,0]	0,867
DAD, mmHg	94,0 [86,5; 102,5]	100,0 [89,25; 113,5]	90,0 [85,0; 101,0]	0,108
Total cholesterol, mmol/l	5,0 [4,3; 5,8]	5,7 [5,4; 6,2]	5,5 [4,5; 5,7]	0,022
Triglycerides, mmol/l	1,5 [1,1; 2,1]	1,4 [1,0; 1,8]	1,4 [0,8; 2,1]	0,348
HDL-C, mmol/l	1,2 [1,0; 1,5]	1,4 [1,1; 1,8]	1,2 [0,9; 1,7]	0,029
LDL-C, mmol/l	3,0 [2,3; 3,7]	3,5 [3,0; 4,0]	2,9 [2,7; 3,4]	0,124
Liver diseases (including NAFLD), n (%)	13 (5%)	3 (12%)	1 (7%)	0,666
Stomach ulcer or duodenal ulcer, n (%)	30 (11%)	3 (12%)	2 (13%)	0,737
Cardiovascular diseases (including coronary heart disease, MI, CHF), n (%)	99 (38%)	4 (16%)	3 (20%)	0,035

Note: BMI — body mass index, NAFLD — a non–alcoholic fatty liver disease, SAD — systolic blood pressure, DAD — diastolic blood pressure, HDL-C — high-density lipoprotein cholesterol, LDL-C — low-density lipoprotein cholesterol, n % — number of respondents in the group (%of the total number of respondents in the group).
* — Kruskal-Wallis criterion for comparing three groups, Pearson criterion for comparing qualitative characteristics

Table 2. *Concentration of plasma fatty acids in groups depending on the status of alcohol consumption, Me [25%; 75%]*

FA, nmol/ml	Group 1 (MC) n=260	Group 2 (ModC) n=25	Group 3 (HC) n=15	p
ALA	105,5 [80,0; 127,0]	96,0 [74,5; 127,0]	116,0 [103,0; 139,0]	**0,041**
EPA	55,0 [37,0; 71,0]	50,0 [33,5; 66,0]	63,0 [58,0; 79,0]	0,136
DHA	178,0 [131,7; 222,5]	159,0 [108,0; 206,5]	196,0 [155,0; 265,0]	0,090
LA	3488,5 [3217,5; 3745,7]	3517,0 [3309,5; 3733,0]	3374,0 [3052,0; 3759,0]	0,224
GLA	85,0 [64,0; 102,0]	97,0 [58,5; 111,5]	89,0 [74,0; 114,0]	0,261
DHGLA	199,5 [132,7; 269,0]	200,0 [120,0; 307,0]	282,0 [253,0; 324,0]	**0,002**
AA	1229,5 [1044,5; 1334,0]	1154,0 [922,0; 1310,5]	1279,47 [1233,0; 1342,0]	0,110
DTA	31,0 [26,0; 34,2]	29,0 [22,0; 36,5]	33,0 [31,0; 37,0]	**0,017**
DPA	34,0 [27,0; 44,0]	35,0 [25,5; 43,0]	42,0 [33,0; 50,0]	**0,023**
GEX	70,0 [60,0; 80,0]	68,0 [62,0; 78,0]	78,0 [61,0; 84,0]	0,431
OL	1993,5 [1544,2; 2462,0]	1677,0 [1237,5; 2457,0]	2130,0 [1761,0; 2676,0]	0,243
MID	24,0 [20,0; 28,0]	23,0 [18,5; 27,5]	23,0 [21,0; 29,0]	0,741
SEL	87,0 [74,0; 104,2]	91,0 [80,5; 108,0]	101,0 [80,0; 115,0]	0,157

Примечание: p — сравнение между 1 и 3 группами
Note: p — is a comparison between groups 1 and 3

Discussion

Some studies evaluate the association between omega-3 FA and alcohol [3, 13, 14]. In the body, α-linolenic FA is a biochemical basis for long-chain omega-3 UFA. The levels of long-chain polyunsaturated omega-3 UFAs, including eicosapentanoic and docosahexaenic acids, are associated with susceptibility to alcohol in vertebrate and invertebrate models [15, 16]. Long-chain polyunsaturated FAs inhibit development of acute functional alcohol tolerance, i.e., omega-3 UFA, particularly eicosapentanoic acid, is essential for the normal response to alcohol [15, 16]. In humans, acute ethanol tolerance is closely associated with susceptibility to alcohol abuse. It has been established that three weeks of supplements containing eicosapentanoic and docosahexaenic UFAs significantly reduced the stress level and cortisone concentrations in alcohol-dependent individuals abstaining from alcohol [17]. Impact of FAs on the response to ethanol can be a result of genetic factors. There is considerable genetic contribution to alcohol response variability in humans [15, 18]. Understanding genetic factors is essential for establishing susceptibility to alcohol and development of an efficient therapy [15].

In this study, we found statistically significant increase in blood concentrations of α-linolenic omega-3 UFA in the group of high alcohol consumption vs. mild alcohol consumption group. The concentration of the long-chain eicosapentanoic and docosahexaenic omega-3 UFAs was also higher in this group, however not statistically significant.

Dihomo-gamma-linolenic UFA is a product of elongation of omega-6 gamma-linolenic acid using delta-6-desaturase, which is a product of linoleic acid desaturation (18:2, omega-6) [19]. Alcohol is known to inhibit delta-6 and delta-5-desaturases, which participate in conversion of omega-6 UFA. Alcohol directly affects cell membrane composition, increasing the level of omega-6 polyunsaturated FA in membranes, thus boosting their flowability and damage to the cells. This pathological effect can be partially mitigated by changing the omega-6/omega-3 FA ratio by adding omega-3 UFA [19].Also, in the CHS study (a study of the cardiovascular health), dihomo-gamma-linolenic UFA is proposed as a potential biomarker in the development of unfavourable events, such as stroke [20].

We established an increase in blood concentrations of dihomo-gamma-linolenic FA by 29% in the high alcohol consumption group. Gamma-linolenic and arachidonic FA concentrations tended to rise in the high alcohol consumption group; however, the increase was not statistically significant. Concentrations of docosatetraenoic and docosapentaenoic omega-6 UFAs, products of arachidonic FA metabolism, varied considerably in mild and high alcohol consumption groups, p=0.017 and p=0.023, respectively. This study design does not allow making assumptions as to the mechanisms of these changes.

Study Limitation

The study enrolled a small sample size; subjects were divided into groups unevenly; and there was no control group (non-drinkers). Also, the questionnaire did not take into account omega-3 FA supplements. The study was cross-sectional, so no follow-up is used.

Conclusion

This study demonstrated that alcohol consumption changes UFA spectrum. It has been shown that in male subjects aged 35–74 years old, living in rural areas of the Novosibirsk Region, from the high alcohol consumption group, the concentrations of α-linolenic omega-3 UFA and dihomo-gamma-linolenic, docosatetraenoic, docosapentaenoic omega-6 UFA were higher than in the mild alcohol consumption group. Therefore, identification of any changes in the unsaturated fatty acid profile can be used as an additional prognostic biomarker, allowing to assess the risk of CVD and their complications in male subjects consuming high amounts of alcohol.

Вклад авторов:

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Шрамко В.С.: сбор, анализ и интерпретация данных; разработка концепции и дизайна исследования

Стахнёва Е.М.: анализ и интерпретация данных, написание и подготовка рукописи к публикации

Каштанова Е.В.: анализ и интерпретация данных; проверка критически важного интеллектуального содержания и утверждение рукописи для публикации

Щербакова Л.В.: анализ и интерпретация данных, статистическая обработка результатов

Полонская Я.В.: анализ и интерпретация данных

Рагино Ю.И.: анализ и интерпретация данных, утверждение рукописи для публикации

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

Shramko V.S.: data collection, analysis and interpretation; development of the research concept and design

Stakhneva E.M.: data analysis and interpretation, writing and preparation of a manuscript for publication

Kashtanova E.V.: data analysis and interpretation; verification of critical intellectual content and approval of the manuscript for publication

Shcherbakova L.V.: data analysis and interpretation, statistical processing of results

Polonskaya Ya.V.: data analysis and interpretation

Ragino Yu.I.: data analysis and interpretation, approval of the manuscript for publication

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