VILNIUS UNIVERSITY

ASTA MAŽEIKIENĖ

ASSESSMENT AND ANALYSIS OF ANTIOXIDANT CAROTENOIDS IN LITHUANIAN POPULATION AND THEIR SIGNIFICANCE IN THE ETIOPATHOGENESIS OF CARDIOVASCULAR DISEASES

Summary of doctoral dissertation

Biomedical Sciences, Medicine (06 B)

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VILNIAUS UNIVERSITETAS

ASTA MAŽEIKIENĖ

ANTIOKSIDANTŲ KAROTENOIDŲ TYRIMAS IR VERTINIMAS LIETUVIŲ POPULIACIJOJE, JŲ SVARBA ŠIRDIES IR KRAUJAGYSLIŲ LIGŲ ETIOPATOGENEZĖJE

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ABBREVIATIONS

ABCG5 – ATP-binding cassette subfamily G member 5

Apo A-I – apolipoprotein A-I

Apo B – apolipoprotein B

BCDO2 – β, β-carotene-9,10'-oxygenase

BCMO1– β, β-carotene-15,15'-monooxygenase

BHT – butylhydroxytoluene

BMI – body mass index

CD36 – cluster of differentiation 36

CI – confidence interval

CINDI – research project "Countrywide Integrated Noncomunicable Disease Intervention"

CRP – C-reactive protein (hs-CRP – high sensitivity CRP)

CVD – cardiovascular disease

CVD – cardiovascular diseases

EH – Eastern Highlanders

FABP – fatty acid-bindig protein

GSTP1 – glutathione S-transferase Pi 1

HCC – healthcare centre

HDL-C – high density lipoprotein cholesterol

HDP – high density polyethylene

HPLC – high-performance liquid chromatography

HR-LBP – human retinal lutein-binding protein

LDL-C – low density lipoprotein cholesterol

LITGEN – VU MF research project "Genetic diversity of the population of Lithuania and changes of its genetic structure associated with evolution and common diseases"

LitHir – the Lithuanian High Cardiovascular Risk primary prevention program

LiVicordia – Linköping–Vilnius coronary disease risk assessment study

LMC – Centre of Laboratory Medicine

Lp (a) – lipoprotein (a)

MAF – minor allele frequency

MeOH – methanol

MF – Faculty of Medicine

MI – myocardial infarction

MONICA – research project "Multinational Monitoring of Trends and Determinants of Cardiovascular Disease"

MTBE – tert-butyl-methylether

NPC1L1 – Niemann–Pick C1 like1

NS – Northern Samogitians

OR – odds ratio

p – the probability of getting a value of the test statistic as extreme as or more extreme than that observed by chance alone, if the null hypothesis is H0

PTFE – polytetrafluoroethylene

SD – standard deviation

SH – Southern Highlanders

SNP – single nucleotide polymorphism

SPSS – Statistical Package for the Social Sciences

SR-BI – scavenger receptor class B type I

SS – Southern Samogitians

TAG – triacylglycerols

Total-C – total cholesterol

VU – Vilnius University

VUH SK – Vilnius University Hospital Santariškių Klinikos

WH – Western Highlanders

WS – Western Samogitians

$\chi2$ – chi-square statistics for the test of independence

CONTENT

1. INTRODUCTION ... 9

1.1. Study object ... 9

1.2. Novelty and practical significance of the study ... 9

1.3. Study aim ... 11

1.4. Study goals ... 11

1.5. Defensive statements ... 11

2. METHODS ... 12

2.1. The study group ... 12

2.2. Study methods ... 15

2.2.1. Study methods for demographic and life-style factors as well as morbidity ... 15

2.2.2. The methodology of lycopene intake assessment ... 15

2.2.3. Collection of blood, sample preparation, storage and transportation 16

2.2.4. Analysis of carotenoid levels in blood serum samples ... 16

2.2.5. Analysis of biochemical blood markers ... 21

2.2.6. Study of genome data ... 21

2.2.7. Statistical data analysis ... 22

3. RESULTS ... 23

3.1. Results of measuring blood serum carotenoid levels ... 23

3.2. Correlations of demographic and life-style factors with blood serum carotenoid levels ... 24

3.3. Study of lycopene consumption and its importance for the assessment of cardiovascular risk ... 35

3.4. Importance of serum carotenoid levels for the assessment of cardiovascular risk ... 39

3.5. Influence of demographic and life-style factors and biochemical blood markers on the blood serum concentration of carotenoids ... 42

3.6. Association between genome markers and blood serum carotenoid concentrations ... 44
4. CONCLUSIONS ... 49
REFERENCES ... 51
SANTRAUKA ... 54
STUDY APPROBATION ... 55
ABOUT THE AUTHOR ... 58
APIE AUTORĘ ... 59

1. INTRODUCTION

1.1. Study object

Studies of the prevalence of traditional risk factors of cardiovascular diseases and their correction have been among the priority areas in Lithuania for several years. MONICA (Multinational Monitoring of trends and Determinants of Cardiovascular Disease) (Domarkienė et al., 2003) and CINDI (Countrywide Integrated Noncomunicable Disease Intervention) (Klumbienė et al., 2002; Grabauskas et al., 2008) regional projects initiated some decades ago were among the originators of such studies. The importance of the problem currently is reflected by the studies carried out by individual research teams as well as by Lithuanian national governmental programmes aiming to reduce morbidity, such as the Lithuanian High Cardiovascular Risk Primary Prevention Programme, LitHir, which has been initiated in 2006 and involved 94.8% of all primary health care centres in Lithuania (Laucevičius et al., 2012, 2013, 2015). Despite the fact that the traditional risk factors of atherosclerosis are prevailing among the cardiovascular risk factors both worldwide and in Lithuania (Rinkūnienė et al., 2012, 2014), high morbidity rates still remain, thus, researches continuously look for non-traditional, new biochemical (Banys et al., 2012, 2014; Coj et al., 2013) and genomic markers (Domarkienė et al., 2013) suitable for assessing the cardiovascular risk, the diagnosis of cardiovascular diseases and at the same time for the reduction of morbidity rates. Attempts have been made to address combinations of risk factors characteristic for the population and to disclose the inherent mechanisms of the disease as well as the causal biological etiopathogenesis.

1.2. Novelty and practical significance of the study

Cardiovascular diseases are multifactorial diseases; the damage caused by the oxidative stress plays an important role in their etiopathogenesis. Oxidation is a process permanently taking place in the human body, resulting in the formation of free radicals (FR), i.e. unstable molecules or their parts having an unpaired electron (Kučinskienė, 2001). Low concentrations of free radicals are required to maintain the normal function of a cell, as they act as secondary signalling molecules; hydrogen peroxide formed in leukocytes and hypochlorous acid are active and strong oxidisers killing microorganisms, nitrogen oxides play a role in the regulation of blood pressure (Didžiapetrienė et al., 2011). Different endogenous processes as well as exogenous factors – stress, inflammation, smoking, alcohol use, environmental pollution or ageing – result in a more intensive formation of free radicals; they react with biomolecules such as DNA,

proteins, lipids present in the cell, and damage them, leading to an oxidative stress. It has been proved that free radicals and other reactive oxygen compounds are involved in the pathogenesis of more than one hundred diseases, including the cardiovascular disease (Montezano et al. 2010; Ashor et al., 2014; Domej et al., 2014; Lobo et al., 2015; Cruickshank et al., 2015). However, a body is able to prevent the effects of the above-mentioned compounds – they are neutralised by various enzymatic and non-enzymatic antioxidative systems. Antioxidants consumed with food also play an important role. Therefore, the consumption of foods of plant origin, especially fruits and vegetables rich in different antioxidants, is promoted in the chronic disease preventive programmes. Antioxidant carotenoids – natural plant pigments–play a particular role in the prevention of harmful effects caused by reactive oxygen compounds and the suppression of oxidative stress (Ginter et al., 2014; Fiedor et al., 2014). More than 95% of carotenoids found in the human body comprise β-carotene, α-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin (Namitha et al., 2010). The biological effects of carotenoids are not restricted to the antioxidative action. These compounds are well known as the precursors of vitamin A, they can affect the interactions among cells, the metabolism of xenobiotics or immune response. Many epidemiological studies have been carried out worldwide, proving that carotenoids are among the most important exogenous lipophilic antioxidants reducing the risk of many chronic non-infectious diseases, but we know very little about the levels of these compounds in the Lithuanian population. Certainly, the most important and probably the only study in this area was the scientific project "LiVicordia", implemented jointly by Lithuanian and Swedish scientists. The study proved that the blood levels of lipid-soluble antioxidants – lycopene and β-carotene – in males living in Vilnius were statistically significantly lower than in study individuals from Linköping (Kristenson et al., 1997). The concentration of antioxidants was tested in Sweden.

The high-performance liquid chromatography method (HPLC) for the analysis of blood carotenoid levels were introduced for the first time in Lithuania during our study; the mean blood concentrations of carotenoids were established in the Lithuanian population, which allowed to assess the obtained results in the context of data of analogous studies carried out in other European countries.

It is worth noting that studies to prove the biological activity of carotenoids usually are performed *in vitro* and using animal models. However, our understanding about the metabolism of these compounds and their biological effects on the human body is limited. It is expected that the resorption of carotenoids, their blood levels and the distribution in the tissues are influenced both by nutrition or other physiological factors and the polymorphism of genes, coding receptors of lipoproteins as well as proteins and enzymes involved in lipid transfer and the metabolism of carotenoids. Only a few studies have been carried out worldwide to establish genetic regions and gene variations associated with the levels of carotenoids in the human body, coding proteins involved in the

metabolism of carotenoids and associated with the diversity of individual response to consumption. In our study, we assessed correlations between the serum concentration of carotenoids and the demographic, life-style, nutrition and other epidemiological factors as well as genotype-dependent features. The study is exceptional, because all study individuals were of Lithuanian origin; this fact provided us with the opportunity to establish certain features characteristic of a specific rather than of a mixed population.

1.3. Study aim

To establish the mean blood concentration of carotenoids, factors predetermining such concentration and the correlation with cardiovascular morbidity as well as with the biochemical blood risk factors for these diseases in the Lithuanian population.

1.4. Study goals

1) to evaluate the mean blood serum concentration of an individual and the total carotenoids in the study population;
2) to analyse the association of life-style and demographic factors with the variation of blood serum concentrations of carotenoids;
3) to estimate the mean lycopene intake, to establish its main food sources and correlations with the serum lycopene concentration;
4) to establish the genome markers associated with the blood serum concentration of carotenoids;
5) to establish the correlations of blood serum carotenoid concentrations and lycopene intake with cardiovascular morbidity and the blood biochemical risk factors of CVD.

1.5. Defensive statements

1. The blood serum concentration of carotenoids is associated with the life-style, nutritional, demographic and genetic factors.
2. The lower blood serum concentration of carotenoids is associated with higher rates of cardiovascular morbidity.

2. METHODS

2.1. The study group

Dissertation-related studies were carried out as a part of the project implemented by the Medicine Faculty of Vilnius University in 2011–2015 under the priority 3 of the Human Resource Development Action Program Strengthening Researchers Abilities. The project title: "Genetic diversity of the population of Lithuania and changes of its genetic structure associated with the evolution and common diseases" (LITGEN, project leader Prof. Habil. Dr. V. Kučinskas). Permission No. 158200-05-329-79 to conduct this study was issued by the Vilnius Regional Biomedical Research Ethics Committee (dated 3 May 2011).

Aiming to ensure the representative study sample, the structure of the Lithuanian population according to ethnolinguistic groups was considered (Jakaitienė et al., 2013).

The study population involved 726 individuals of Lithuanian origin, 4 to 85 years old, randomly selected from six ethnolinguistic regions (29 cities and boroughs): 64 (8.9%) Eastern Highlanders (EH), 70 (9.6%) – Southern Highlanders (SH), 97 (13.4%) – Western Highlanders (WH), 247 (34%) – Northern Samogitians (NS), 181 (24.9%) – Southern Samogitians (SS), and 67 (9.2%) – Western Samogitians (WS) (**Fig. 1**).

(a) (b)

Figure 1. Distribution of study population according to (a) Lithuanian cities, (b) ethnolinguistic regions (according to the classification of dialects proposed by A. Girdenis and Z. Zinkevičius (based on "Lietuvių kalbos atlasas I" map No. 1 (1977)).

Study individuals visited primary health care centres located in the cities or districts they live. The distribution of the study group according to age and gender is presented in **Figure 2.** 50.3% individuals of the study group were females and 49.7% were males; 66.9% of them lived in cities and boroughs and 33.1% in rural areas. The Lithuanian origin of the participants was proved in three generations, i.e. parents and grandparents of the study individuals were of Lithuanian origin.

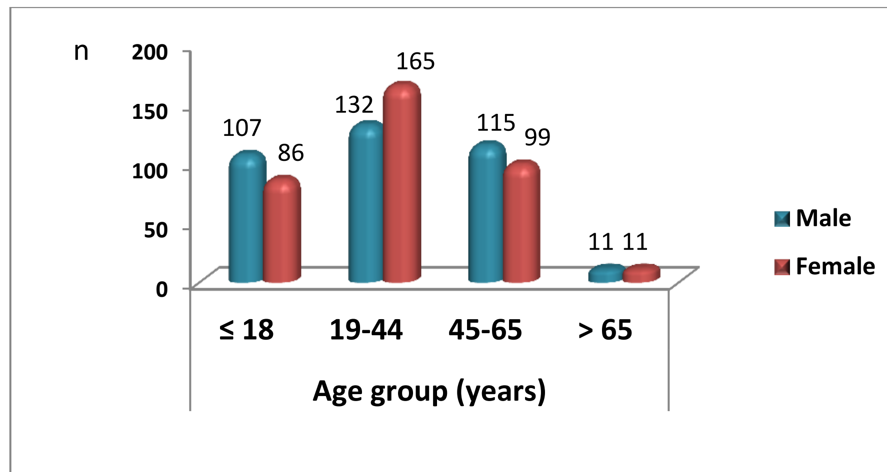


Figure 2. The structure of the study group according to age and gender

Characteristics of study individuals are presented in **Table 1**.

Table 1. Characteristics of the study group

Characteristic	Age groups (years)							
	≤ 18		19–44		45–65		> 65	
	n = 193		n = 297		n = 214		n = 22	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (y)	14	3	35	9	52	5	74	7
Height (cm)	160	34	170	24	168	25	167	9
Weight (kg)	56	19	76	22	87	48	81	17
Body mass index (BMI) (kg/m^2)	20	4	26	6	30	18	29	4
Glucose (mmol/l)	5.06	0.79	5.31	1	5.71	1.45	5.43	0.63
Total-C (mmol/l)	4.45	0.88	5.69	1.18	6.2	1.19	6.42	1.23
TAG (mmol/l)	0.83	0.4	1.39	1.01	1.6	1.67	1.43	0.52
HDL-C (mmol/l)	1.45	0.3	1.44	0.36	1.4	0.36	1.36	0.26
LDL-C (mmol/l)	2.62	0.74	3.6	1.01	4.08	1.05	4.4	1.09
*hs*CRP (mg/l)	1.02	2.53	2.04	3.37	2.35	3.44	6.05	9.47
Apo A-I (g/l)	1.49	0.24	1.61	0.29	1.63	0.28	1.56	0.29
Apo B (g/l)	0.67	0.18	0.93	0.28	1.07	0.26	1.07	0.23
Lp (a) (g/l)	0.16	0.24	0.19	0.24	0.22	0.28	0.17	0.24

Aiming to establish the correlations of the mean blood serum concentration of carotenoids with cardiovascular morbidity as well as with biochemical blood risk factors for these diseases, individuals with cardiovascular diseases (myocardium infarction and/or stroke) were selected from the total study sample – 50 study individualss in total – and, considering the age of the individuals, a control group consisting of 316 study individualss without a cardiovascular disease was formed. The characteristics of the above-mentioned groups are presented in **Table 2**.

For the study of genome markers, 220 non-related individuals 40 to 65 years old were randomly selected from the study sample (50.0% of study individuals in this group were females and 49.5% males).

Table 2. Characteristics of study individuals with and without cardiovascular diseases

Characteristic			Individuals with CVD		Individuals without CVD	
			n = 316		n = 50	
Gender	Male	n	164		21	
	Female	n	152		29	
			Mean	SD	Mean	SD
Age (y)			53	9	58	7
Height (cm)			169	23	163	36
Weight (kg)			85	42	83	21
Body mass index (BMI) (kg/m^2)			29	15	29	4
Glucose (mmol/l)			5.66	1.37	5.63	1.41
Total-C (mmol/l)			6.17	1.09	6.15	1.40
TAG (mmol/l)			1.55	1.48	1.63	1.09
HDL-C (mmol/l)			1.41	0.36	1.42	0.42
LDL-C (mmol/l)			4.06	0.99	3.98	1.15
*hs*CRP (mg/l)			2.16	2.83	4.57	8.28
Apo A-I (g/l)			1.62	0.28	1.63	0.34
Apo B (g/l)			1.05	0.25	1.08	0.31
Lp (a) (g/l)			0.19	0.24	0.26	0.32

2.2. Study methods

2.2.1. Study methods for demographic and life-style factors as well as morbidity

An interview using a validated questionnaire (Kristenson M., 1997; Mažeikienė A., 2008) was applied to assess the life-style, family history, nutrition habits and health status. Questions about the demographic data of study individualss, their life-style and dietary habits as well as health status were included in the questionnaire.

Study individuals were asked to assess their physical activity and movement at work and during their leisure time (during several recent years) according to the presented examples representing four groups. Habits of smoking (4 groups were specified: smoking every day, smoking rarely, discontinued smoking and never smoking) and alcohol use (4 groups were specified: using often, using occasionally, never drinking alcohol, and drinking alcohol in the past) were assessed using questionnaires.

Data about cardiovascular morbidity (myocardial infarction, stroke) were presented by the study individuals in the questionnaire. Information on health status presented in the questionnaires of randomly selected 8% of study individualss was checked with the medical records available at patients' primary health care establishments. Diagnoses of all study individuals who indicated cardiovascular diseases in the questionnaire matched the information in the medical records.

The study individuals were asked to specify their height and weight in the questionnaire. The BMI was calculated using the formula:

$$\text{BMI (Kettle's index)} = \text{weight (kg)} / \text{height (m)}^2.$$

The study individuals were assigned into four groups on the basis of their BMI: too low weight (BMI < 18.5 kg/m^2), normal weight (BMI 18.5–24.9 kg/m^2), overweight (BMI 25–29.9 kg/m^2) and obesity (BMI ≥30 kg/m^2) (Tutkuvienė et al., 2004).

2.2.2. The methodology of lycopene intake assessment

72-hour recall method was used to evaluate mean lycopene intake and its dietary sources; the main focus was on the consumption of lycopene-rich products such as tomatoes and their products (fresh tomatoes, cooked tomatoes, canned tomatoes, tomato sauce, tomato soup (thickened), tomato juice, tomato ketchup), apricots (dried), pink grapefruit (fresh), watermelons (fresh), papaya, guava. Study induviduals were asked to specify the consumed amount of the above-mentioned foods or the size of serving as precisely as possible. The amount of lycopene obtained from these products was calculated on the basis of data for European Carotenoids Database (O'Neill et al., 2001) containing summarised data from

different European countries on the amount of lycopene present in different alimentary products (in milligrams for 100 g of product weight).

2.2.3. Collection of blood, sample preparation, storage and transportation

Study individuals had to arrive into the primary health care establishment located in the city or district they live from 7:30 to 10:00 a.m. in fasting condition for at last 12 hours, abstaining from smoking, use of alcohol and medications; in the facility, venous blood samples were taken from the study individuals (a 5 ml vacuum tube was used (*BD Vacutainer SST II Advance* (*Becton Dickinson,* USA)). 40 minutes later tubes with collected blood samples were centrifuged for 10 min at a speed of 3000 rpm (centrifuge LMC-3000 was used (*Biosan*, Latvia). Samples were stored at +2 – + 8° C (in refrigerator) up to transportation. To avoid temperature variations, the cooled samples were transported in thermostable containers (polystyrene foam boxes). Serum samples were transported to the Centre of Laboratory Medicine of the Santariškių Clinics, Vilnius University Hospital within 3–6 hours and were kept there in a deep-freezer at –80° C. The carotenoid concentration was analysed using HPLC within 6–12 months after the sample collection. Blood sample collection, preparation, transportation of a sample and all further stages of the analysis were carried out avoiding direct sun or intensive artificial light, to prevent the dissociation or isomerisation of carotenoids.

2.2.4. Analysis of carotenoid levels in blood serum samples

The levels of six blood serum carotenoids – lutein, zeaxanthin, α- and β-carotene, β-cryptoxanthin and lycopene – were tested in the blood serum of a study subject using HPLC.

2.2.4.1. Sample preparation

Reagents:

- echinenone (β,β-carotene-4-one), (*Chromadex*, USA)
- n-hexane for HPLC (*Merck*, Germany)
- ethanol for HPLC (*Merck*, Germany)
- butylhydroxytoluene (*Merck*, Germany)
- methanol for HPLC, 99.9% purity (*Sigma–Aldrich*, Germany)
- tert-butyl-methylether for HPLC, 99.8% purity (*Carl Roth*, Germany)
- nitrogen gas (> 99.996%)

Equipment and laboratory instruments:

- Vortex RS-VF10 (*Phoenix instrument GmbH*, Germany)
- Micro-centrifuge Velocity 13μ (*Dynamica*, UK)
- Dry-Bath Incubator, model DB28125 (*Barnstead Thermolyne*, USA)
- Adjustable volume micropipettes ACURA, 20–200 ml and 100–1000 ml volume (*Socorex*, Switzerland)

- microtubes (2 ml volume)
- screw-caped chromatography bottles, brown glass, for HPLC (*Agilent Technologies*, USA)
- screw-caps with PTFE / silicone seal for HPLC (*Agilent Technologies*, USA)
- inserts for open-mouthed bottles, 250 µl volume for HPLC (*Agilent Technologies*, USA)

Carotenoid extraction and HPLC analysis were carried out according to the Boehm et al. methodology (Boehm et al., 2006)**.** An ethanolic echinenone solution (50 µl of echinenone standard solution in 10 ml of ethanol) was used as the internal standard. A microtube with 1 ml of serum and 500 µl of internal standard was vortexed for 30 seconds. Then 400 µl of hexane with 0.1% of butylhydroxytoluene was added and vortexed for 1 minute; the sample was subsequently centrifuged for 2 minutes. The centrifugation speed was 14 000 rpm. The extraction steps were repeated twice for each sample. The supernatant (hexane layer) was transferred after each centrifugation to a new tube and finally completely dried applying a gentle flow of the nitrogen gas at a temperature of 30 ± 1° C. The residue was dissolved in 250 µl of MeOH and MTBE (1:1, v/v) mixture and centrifuged for 4 min at a speed of 14 000 rpm. The superficial layer was transferred into a chromatography bottle.

2.2.4.2. Preparation of standard solutions of carotenoids

Reagents:

- standard carotenoids (crystal form) for HPLC, purity 95–98% (*Chromadex*, USA):
- β-carotene (β, β-carotene) 96%, β-cryptoxanthin (3*R*)–β, β-carotene-3-ol) 97 %, α-carotene (6'R)-β, ε-carotene) 97%, echinenone (β, β-carotene-4-one) 98%, lutein (xanthophyll (3*R*,3'*R*,6'*R*)–β, ε-carotene-3,3'-diol) 96%, lycopene (ψψ-carotene) 95%, zeaxanthin (3*R*,3'*R*)-β, β-carotene-3,3'-diol) 97%
- toluene (*Merck*, Germany)
- cyclohexane (*Merck*, Germany)

Equipment and laboratory instruments:

- membrane filters, pore size 0.2 µm (*Macherey-Nagel*, Germany)
- ultrasound bath USC100T (*VWR*, Belgium)
- brown plastic HDP bottle, 15 ml

Carotenoids in the study sample were identified using standard carotenoids (crystal form, purity 95–98%) for HPLC. Crystals of standard carotenoids were dissolved in a toluene–cyclohexane mixture (1:4 v/v), standard lutein and zeaxanthin were dissolved in ethanol (1:10) and dispensed into brown plastic HDP

bottles. The sediment was removed using an ultrasound bath and membrane filters. Standard carotenoid solutions were stored in a refrigerator at a temperature of –80° C.

The working solutions of standard carotenoids were prepared before each test. For this purpose, the above-mentioned standard solutions were diluted with a mobile phase (MeOH: MTBE (1:1, v/v)) in proportion of 1:50, 1:100, 1:250 and 1:500. For each chromatography test, 20 µl of these working solutions were used.

2.2.4.3. Spectrophotometrical testing of the standard carotenoid solutions

Reagents:

- solutions of carotenoid standard (~40-150 µg/ml): standard carotenoids in the toluene–cyclohexane mixture (1:4); lutein and zeaxanthin standards in ethanol (1:10)
- n-hexane for HPLC (*Merck*, Germany)
- ethanol for HPLC (*Merck*, Germany)
- petrol ether for HPLC (*B.D.H.*, Great Britain)

Equipment and laboratory instruments:

- quartz glass microcuvette, 1 cm light path (10 mm, 45x12.5x12.5 mm, 700 µl) (*Hellma*, Germany)
- ground volumetric transparent glass flasks with a glass stopper (*Brand*, Germany)
- Pasteur pipettes, glass, 150 mm length, not graduated
- spectrophotometer Agilent 8453*(Agilent Technologies,* USA)
- ultrasound bath USC100T (*VWR*, Belgium)
- purified deionised water (prepared in a water-purifying system *(Millipore*, USA),
- Sartorius balances (*CP64-OCE*, Germany)

The concentrations of standard carotenoid solutions were tested periodically using spectrophotometry. Solutions of standard carotenoids were diluted 1:10, 1:20 or 1:50 with ethanol, hexane or petroleum ether, and the extinction coefficient was established by spectrophotometry using an appropriate wave length. Wave length and $E_{1\%,\ 1cm}$ are presented in **Table 3**.

Carotenoid	**Solvent**	**λmax (nm)**	**$E_{1\%,1cm}$**	**Dilution**
(*E*)- lutein	Ethanol	445	2550	1:10
(*E*)- zeaxanthin	Ethanol	450	2480	1:10
(*E*)- β-cryptoxanthin	Petroleum ether	449	2400	1:20
(*E*)-β- carotene	n-hexane	450	2590	1:20
(*E*)-α- carotene	n-hexane	445	2710	1:20
(*E*)-lycopene	Petroleum ether	470	3450	1:50

Table 3. Solvents, wave length and $E_{1\%,\ 1cm}$ values used to calculate the concentration of carotenoid standard solutions (Craft et al., 1988; Olmedilla et al., 1990; Hart et al., 1995; Britton et al., 2004)

Calculation:

$c\ [g/100ml] = ((E \times 1g/100ml)/\ E_{1\%,1cm}) \times \text{dilution factor}$
in our study: $E = E_{\text{carotenoid standard solution}} - E_{\text{solvent}}$
$c\ [\mu g/ml] = E \times 1000/E_{1\%,1cm} \times 10 \times \text{dilution factor}\ (10, 20 \text{ or } 50)$

The specific extinction coefficient $E_{1\%,\ 1cm}$ is defined as the theoretical absorption value of 1% (i.e. 1 g in 100 ml) concentration in a quartz glass microcuvette, 1 cm light path.

2.2.4.4. Chromatographic separation and identification of carotenoids

Reagents: ▪ methanol for HPLC, 99.9% purity (*Sigma-Aldrich*, Germany) ▪ tert-butyl-methylether for HPLC, 99.8% purity (*Carl Roth*, Germany)

Equipment
▪ High-performance liquid chromatography system (*Shimadzu Prominence*, Japan)
    ▪ column oven CTO20AC
    ▪ absorbance detector SPD-M20A
    ▪ system controller CBM-20A
    ▪ solvents delivery unit LC-30AD
    ▪ online degasser DGU-20A5R
    ▪ auto-sampler SIL-30AC
▪ HPLC column, Stabilty 100 C30, 250 mm × 4.6 mm; particle size 5 μm (*Dr.Maisch GmbH*, Germany)
▪ HPLC pre-columns, C30, 20 mm × 4.6 mm (*Dr.Maisch GmbH*, Germany)
▪ a holder for protecting HPLC pre-columns (*Dr.Maisch GmbH*, Germany)

Chromatographic separation was performed using the system for high-performance liquid chromatography (*Shimadzu Prominence,* Japan), with C30 (250 mm × 4.6 mm; particle size 5 μm) HPLC column and protective pre-columns (C30, 20 mm × 4.6 mm). The temperature in the column: 23 ± 1° C. The volume of sample injected into HPLC during the test: 30 μl. A two-component mobile phase of methyl-tert-butyl ether and methanol was used, the flow rate was 1.3 ± 1 ml/min; change in gradient conditions during the test is presented in **Table 4**.

Table 4. Parameters of mobile phase composition and gradient elution formation in HPLC

Time (min.)	MeOH (%)	MTBE (%)
0.01	90	10
35	55	45
45	40	60
50	90	10

A UV-visible spectrophotometer was used for detection, wave length 450 nm (470 nm – for lycopene concentration measurements). An example of the serum carotenoids chromatogram is presented in **Figure 3**. Lutein and zeaxanthin are isomers with identical chemical formula and characterised by common biological functions. Therefore, the total concentration of these compounds was calculated in the study.

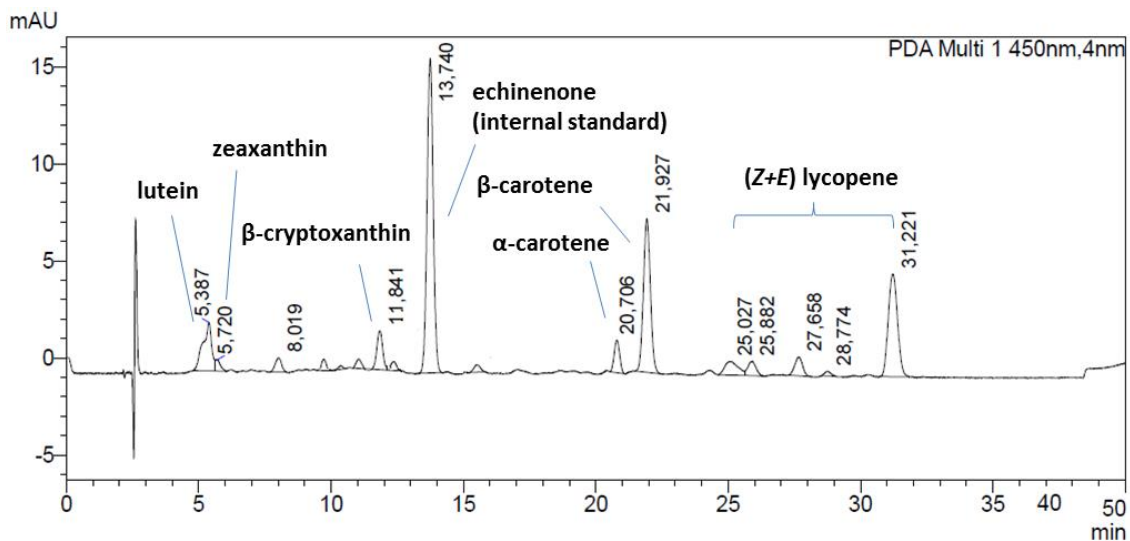


Figure 3. An example of serum carotenoid separation using the HPLC method

2.2.4.5. Quality control

Good laboratory practice principles have been followed at the laboratory during testing, as well as requirements for external quality control, laboratory surveillance, preparation for laboratory testing and protocol.

At least two blood serum samples were tested for carotenoid concentrations for each study individual. The reproducibility of the internal standard was 92 ± 12%.

The concentrations of standard carotenoid solutions were tested periodically using spectrophotometry, and the calibration of the instrument was performed.

2.2.5. Analysis of biochemical blood markers

The biochemical analysis was performed in the Biochemistry Laboratory of the Centre of Laboratory Medicine of the Santariškių Clinics Hospital of Vilnius University. The list of methods and analysers is presented in **Table 5**.

Test	Method	Analyser
Total-C	Enzymatic colorimetric assay	Architect ci8200 (Abbott, USA)
HDL-C	Enzymatic colorimetric assay	Architect ci8200 (Abbott, USA)
LDL-C	Estimated using the Friedewald equation *in case of triglyceride concentration >4.5 mmol/l, direct enzymatic clearance assay was used	________ *Architect ci8200 (Abbott, USA)
TAG	Enzymatic colorimetric assay	Architect ci8200 (Abbott, USA)
hsCRP	Immunoturbidimetric assay	Architect ci8200 (Abbott, USA)
Glucose	Immunoturbidimetric assay	Architect ci8200 (Abbott, USA)
Apo A-I	Immunonephelometric assay	BN II (Siemens, Germany)
Apo B	Immunonephelometric assay	BN II (Siemens, Germany)
Lp (a)	Immunonephelometric assay	BN II (Siemens, Germany)

Table 5. Methods and analysers used to perform biochemical blood tests at the Biochemistry Laboratory of the Centre of Laboratory Medicine of Santariškių Clinics Hospital of Vilnius University

2.2.6. Study of genome data

The genotyping was performed using *Illumina Infinium® HD* SNP lusts (~770 000 SNP): *HumanOmniExpress-12 v1.1* (at the Laboratory of Molecular Genetics and Cytogenetics of Medicine Genetics Centre, Santariškių Clinics Hospital of Vilnius University) and *HumanOmniExpress-12 v1.0.* (at the Institute of Molecular and Cell Biology of Tartu University, Estonia).

Study individuals were genotyped for 91 SNP in 9 genes-candidates: *APOE, BCMO1, NPC1L1, CD36, GSTP1, ABCG5, FABP1, FABP2,* and *LIPC.*

The extraction of DNA, measurements of DNA concentration and purity, genotyping and bioinformation – statistical quality analysis of primary data – were performed by the staff of the Department of Human and Medicine Genetics of Medicine Faculty, Vilnius University. The equipment used and a detailed description of the procedures have been presented in the doctoral dissertation of Dr. Ingrida Domarkienė (Domarkienė I., 2015).

2.2.7. Statistical data analysis

Descriptive and analytical statistical methods were applied in the study. For quantitative variables, descriptive statistics was presented as the arithmetic mean, median, standard deviation (SD) with a 95% confidence interval (CI) and quartiles; qualitative variables were expressed as absolute numbers (n) and percentage (%). Histograms were used to assess the distribution of quantitative variables in case of a normal distribution curve, and the Kolmogorov–Smirnov test was used to verify a specific statistical hypothesis. If the distribution of variables did not match the normal distribution curve, non-parametric criteria were used to assess the differences between the distributions of variables (Mann–Whitney U test and Kruskal–Wallis test). Statistical correlations between the variables were assessed applying Pearson's (when normality assumption had been met) or Spearmen's correlation coefficient (r). The correlation was considered as week ($r < 0.3$), moderate ($0.3 \leq r \leq 0.7$) or strong (> 0.7). Results were considered as statistically significant if the value of error likelihood was $p < 0.05$. The logarithm values of quantitative variables enabled the use of the dispersion analysis (ANOVA) at the beginning for the variables not meeting the normal distribution model and to calculate the differences of mean values among the study samples. To identify statistically significant differences among the study groups, the Bonferroni test was used for multiple comparisons. For the prognosis of values of a dependent variable according to the values of independent variables, the multifactorial linear regression test was applied. An independent variable was entered into the regression equation if its *p* value did not exceed 0.05. Pearson's χ^2 (chi square) test was used to assess a statistical correlation between the qualitative variables (to verify the hypothesis of independence). Data were described in a four-field (2×2) frequency table; if at least one potential number of observations was less than 5, the exact Fisher's test was calculated additionally. When the risk variable was analysed according to the causal (independent) variable, the odds ratio (OD) was assessed; the latter was considered as statistically significant, if the value was not within the 95% of the confidence interval.

The *Microsoft Excel* and *SPSS* (IBM SPSS v21.0) software were used for statistical data analysis. The *Nutrisurvey* software was used to calculate lycopene amounts in food products. The Hardy–Weinberg equilibrium and allele frequencies were calculated with the *PLINK v1.07* software.

3. RESULTS

3.1. Results of measuring blood serum carotenoid levels

Mean concentrations of individual carotenoids and their total concentration in the blood serum were measured (μmol/l): lutein + zeaxanthin 0.278 ± 0.131, β-cryptoxanthin 0.15 ± 0.168, α-carotene 0.153 ± 0.121, β-carotene 0.522 ± 0.37, lycopene 0.557 ± 0.299, total 1.60 ± 0.72. The prevailing blood carotenoids (65%) were lycopene and β-carotene (**Figure 4**).

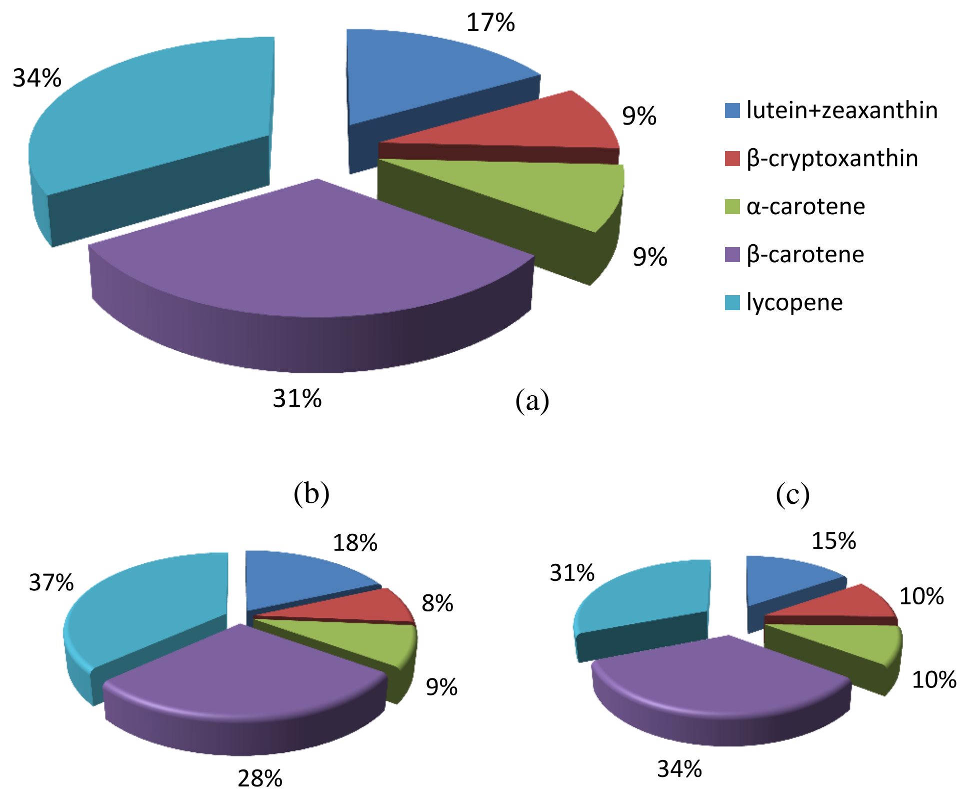


Figure 4. Blood serum carotenoids in individuals of the study group: (a) total population, (b) group of males, (c) group of females

3.2. Correlations of demographic and life-style factors with blood serum carotenoid levels

Differences of mean concentrations of individual carotenoids and their total concentration in the blood serum according to gender were assessed in this study (**Table 6**). The mean total blood serum concentration of carotenoids in females was statistically significantly higher ($p = 0.0015$) than in males (1.75 ± 0.75 and 1.46 ± 0.66 μmol/l, accordingly). The serum β-cryptoxanthin concentration in the group of females was higher 1.5 times and of α-carotene and β-carotene 1.4 times than in males.

Table 6. Comparison of mean concentrations of individual carotenoids and their total concentration in blood serum (μmol/l) in the study groups of different genders

Carotenoid concentration (μmol / l)		Gender		ANOVA (p value)
		Male	Female	
		n = 365	n = 361	
Lutein + zeaxanthin	Mean	0.28	0.28	0.653
	SD	0.13	0.14	
β-cryptoxanthin	Mean	0.12	0.18	0.0000*
	SD	0.11	0.2	
α-carotene	Mean	0.13	0.18	0.0000*
	SD	0.1	0.14	
β-carotene	Mean	0.43	0.61	0.0000*
	SD	0.32	0.4	
Lycopene	Mean	0.56	0.56	0.45
	SD	0.32	0.28	
Total carotenoid conc.	Mean	1.46	1.75	0.0015*
	SD	0.66	0.75	

* — statistically significant difference ($p < 0.05$).

Statistically significant differences ($p < 0.01$) in the mean concentrations of all tested carotenoids except β-carotene were discovered in the groups of study individuals of different age. The lowest concentrations of individual carotenoids and their total concentration in blood serum were found in the group of individuals above 65 years old. The mean concentration of β-cryptoxanthin in this group was lower even by 55% to compare with the group of children (**Table 7**).

Table 7. Comparison of mean concentrations of individual carotenoids and their total concentration in blood serum (μmol/l) in the groups of different age

Carotenoid concentration (µmol / l)		Age groups (years)				ANOVA (p value)
		≤ 18	19–44	45–65	> 65	
		n = 93	n = 297	n = 214	n = 22	
Lutein + zeaxanthin	Mean	0.35	0.29	0.28	0.25	0.019*
	SD	0.09	0.13	0.15	0.20	
β-cryptoxanthin	Mean	0.20	0.14	0.12	0.09	0.000*
	SD	0.25	0.11	0.13	0.07	
α-carotene	Mean	0.14	0.16	0.16	0.10	0.001*
	SD	0.11	0.13	0.13	0.07	
β-carotene	Mean	0.53	0.52	0.53	0.38	0.261
	SD	0.35	0.35	0.42	0.17	
Lycopene	Mean	0.49	0.64	0.53	0.34	0.000*
	SD	0.26	0.30	0.31	0.23	
Total carotenoid conc.	Mean	1.56	1.68	1.57	1.22	0.004*
	SD	0.69	0.71	0.77	0.45	

* — statistically significant difference ($p < 0.05$).

A comparison of concentrations of the tested carotenoids among the groups of different age revealed statistically significant differences in mean concentrations of lycopene, α-carotene and β-cryptoxanthin (**Table 8**).

Table 8. Differences of carotenoid concentrations among the age groups (p-values (Bonferroni test))

Lutein + zeaxanthin					**β-carotene**				
Age (y.)	≤ 18	19–44	45–65	>65	Age (y.)	≤ 18	19–44	45–65	>65
≤ 18		0.154	1.000	0.044	≤ 18		1.000	1.000	0.462
19–44	0.154		1.000	0.434	19–44	1.000		1.000	1.000
45–65	1.000	1.000		0.178	45–65	1.000	1.000		1.000
> 65	0.044	0.434	0.178		>65	0.462	1.000	1.000	
β- cryptoxanthin					**Lycopene**				
Age (y.)	≤ 18	19–44	45–65	>65	Age (y.)	≤ 18	19–44	45–65	>65
≤ 18	19–44	45–65	>65	0.000	≤ 18		0.000	1.000	0.001
19–44	0.002		0.088	0.015	19–44	0.000		0.000	0.000
45–65	0.000	0.088		0.256	45–65	1.000	0.000		0.000
> 65	0.000	0.015	0.256		> 65	0.001	0.000	0.000	
α-carotene					**Total carotenoid concentration**				
Age (y.)	≤ 18	19–44	45–65	>65	Age (y.)	≤ 18	19–44	45–65	>65
≤ 18		0.034	0.042	0.351	≤ 18		0.473	1.000	0.095
19–44	0.034		1.000	0.012	19–44	0.473		0.151	0.009
45–65	0.042	1.000		0.012	45–65	1.000	0.151		0.144
> 65	0.351	0.012	0.012		> 65	0.095	0.009	0.144	

— statistically significant difference ($p < 0.05$).

Table 9. Comparison of mean concentrations of individual carotenoids and their total concentration in blood serum (μmol/l) in the study groups according body mass index

Carotenoid concentration (μmol/l)		BMI categories				ANOVA (p value)
		too small weight	normal weight	overweight	obesity	
		BMI				
		<18.5	18.5–24.9	25–29.9	≥30	
		n = 65	n = 280	n = 167	n = 214	
Lutein+ zeaxanthin	Mean	0.27	0.28	0.29	0.27	0.782
	SD	0.09	0.13	0.15	0.13	
β-cryptoxanthin	Mean	0.21	0.17	0.13	0.12	0.000*
	SD	0.20	0.20	0.11	0.12	
α-carotene	Mean	0.12	0.17	0.16	0.14	0.008*
	SD	0.09	0.13	0.12	0.11	
β-carotene	Mean	0.56	0.56	0.55	0.44	0.000*
	SD	0.38	0.37	0.40	0.33	
Lycopene	Mean	0.54	0.57	0.59	0.47	0.012*
	SD	0.25	0.27	0.33	0.32	
Total carotenoid concentration	Mean	1.58	1.68	1.66	1.46	0.002*
	SD	0.70	0.73	0.75	0.68	

* — statistically significant difference ($p < 0.05$).

Statistically significant differences ($p < 0.05$) in the concentrations of all tested carotenoids, except lutein and zeaxanthin, were discovered among the BMI categories (**Table 9**).

A lower total concentration of carotenoids, β-carotene and β-cryptoxanthin was found in the group of obese study individuals to compare with the study individuals with a normal body weight and overweight; lower levels of α-carotene were reported in obese study individuals as compared with those having a normal body weight (**Table 10**).

Table 10. Differences of carotenoid concentrations among BMI categories (p-values (Bonferroni test))

Lutein + zeaxanthin					β-carotene				
BMI	<18.5	18.5–24.9	25–29.9	≥30	**BMI**	<18.5	18.5–24.9	25–29.9	≥30
<18.5		1.000	1.000	1.000	<18.5		1.000	1.000	0.005
18.5–24.9	1.000		1.000	1.000	18.5–24.9	1.000		1.000	0.000
25–29.9	1.000	1.000		1.000	25–29.9	1.000	1.000		0.005
≥30	1.000	1.000	1.000		≥30	0.005	0.000	0.005	
β-cryptoxanthin					**Lycopene**				
BMI	<18.5	18.5–24.9	25–29.9	≥30	**BMI**	<18.5	18.5–24.9	25–29.9	≥30
<18.5		0.452	0.002	0.000	<18.5		0.058	0.099	1.000
18.5–24.9	0.452		0.019	0.000	18.5–24.9	0.058		1.000	0.142
25–29.9	0.002	0.019		1.000	25–29.9	0.099	1.000		0.318
≥30	0.000	0.000	1.000		≥30	1.000	0.142	0.318	
α-carotene					**Total carotenoid concentration**				
BMI	<18.5	18.5–24.9	25–29.9	≥30	**BMI**	<18.5	18.5–24.9	25–29.9	≥30
<18.5		0.101	0.095	1.000	<18.5		1.000	1.000	0.562
18.5–24.9	0.101		1.000	0.038	18.5–24.9	1.000		1.000	0.001
25–29.9	0.095	1.000		0.101	25–29.9	1.000	1.000		0.031
≥30	1.000	0.038	0.101		≥30	0.562	0.001	0.031	

[shaded] — statistically significant difference ($p < 0.05$).

Probably the highest differences in carotenoid levels were detected in different seasons of the year: mean concentrations of all carotenoids were statistically significantly different ($p < 0.0000$) (**Table 11**). The lowest mean total concentration of carotenoids was reported in spring (1.42 ± 0.67 µmol/l) and the highest in autumn (1.72 ± 0.73 µmol/l) (**Fig. 5**).

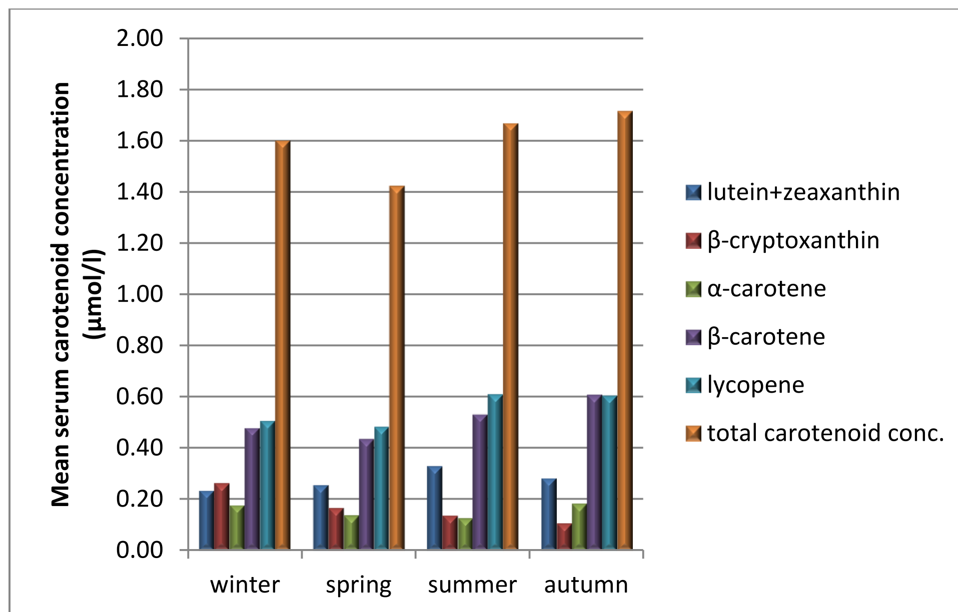


Figure 5. Mean concentration of carotenoids (µmol/l) in different seasons

Table 11. Comparison of mean concentrations of individual carotenoids and their total concentration in blood serum (µmol/l) in different seasons

Carotenoid concentration (µmol/l)		Season				ANOVA (p value)
		Winter	Spring	Summer	Autumn	
		n = 101	n = 205	n = 172	n = 248	
Lutein + zeaxanthin	Mean	0.23	0.25	0.33	0.28	0.000*
	SD	0.12	0.12	0.14	0.13	
β-cryptoxanthin	Mean	0.26	0.16	0.13	0.10	0.000*
	SD	0.31	0.14	0.11	0.07	
α-carotene	Mean	0.17	0.14	0.12	0.18	0.000*
	SD	0.16	0.11	0.09	0.12	
β-carotene	Mean	0.48	0.43	0.53	0.61	0.000*
	SD	0.34	0.33	0.35	0.40	
Lycopene	Mean	0.51	0.48	0.61	0.60	0.000*
	SD	0.28	0.29	0.32	0.29	
Total carotenoid concentration	Mean	1.60	1.42	1.67	1.72	0.000*
	SD	0.82	0.67	0.67	0.73	

* — statistically significant difference ($p < 0.05$).

Table 12. Differences of carotenoid concentrations in different seasons (p-values (Bonferroni test))

Lutein + zeaxanthin					β-carotene				
	Winter	Spring	Summer	Autumn		Winter	Spring	Summer	Autumn
Winter		0.743	0.000	0.003	Winter		0.398	0.988	0.013
Spring	0.743		0.000	0.107	Spring	0.398		0.001	0.000
Summer	0.000	0.000		0.001	Summer	0.988	0.001		0.349
Autumn	0.003	0.107	0.001		Autumn	0.013	0.000	0.349	
β- cryptoxanthin					**Lycopene**				
	Winter	Spring	Summer	Autumn		Winter	Spring	Summer	Autumn
Winter		0.000	0.000	0.000	Winter		1.000	0.203	0.027
Spring	0.000		0.283	0.000	Spring	1.000		0.000	0.000
Summer	0.000	0.283		0.070	Summer	0.203	0.000		1.000
Autumn	0.000	0.000	0.070		Autumn	0.027	0.000	1.000	
α-carotene					**Total carotenoid concentration**				
	Winter	Spring	Summer	Autumn		Winter	Spring	Summer	Autumn
Winter		0.025	0.004	1.000	Winter		0.160	1.000	0.574
Spring	0.025		1.000	0.000	Spring	0.160		0.001	0.000
Summer	0.004	1.000		0.000	Summer	1.000	0.001		1.000
Autumn	1.000	0.000	0.000		Autumn	0.574	0.000	1.000	

* — statistically significant difference ($p < 0.05$).

Table 12 shows p-values for statistically significant differences in the mean concentrations of carotenoids in the groups of study individuals in different seasons.

The study results revealed that the mean concentrations of β-carotene, lycopene and the total mean concentration of carotenoids were statistically significantly different ($p < 0.02$) in the groups of different physical activity – the lowest levels were reported in the individuals spending leisure time at intensive workouts and participating in sport competitions (**Table 13**).

Table 13. Comparison of mean concentrations of individual carotenoids and their total concentration in blood serum (µmol / l) in the study groups characterised by different physical activity at leisure time

Carotenoid concentration (µmol / l)		Physical activity (at leisure time)				ANOVA (p value)
		group 1	group 2	group 3	group 4	
		n = 220	n = 342	n = 97	n = 23	
Lutein+ zeaxanthin	Mean	0.27	0.28	0.29	0.25	0.263
	SD	0.14	0.12	0.15	0.11	
β-cryptoxanthin	Mean	0.13	0.16	0.16	0.15	0.073
	SD	0.12	0.18	0.19	0.09	
α-carotene	Mean	0.15	0.16	0.15	0.14	0.137
	SD	0.13	0.12	0.11	0.13	
β-carotene	Mean	0.53	0.56	0.54	0.51	0.005*
	SD	0.36	0.37	0.34	0.33	
Lycopene	Mean	0.53	0.58	0.58	0.51	0.026*
	SD	0.30	0.30	0.29	0.25	
Total carotenoid concentration	Mean	1.53	1.69	1.67	1.52	0.002*
	SD	0.73	0.71	0.69	0.65	

* — statistically significant difference ($p < 0.05$).

Significant differences in the mean concentrations of β-carotene, lycopene and the total mean concentration of carotenoids were detected among the study individuals from the groups of the lowest physical activity (group 1) and the moderate physical activity (group 2) (**Table 14**).

Table 14. Differences of carotenoid concentrations among the groups of study individuals characterised by different physical activity at leisure time (p-values (Bonferroni test))

Lutein + zeaxanthin					β-carotene				
Physical activity (at leisure time)	group 1	group 2	group 3	group 4	**Physical activity (at leisure time)**	group 1	group 2	group 3	group 4
group 1		0.554	1.000	1.000	group 1		0.004	0.096	1.000
group 2	0.554		1.000	1.000	group 2	0.004		1.000	1.000
group 3	1.000	1.000		1.000	group 3	0.096	1.000		1.000
group 4	1.000	1.000	1.000		group 4	1.000	1.000	1.000	

β- cryptoxanthin					Lycopene				
Physical activity (at leisure time)	group 1	group 2	group 3	group 4	**Physical activity (at leisure time)**	group 1	group 2	group 3	group 4
group 1		0.073	1.000	0.972	group 1		0.035	0.137	1.000
group 2	0.073		1.000	1.000	group 2	0.035			1.000
group 3	1.000	1.000		1.000	group 3	0.137	1.000	1.000	1.000
group 4	0.972	1.000	1.000		group 4	1.000	1.000	1.000	

α-carotene					Total carotenoid concentration				
Physical activity (at leisure time)	group 1	group 2	group 3	group 4	**Physical activity (at leisure time)**	group 1	group 2	group 3	group 4
group 1	group 2	group 3	group 4	1.000	group 1		0.002	0.110	1.000
group 2	0.252		1.000	0.882	group 2	0.002		1.000	1.000
group 3	1.000	1.000		1.000	group 3	0.110	1.000		1.000
group 4	1.000	0.882	1.000		group 4	1.000	1.000	1.000	

* — statistically significant difference ($p < 0.05$).

Aiming to detect differences in the blood serum concentration of individual carotenoids and their total mean concentration, which are influenced by physical activity at work, younger than 18-year-old individuals and those who could not assign themselves to any of the above-mentioned groups of physical activity at work were excluded from the study sample. The Study data demonstrated that the mean concentration of β-carotene and the mean total concentration of carotenoids were statistically significantly different among specific groups of physical activity at work ($p = 0.015$) – the lowest mean concentration of β-carotene was reported in the group of physically most active individuals (**Table 15**).

When carotenoid concentrations were compared among individual groups of physical activity, statistically significant differences in the mean concentration of β-carotene and in the total mean concentration of carotenoids were reported in the group of individuals involved in very heavy working activities (group 1) and the group of individuals engaged in moderately heavy work (**Table 16**).

Table 15. Comparison of the mean concentrations of individual carotenoids and their total concentration in the blood serum (μmol/l) of the study groups characterised by different physical activity at work

Carotenoid concentration (μmol/l)		Physical activity (at work)				ANOVA (p value)
		1 group	2 group	3 group	4 group	
		n = 162	n = 189	n = 90	n = 56	
Lutein+ zeaxanthin	Mean	0.27	0.30	0.30	0.26	0.074
	SD	0.12	0.15	0.14	0.14	
β-cryptoxanthin	Mean	0.13	0.15	0.13	0.12	0.068
	SD	0.10	0.13	0.10	0.12	
α-carotene	Mean	0.17	0.18	0.15	0.13	0.110
	SD	0.13	0.14	0.10	0.08	
β-carotene	Mean	0.56	0.59	0.43	0.40	0.003*
	SD	0.41	0.43	0.24	0.23	
Lycopene	Mean	0.59	0.60	0.58	0.55	0.657
	SD	0.30	0.32	0.29	0.31	
Total carotenoid concentration	Mean	1.66	1.75	1.53	1.41	0.028*
	SD	0.73	0.84	0.52	0.58	

* — statistically significant difference (p < 0.05).

Table 16. Differences of carotenoid concentrations among the groups of study individuals characterised by different physical activity at work (p-values (Bonferroni test))

Lutein + zeaxanthin					**β-carotene**				
Physical activity (at work)	group 1	group 2	group 3	group 4	**Physical activity (at work)**	group 1	group 2	group 3	group 4
group 1		1.000	0.488	1.000	group 1		1.000	0.402	0.034
group 2	1.000		1.000	0.287	group 2	1.000		0.094	0.006
group 3	0.488	1.000		0.145	group 3	0.402	0.094		1.000
group 4	1.000	0.287	0.145		group 4	0.034	0.006	1.000	
β- cryptoxanthin					**Lycopene**				
Physical activity (at work)	group 1	group 2	group 3	group 4	**Physical activity (at work)**	group 1	group 2	group 3	group 4
group 1		1.000	1.000	0.121	group 1		1.000	1.000	1.000
group 2	1.000		1.000	0.056	group 2	1.000		1.000	1.000
group 3	1.000	1.000		0.476	group 3	1.000	1.000		1.000
group 4	0.121	0.056	0.476		group 4	1.000	1.000	1.000	

α-carotene					Total carotenoid concentration				
Physical activity (at work)	group 1	group 2	group 3	group 4	**Physical activity (at work)**	group 1	group 2	group 3	group 4
group 1		1.000	1.000	0.422	group 1		1.000	1.000	0.120
group 2	1.000		0.753	0.182	group 2	1.000		1.000	0.020
group 3	1.000	0.753		1.000	group 3	1.000	1.000		0.594
group 4	0.422	0.182	1.000		group 4	0.120	0.020	0.594	

— statistically significant difference ($p < 0.05$).

Concentrations of all studied carotenes were statistically significantly different ($p < 0.05$) between the groups of smokers and non-smokers (**Table 17**).

Table 17. Comparison of mean concentrations of individual carotenoids and their total concentration in blood serum (μmol/l) in the study groups with different smoking habits

Carotenoid concentration (μmol/l)		Smoking habits				ANOVA (p value)
		Everyday	Rarely	Never	Formerly	
		n = 77	n = 52	n = 500	n = 76	
Lutein + zeaxanthin	Mean	0.26	0.28	0.28	0.29	0.188
	SD	0.14	0.16	0.13	0.11	
β-cryptoxanthin	Mean	0.10	0.13	0.16	0.15	0.000*
	SD	0.08	0.12	0.18	0.13	
α-carotene	Mean	0.12	0.16	0.16	0.16	0.009*
	SD	0.08	0.11	0.13	0.56	
β-carotene	Mean	0.35	0.54	0.56	0.44	0.000*
	SD	0.26	0.39	0.38	0.31	
Lycopene	Mean	0.54	0.65	0.54	0.63	0.018
	SD	0.29	0.28	0.29	0.36	
Total carotenoid concentration	Mean	1.32	1.69	1.65	1.60	0.001*
	SD	0.53	0.66	0.75	0.69	

* — statistically significant difference ($p < 0.05$).

Table 18. Differences of carotenoid concentrations between the groups of smokers and non-smokers (p-values (Bonferroni test))

Lutein + zeaxanthin				
Smoking	Often	Rarely	Never	Formerly
Often		1.000	0.438	0.245
Rarely	1.000		1.000	1.000
Never	0.438	1.000		1.000
Formerly	0.245	1.000	1.000	

β-carotene				
Smoking	Often	Rarely	Never	Formerly
Often		0.001	0.000	0.126
Rarely	0.001		1.000	0.435
Never	0.000	1.000		0.033
Formerly	0.126	0.435	0.033	

β- cryptoxanthin				
Smoking	Often	Rarely	Never	Formerly
Often		0.084	0.000	0.003
Rarely	0.084		1.000	1.000
Never	0.000	1.000		1.000
Formerly	0.003	1.000	1.000	

Lycopene				
Smoking	Often	Rarely	Never	Formerly
Often		0.066	1.000	0.782
Rarely	0.066		0.053	1.000
Never	1.000	0.053		0.707
Formerly	0.782	1.000	0.707	

α-carotene				
Smoking	Often	Rarely	Never	Formerly
Often		0.016	0.017	0.094
Rarely	0.016		1.000	1.000
Never	0.017	1.000		1.000
Formerly	0.094	1.000	1.000	

Total carotenoid concentration				
Smoking	Often	Rarely	Never	Formerly
Often		0.006	0.001	0.057
Rarely	0.006		1.000	1.000
Never	0.001	1.000		1.000
Formerly	0.057	1.000	1.000	

— statistically significant difference ($p < 0.05$).

The concentrations of α-carotene and β-carotene circulating in the blood of actively smoking persons were by 25% and even by 37.5% lower than in non-smokers. The concentration of β-cryptoxanthin in the group of ex-smokers was lower 1.5 times as compared with the study individuals who never smoked. The concentration of carotenoids, which do not have properties of vitamin A precursors – lycopene, lutein and zeaxanthin – in the blood of smokers and non-smokers did not reach a statistically significant difference (**Table 18**).

The groups of individuals with different habits of alcohol use were statistically significantly different ($p < 0.002$) by mean concentrations of α-carotene, lycopene and β-cryptoxanthin (**Table 19**).

The serum concentration of β-cryptoxanthin was almost two times (1.8) higher and the concentration of lycopene – 1.4 times higher in individuals abstaining from alcohol, to compare with the study individuals often drinking alcohol. The concentration of α-carotene was insignificantly (by 12.5%) higher in the blood serum of individuals sometimes drinking alcohol than in those who never used it (**Table 20**).

Table 19. Comparison of the mean concentrations of individual carotenoids and their total concentration in the blood serum (μmol/l) of the study groups with different habits of alcohol use

Carotenoid concentration (μmol / l)		Alcohol use				ANOVA (p value)
		Often	Rarely	Never	Formerly	
		n = 27	n = 432	n = 221	n = 27	
Lutein + zeaxanthin	Mean	0.27	0.28	0.26	0.32	0.127
	SD	0.13	0.13	0.12	0.14	
β-cryptoxanthin	Mean	0.10	0.14	0.18	0.13	0.003*
	SD	0.07	0.12	0.23	0.10	
α-carotene	Mean	0.15	0.16	0.14	0.15	0.002*
	SD	0.14	0.12	0.12	0.12	
β-carotene	Mean	0.43	0.52	0.54	0.51	0.108
	SD	0.34	0.37	0.38	0.44	
Lycopene	Mean	0.48	0.60	0.67	0.53	0.000*
	SD	0.38	0.30	0.26	0.36	
Total carotenoid concentration	Mean	1.54	1.65	1.56	1.57	0.195
	SD	0.84	0.72	0.71	0.85	

* — statistically significant difference ($p < 0.05$).

Table 20. Differences in carotenoid concentrations among the study groups with different habits of alcohol use (p-values (Bonferroni test))

Lutein + zeaxanthin

Alcohol use	Often	Rarely	Never	Formerly
Often		1.000	1.000	1.000
Rarely	1.000		0.389	1.000
Never	1.000	0.389		0.308
Formerly	1.000	1.000	0.308	

β-carotene

Alcohol use	Often	Rarely	Never	Formely
Often		0.394	0.169	1.000
Rarely	0.394		1.00	1.000
Never	0.169	1.000		1.000
Formerly	1.000	1.000	1.00	

β- cryptoxanthin

Alcohol use	Often	Rarely	Never	Formerly
Often		0.123	0.008	1.000
Rarely	0.123		0.119	1.000
Never	0.008	0.119		0.476
Formerly	1.000	1.000	0.476	

Lycopene

Alcohol use	Often	Rarely	Never	Formerly
Often		1.000	0.066	0.258
Rarely	1.000		0.000	0.177
Never	0.066	0.000		1.000
Former	0.258	0.177	1.000	

α-carotene

Alcohol use	Often	Rarely	Never	Formerly
Often		1.000	1.000	1.000
Rarely	1.000		0.001	1.000
Never	1.000	0.001		1.000
Formerly	1.000	1.000	1.000	

Total carotenoid concentration

Alcohol use	Often	Rarely	Never	Formerly
Often		1.000	1.000	1.000
Rarely	1.000		0.455	1.000
Never	1.000	0.455		1.000
Formerly	1.000	1.000	1.000	

— statistically significant difference ($p < 0.05$).

3.3. Study of lycopene consumption and its importance for the assessment of cardiovascular risk

Analysis of nutrition of the study individuals has revealed that the main sources of lycopene are tomatoes and their products (78%), watermelons (15%), pink grapefruits (7%). The biggest amounts of lycopene – 2.09 [1.85; 2.33] mg/day, i.e. 36% – were obtained by consuming fresh tomatoes; the intake of tomatoes per day on average was 69.29 [61.47; 77.11] g. Rather big amounts of this anti-oxidant are also received with tomato juice (1.23 [0.84; 1.,62] mg/day, i.e. 21%) (**Figure 6, Table 21**).

It has been estimated that the mean amount of lycopene consumed by one individual per day was 5.77 [5.09; 6.45] mg, median 2.36 mg, i.e. 0.08 milligrams for one kilogram of body mass per day.

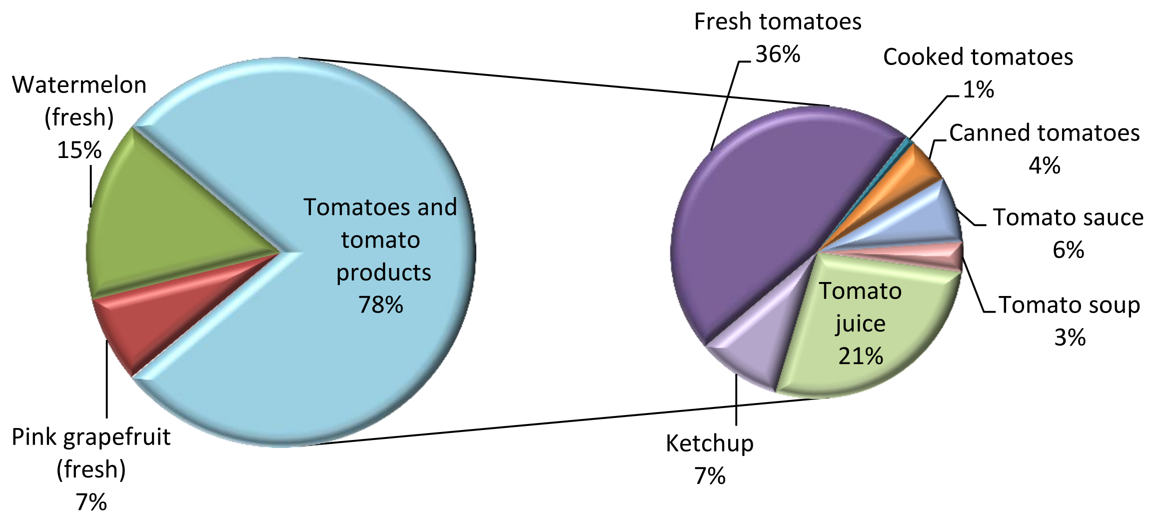


Figure 6. Mean amount of lycopene consumed per day with different food products, percentage

Table 21. Mean consumption of food products containing lycopene (g per day) and mean amount of lycopene consumed per day with different food products (mg per day)

Food product	Overall intake (g/d)			Lycopene intake (mg/d)		
	Mean	Mean CI		Mean	Mean CI	
Tomatoes and tomato products	100.32	90.12	110.53	4.47	3.94	5.00
Fresh tomatoes	69.29	61.47	77.11	2.09	1.85	2.33
Cooked tomatoes	1.24	0.36	2.12	0.05	0.01	0.08
Canned tomatoes	4.55	3.02	6.09	0.23	0.15	0.30
Tomato sauce	5.26	4.54	5.99	0.33	0.28	0.37
Tomato soup	1.84	0.52	3.16	0.15	0.04	0.25
Tomato juice	15.38	10.52	20.24	1.23	0.84	1.62
Ketchup	3.35	2.77	3.93	0.41	0.34	0.49
Apricot (dried)	4.00	2.04	5.96	0.005	0.003	0.01
Pink grapefruit (fresh)	12.56	8.66	16.47	0.41	0.28	0.55
Watermelon (fresh)	21.69	12.15	31.23	0.87	0.49	1.25

Females receive this antioxidant with food products 5.97 [4.85; 6.29] mg/day on average. This amount is statistically significantly ($p = 0.000$) higher than that consumed by males (5.57 [5.33; 6.62] mg/day). Lycopene consumption was also statistically significantly ($p = 0.000$) different in the subgroups of the study individuals of different age. The lowest consumption of lycopene with food was reported among the more than 65 years old individuals (2.92 [2.53; 3.31] mg/day) (**Figure 7**).

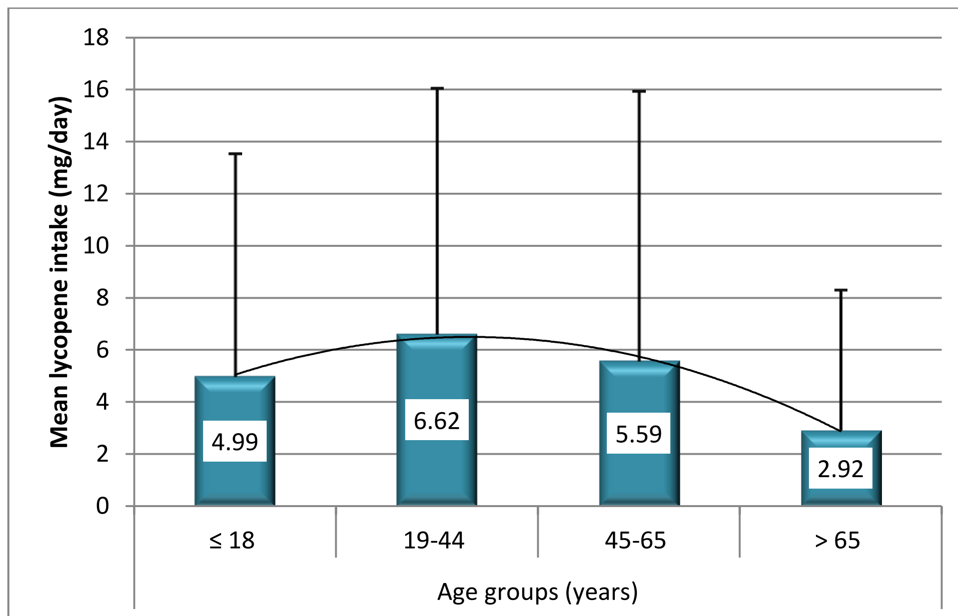


Figure 7. Consumption of lycopene in different age groups the (Histogram illustrates the mean consumption of lycopene (mg/ day) in each age group).

Seasonal changes influence lycopene consumption (**Figure 8**) – the consumed amount in autumn is 2.1 times higher than in spring. Mean lycopene intake among individuals living in rural areas and in cities (5.52 [5.22; 6.62] and 5.92 [4.87; 6.17] mg/day) did not reach a statistically significant difference. No statistically significant difference was discovered when lycopene consumption was compared between the groups of study individuals with and without CVD ($p = 0.393$). Differences in lycopene consumption between the above-mentioned groups are presented in **Table 22**.

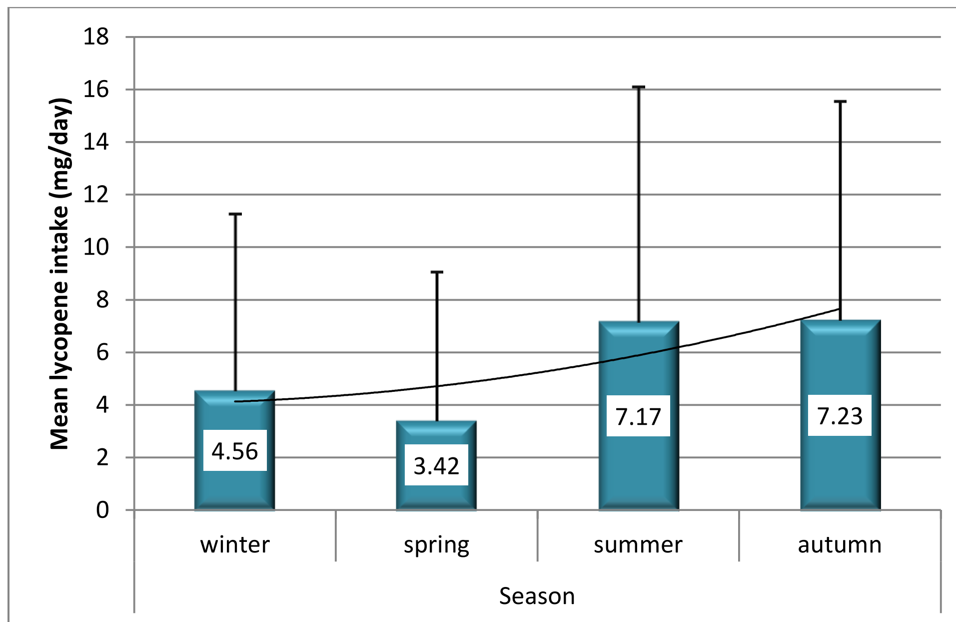


Figure 8. Consumption of lycopene in different seasons the (histogram illustrates the mean consumption of lycopene (mg/day))

The correlation of lycopene intake with biochemical blood markers is illustrated in **Table 23**. A positive statistically significant correlation was established between the consumed amount of lycopene and increased levels of total cholesterol and high-density lipoprotein cholesterol ($r = 0.095$; $p < 0.011$ and $r = 0.084$; $p < 0.024$, respectively). A positive statistically significant correlation was also established between the serum concentrations of lutein, zeaxanthin, lycopene, α- and β-carotene, β-cryptoxanthin as well as the total levels of carotenoids and lycopene consumption.

Table 22. Differences in mean lycopene consumption (mg/day) predetermined by seasonal changes, age, gender and place of residence as well as cardiovascular morbidity

		n	Mean lycopene intake (mg/day)				p value*
			Median	Mean	Mean 95 % CI		
Gender	Male	365	1.80	5.57	4.85	6.29	0.01**
	Female	361	3.03	5.97	5.33	6.62	
Age group (years)	≤18	193	1.80	4.99	4.37	5.61	0.000**
	19–44	297	3.31	6.62	5.93	7.31	
	45–65	214	1.86	5.59	4.84	6.34	
	>65	22	0.16	2.92	2.53	3.31	
Seson	Winter	101	1.72	4.56	4.07	5.05	0.000**
	Spring	205	1.17	3.42	3.02	3.83	
	Summer	172	4.00	7.17	6.53	7.82	
	Autumn	248	3.00	7.23	6.33	8.12	
Living area	Urban	484	2.42	5.92	5.22	6.62	0.393
	Rural	239	2.30	5.52	4.87	6.17	
CVD	Non-diseased	316	2.50	5.89	4.91	6.87	0.075
	Diseased	50	1.67	4.29	3.59	4.99	

* Mann–Whitney U or Kruskal Wallis test; **— statistically significant difference ($p < 0,01$).

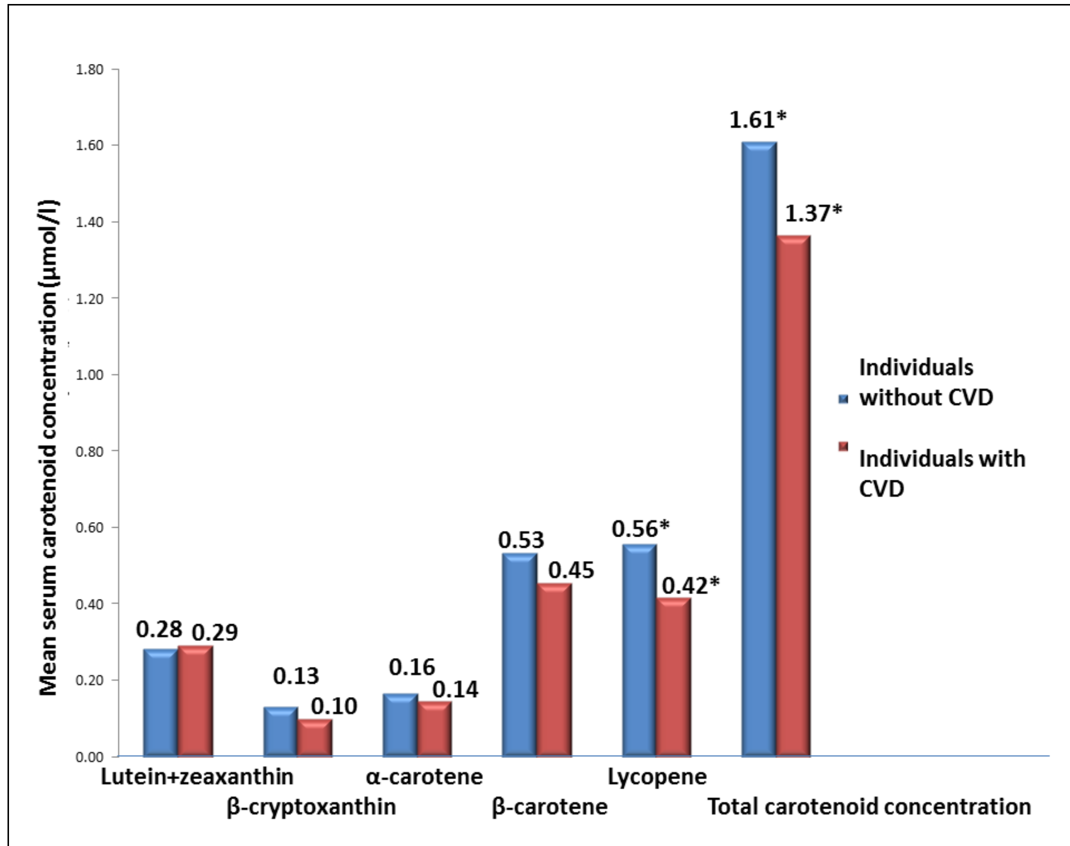
Table 23. Correlation of lycopene consumption with different biochemical blood markers

Biochemical parameter	Spearman correlation cofficient (r)	p value
	n = 736	
Glucose (mmol/l)	0.029	0.428
Total-C (mmol/l)	0.095	0.011*
TAG (mmol/l)	0.061	0.103
HDL-C (mmol/l)	0.084	0.024*
LDL-C (mmol/l)	0.062	0.097
*hs*CRB (mg/l)	0.051	0.170
Apo A-I (g/l)	0.069	0.068
Apo B (g/l)	0.030	0.428
Lp (a) (g/l)	0.096	0.091
Lutein+ zeaxanthin (μmol/l)	0.127	0.001**
β-cryptoxanthin (μmol/l)	0.004	0.921
α-carotene (μmol/l)	0.077	0.039*
β-carotene (μmol/l)	0.144	0.000**
Lycopene (μmol/l)	0.361	0.000**
Total carotenoid concentration (μmol/l)	0.240	0.000**

— statistically significant correlation ($p < 0.05$) (**$p < 0.01$).

3.4. Importance of serum carotenoid levels for the assessment of cardiovascular risk

It has been established that the mean concentration of lycopene and the mean total carotenoid concentrations were statistically significantly lower in the group of study individuals with CVD to compare with disease-free individuals (**Table 24, Figure 9**).



*— statistically significant difference ($p < 0.05$).

Figure 9. Mean serum concentration of carotenoids (μmol/l) in the groups of study individuals with and without cardiovascular diseases. The mean concentration (μmol/l) of carotenoids was presented in the histogram

Table 24. Comparison of the mean concentrations of individual carotenoids and their total concentration in blood serum (μmol/l) in the study groups of individuals with and without cardiovascular diseases

Carotenoid concentration (μmol/l)		Individuals without CVD	Individuals with CVD	ANOVA (p value)
		n = 316	n = 50	
Lutein+zeaxanthin	Mean	0.28	0.29	0.871
	SD	0.14	0.18	
β-cryptoxanthin	Mean	0.13	0.10	0.059
	SD	0.12	0.08	
α-carotene	Mean	0.16	0.14	0.118
	SD	0.12	0.14	
β-carotene	Mean	0.53	0.45	0.448
	SD	0.40	0.32	
Lycopene	Mean	0.56	0.42	0.001*
	SD	0.30	0.24	
Total carotenoid concentration	Mean	1.61	1.37	0.03*
	SD	0.75	0.61	

*— statistically significant difference ($p < 0.05$).

The risk of CVD development was assessed after the evaluation of differences in the concentrations of the above-mentioned compounds: this risk was 2.6 times higher in individuals having the lowest total serum concentration of carotenoids (the lowest quartile) as compared with the risk for individuals having the highest concentration (the highest quartile). In case of lycopene, this risk increases almost three times (**Table 25**).

Table 25. Risk of developing cardiovascular diseases according to quartiles of lycopene and total serum concentration of carotenoids

		Total carotenoid conc. (μmol/l)		Lycopene conc. (μmol/l)	
Quartile	n	OR	95% CI	OR	95% CI
Q1 (lowest)	93	2.63	1.09; 6.37	2.99	1.18; 7.57
Q2	90	2.08	0.83; 5.18	2.37	0.91; 6.11
Q3	93	0.97	0.35; 2.7	1.5	0.55; 4.13
Q4 (highests)	90	1		1	
p value		0.03		0.01	

The assessment of links with biochemical markers of CVD risk demonstrated that the concentrations of individual tested carotenoids (except lutein and zeaxanthin) significantly correlated with the levels of total cholesterol and HDL cholesterol. A positive correlation with the LDL cholesterol was detected only for lycopene. A negative correlation was found between α-carotene, β-carotene, β-cryptoxanthin as well as the total concentration of carotenoids and an inflammation marker – the high sensitivity C-reactive protein. A positive correlation was discovered also between apolipoprotein A-I and α-carotene, β-carotene as well as the total concentration of carotenoids (**Table 26**).

Table 26. Correlation between carotenoid concentration and blood biochemical markers

Biochemical parameter	Mean serum carotenoid concentration (μmol/l)					
	Lutein + zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	lycopene	Total carotenoid conc.
	(r)- Spearmen or Pearson correlation coefficient					
Glucose (mmol/l)	-0.040	0.023	-0.080	-0.104*	0.038	-0.046
Total-C (mmol/l)	0.172*	0.196**	0.123*	0.115*	0.182**	0.216**
TAG (mmol/l)	0.100	0.046	-0.072	-0.114*	0.157	0.024
HDL-C (mmol/l)	-0.013	0.105*	0.129*	0.160**	0.118*	0.124*
LDL-C (mmol/l)	0.18	0.164	0.147	0.151	0.154*	0.206
*hs*CRB (mg/l)	-0.017	-0.103*	-0.20**	-0.184**	-0.070	-0.150**
Apo A-I (g/l)	0.004	0.067	0.140**	0.127*	0.007	0.110*
Apo B (g/l)	0.12	0.189	0.006	-0.025	0.138	0.096
Lp (a) (g/l)	0.026	0.018	0.111	.076	0.056	0.080

*— statistically significant correlation (*p < 0.05) (**p < 0.005).

3.5. Influence of demographic and life-style factors and biochemical blood markers on the blood serum concentration of carotenoids

The multiple regression method was applied to assess the influence of demographic and life-style factors and biochemical blood markers on the serum levels of carotenoids. The factors having statistically significant ($p < 0.05$) influence of the concentrations of individual carotenoids and the total concentration of carotenoids are presented in **Table 27**.

Gender, age, season and smoking habits had a significant influence on the total serum levels of carotenoids and the concentrations of individual carotenoids. High physical activity both at work and in leisure time has been linked with lower levels of β-carotene. A higher consumption of lycopene with foods increased serum lycopene levels, and the frequent alcohol use resulted in reduced serum lycopene concentrations. Increasing concentrations of total cholesterol were associated with increased levels of carotenoids, and the inflammation marker – *hs*CRP – was associated with a lowered blood concentration of carotenoids.

Table 27. Influence of demographic and life-style factors and biochemical blood markers on the serum concentration of carotenoids (multiple regression method)

	Lutein +zeaxanthin**			β-cryptoxanthin**			α-carotene**			β-carotene**			Lycopene**			Total carotenoid concentration**		
	$R^2 = 0.201$; $p = 0.000$			$R^2 = 0.242$; $p = 0.000$			$R^2 = 0.170$; $p = 0.000$			$R^2 = 0.242$; $p = 0.000$			$R^2 = 0.219$; $p = 0.000$			$R^2 = 0.202$; $p = 0.000$		
	β	p	VIF	β	p	VIF	β	p	VIF	β	p	VIF	β	p	VIF	β	p	VIF
Season*	0.123	0.003	1.013	-0.300	0.000	1.01							0.139	0.000	1.01	0.121	0.001	1.006
Gender * (male / female)	0.106	0.014	1.108	0.189	0.000	1.020	0.196	0.000	1.12	0.169	0.000	1.06				0.174	0.000	1.019
Age (y.)				-0.330	0.000	1.388							-0.335	0.000	1.44	-0.228	0.000	1.442
Smoking*	-0.128	0.002	1.046	-0.104	0.007	1.044				-0.109	0.006	1.09				-0.108	0.002	1.036
BMI	-0.106	0.012	1.072							-0.415	0.000	6.71						
Total-C (mmol / l)	0.240	0.000	1.079	0.190	0.000	1.364	0.628	0.000	7.59	0.266	0.000	1.13				0.378	0.000	1.400
HDL-C (mmol / l)				0.120	0.006	1.132	-0.339	0.000	5.49	-0.14	0.004	1.66	0.306	0.000	1.37			
*hs*CRB (mg / l)				-0.075	0.042	1.043	-0.132	0.001	1.06	-0.14	0.008	1.96				-0.121	0.001	1.064
Apo A-I (g / l)							0.197	0.011	3.86									
TAG (mmol / l)							-0.117	0.024	1.73	0.641	0.000	7.32						
Alcohol use*													-0.178	0.000	1.12			
Physical activity (at work)*										-0.12	0.002	1.05						
Physical activity (at leisure time)*										-0.102	0.01	1.12						
Mean lycopene intake (mg / p)													0.199	0.000	1.02			

*Categorical variables were included in the regression model as pseudovariables; **logarithmic transformation of carotenoid concentrations values are used in the model. Only statistically significant ($p < 0.05$) factors are presented.

3.6. Association between genome markers and blood serum carotenoid concentrations

The study was designed to assess the association of the serum concentration of carotenoids not only with demographic, life-style or other environment-related epidemiological factors, but also with genotype-related individual properties: an emphasis was made on proteins involved in the metabolism of carotenoids, especially in their resorption.

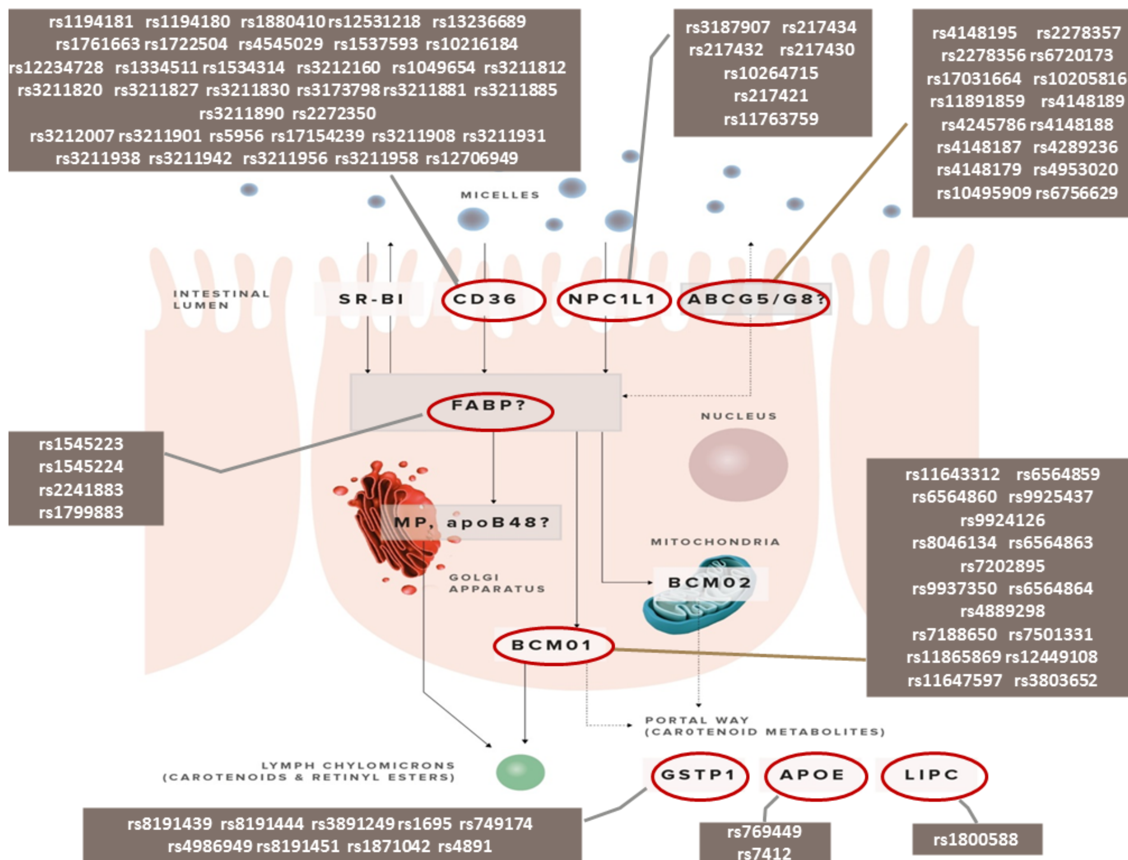


Figure 10. SNP were analysed in the genes coding APOE, BCMO1, NPC1L1, CD36, GSTP1, ABCG5, FABP1, FABP2 and LIPC proteins

For this study we selected not only SNPs statistically significantly associated with carotenoid concentrations in other populations, but also new, not yet studied genomic markers, aiming to detect new statistically significant regions linkable to blood serum carotenoid levels in the Lithuanian population.

Table 28. Genes encoding proteins involved in carotenoid metabolism selected for the study, genetic regions and their annotation

Gene	Region	Annotation	Function
BCMO1	16q11.2	*beta-carotene oxygenase 1*	Symmetric cleavage of α- and β-carotene and β-cryptoxanthin into retinol
NPC1L1	7q21.1	*NPC1-like 1, multi-pass membrane protein*	Transfer of different molecules, including cholesterol and α-tocopherol, through the cell membranes
CD36	7q11.2	*CD36 molecule (thrombospondin receptor)*	Interaction with different ligands, receptor of thrombospondin
GSTP1	11q13	*glutathione S-transferase pi 1*	Inactivation of hydrophobic and electrophilic compounds by conjugation with glutathione
ABCG5	2q14.1	*ATP-binding cassette, sub-family G (WHITE), member 5*	Transfer of different molecules through the cell membranes, limitation of cholesterol resorption
FABP1	2q14.1	*fatty acid binding protein 1, liver*	Involved in assimilation, transfer of fatty acids and other hydrophobic ligands and their metabolism in the liver
FABP2	4q22	*fatty acid binding protein 2, intestinal*	Involved in the metabolism of long-chain fatty acids in epithelial cells of the small intestine; importance in cell proliferation modulation
APOE	19q12	*apolipoprotein E*	Metabolism of triacylglycerol-rich lipids
LIPC	15q21.1	*lipase, hepatic*	Hydrolysis of triacylglycerol involved in the receptor-related mechanism of lipoprotein transfer

An analysis of 91 SNP was performed. They were located in 9 genes encoding proteins involved in carotenoid transfer through the apical membrane of enterocyte and in their further metabolism: APOE, BCMO1, NPC1L1, CD36, GSTP1, ABCG5, FABP1, FABP2 and LIPC (**Figure 10, Table 28**). **The** concentrations of carotenoids were distributed into quartiles for the further analysis (**Table 29**).

Table 29. Quartiles of carotenoid concentrations

Quartile	Lutein + zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	lycopene
	Concentration (μmol/l)				
Q1	≤0.192	≤ 0.06	≤0.248	≤0.078	≤0.327
Q2	0.1921-0.2675	0.061–0.1045	0.2481–0.417	0.0781–0.126	0.3271–0.494
Q3	0.26751–0.371	0.10451–0.171	0.4171–0.689	0.126–0.208	0.4941–0.757
Q4	>0.371	>0.171	>0.689	>0.208	>0.757

Out of 91 SNP, 28 SNP were found in genes *BCMO1, CD36, NPC1L1, GSTP1, ABCG5, FABP1 and FABP2*, when genotype frequencies were statistically significantly different by quartiles of the concentration of carotenoids (χ^2 p < 0,05) (the above-mentioned SNPs are marked in red in Table 30). Allele frequencies were calculated for the defined SNP. The frequencies of minor alleles are presented in **Table 30**.

Table 30. Minor allele frequencies for studied SNPs

Gene	Chr	SNP	M	MAF	HW p	*Gene*	Chr	SNP	M	MAF	HW p
ABCG5	2q14.1	rs10205816	G	0.25	0.11	*CD36*	7q11.2	rs1537593	A	0.11	0.32
ABCG5	2q14.1	rs11891859	A	0.10	0.05	*CD36*	7q11.2	rs17154239	-	0.00	1.00
ABCG5	2q14.1	rs17031664	G	0.06	0.51	*CD36*	7q11.2	rs1722504	A	0.01	1.00
ABCG5	2q14.1	rs2278356	C	0.28	0.18	*CD36*	7q11.2	rs1761663	G	0.35	0.66
ABCG5	2q14.1	rs2278357	A	0.11	0.15	*CD36*	7q11.2	rs1880410	-	0.00	1.00
ABCG5	2q14.1	rs4148179	G	0.40	0.78	*CD36*	7q11.2	rs2272350	-	0.00	1.00
ABCG5	2q14.1	rs4148187	A	0.41	0.49	*CD36*	7q11.2	rs3173798	G	0.10	1.00
ABCG5	2q14.1	rs4148188	A	0.40	0.49	*CD36*	7q11.2	rs3211812	-	0.00	1.00
ABCG5	2q14.1	rs4148189	A	0.10	0.05	*CD36*	7q11.2	rs3211820	A	0.35	1.00
ABCG5	2q14.1	rs4148195	A	0.11	0.16	*CD36*	7q11.2	rs3211827	C	0.34	1.00
ABCG5	2q14.1	rs4245786	G	0.20	0.83	*CD36*	7q11.2	rs3211830	A	0.10	1.00
ABCG5	2q14.1	rs4289236	A	0.19	0.18	*CD36*	7q11.2	rs3211881	G	0.07	0.29
ABCG5	2q14.1	rs4953020	A	0.38	0.89	*CD36*	7q11.2	rs3211885	A	0.44	0.34
ABCG5	2q14.1	rs6720173	C	0.11	0.15	*CD36*	7q11.2	rs3211890	-	0.00	1.00
ABCG5/8	2q14.1	rs10495909	-	0.00	1.00	*CD36*	7q11.2	rs3211901	-	0.00	1.00
ABCG5/8	2q14.1	rs6756629	A	0.05	1.00	*CD36*	7q11.2	rs3211908	A	0.09	0.69
APOE	19q12	rs7412	A	0.07	0.31	*CD36*	7q11.2	rs3211931	A	0.46	0.42
APOE	19q12	rs769449	A	0.10	1.00	*CD36*	7q11.2	rs3211938	-	0.00	1.00
BCMO1	16q11.2	rs11643312	A	0.39	0.89	*CD36*	7q11.2	rs3211942	-	0.00	1.00

BCMO1	16q11.2	rs11647597	C	0.19	1.00	*CD36*	7q11.2	rs3211956	C	0.10	0.70
BCMO1	16q11.2	rs11865869	G	0.25	0.30	*CD36*	7q11.2	rs3211958	G	0.46	0.34
BCMO1	16q11.2	rs12449108	A	0.18	0.66	*CD36*	7q11.2	rs3212007	A	0.01	1.00
BCMO1	16q11.2	rs3803652	-	0.00	1.00	*CD36*	7q11.2	rs3212160	A	0.45	0.28
BCMO1	16q11.2	rs4889298	G	0.43	0.22	*CD36*	7q11.2	rs4545029	A	0.46	0.42
BCMO1	16q11.2	rs6564859	G	0.39	0.89	*CD36*	7q11.2	rs5956	A	0.08	1.00
BCMO1	16q11.2	rs6564860	G	0.42	1.00	*FABP1*	2q14.1	rs1545223	G	0.49	0.03
BCMO1	16q11.2	rs6564863	A	0.30	0.52	*FABP1*	2q14.1	rs1545224	G	0.14	0.77
BCMO1	16q11.2	rs6564864	C	0.44	0.22	*FABP1*	2q14.1	rs2241883	G	0.49	0.03
BCMO1	16q11.2	rs7188650	G	0.29	1.00	*FABP2*	4q22	rs1799883	A	0.28	0.74
BCMO1	16q11.2	rs7202895	G	0.04	0.33	*GSTP1*	11q12	rs1695	G	0.26	0.30
BCMO1	16q11.2	rs7501331	A	0.23	0.85	*GSTP1*	11q12	rs1871042	A	0.27	0.17
BCMO1	16q11.2	rs8046134	A	0.18	0.25	*GSTP1*	11q12	rs3891249	-	0.00	1.00
BCMO1	16q11.2	rs9924126	G	0.48	0.89	*GSTP1*	11q12	rs4891	G	0.27	0.17
BCMO1	16q11.2	rs9925437	-	0.00	1.00	*GSTP1*	11q12	rs4986949	-	0.00	1.00
BCMO1	16q11.2	rs9937350	G	0.27	1.00	*GSTP1*	11q12	rs749174	A	0.27	0.17
CD36	7q11.2	rs10216184	G	0.35	0.88	*GSTP1*	11q12	rs8191439	-	0.00	1.00
CD36	7q11.2	rs1049654	A	0.45	0.34	*GSTP1*	11q12	rs8191444	-	0.00	1.00
CD36	7q11.2	rs1194180	G	0.01	1.00	*GSTP1*	11q12	rs8191451	-	0.00	1.00
CD36	7q11.2	rs1194181	A	0.11	0.32	*LIPC*	15q21.1	rs1800588	A	0.25	0.86
CD36	7q11.2	rs12234728	A	0.00	1.00	*NPC1L1*	7q21.1	rs10264715	A	0.28	0.31
CD36	7q11.2	rs12531218	-	0.00	1.00	*NPC1L1*	7q21.1	rs11763759	G	0.23	0.85
CD36	7q11.2	rs12706949	A	0.46	0.70	*NPC1L1*	7q21.1	rs217421	G	0.31	0.16
CD36	7q11.2	rs13236689	C	0.43	0.49	*NPC1L1*	7q21.1	rs217430	G	0.30	0.52
CD36	7q11.2	rs1334511	G	0.10	1.00	*NPC1L1*	7q21.1	rs217432	G	0.25	0.86
CD36	7q11.2	rs1534314	A	0.10	1.00	*NPC1L1*	7q21.1	rs217434	G	0.23	0.57
						NPC1L1	7q21.1	rs3187907	G	0.23	0.57

The association between the genome markers and serum carotenoid levels was established by comparing the first concentration quartile with the third and fourth quartiles, i.e. with the quartiles in which the concentration of carotenoids is above the median. We assessed the likelihood (as an odds ratio) that the concentration of an individual carotenoid would appear in the first quartile when a rare allele is found. We found 13 alleles in two genes, linkable with lower serum concentrations of lutein / zeaxanthin, lycopene (in the group of males) and alpha-carotene (in the group of females).

After the calculation of odds ratios for all studied SNPs, it has been discovered that *CD 36* gene variations rs4545029, rs1334511, rs1534314, rs3212160, rs1049654, rs3211885, rs3211908, rs3173798, rs3211931, rs3211956, rs3211830 and rs6564863 in gene *BCMO1* (OR ≥ 1.5; CI (1.08–6.16), $p < 0.05$) are significantly associated whit a lower concentration of individual carotenoids (**Table 31**).

Table 31. Variations of genes associated with lower blood serum concentrations of individual carotenoids

Gene	SNP	Alleles associated with a lower concentration of carotenoids	Carotenoids associated	OR	95% CI	
CD 36	rs4545029	T	Lutein + zeaxanthin	1.52	1.10	2.11
	rs1334511	G	Lutein + zeaxanthin	2.80	1.59	4.94
			Lycopene (M)	2.50	1.26	4.60
	rs1534314	A	Lutein+zeaxanthin	2.90	1.60	4.74
			Lycopene (M)	2.40	1.27	4.55
	rs3212160	A	Lutein + zeaxanthin	1.50	1.08	2.08
	rs1049654	A	Lutein + zeaxanthin	1.50	1.08	2.08
	rs3211885	T	Lutein + zeaxanthin	1.65	1.18	2.29
	rs3211908	C	Lutein + zeaxanthin	3.40	1.87	6.16
		T	Lycopene (M)	2.99	1.52	5.85
	rs3173798	T	Lutein + zeaxanthin	3.01	1.72	5.27
	rs3211931	T	Lutein + zeaxanthin	1.93	1.39	2.69
	rs3211956	T	Lutein + zeaxanthin	3.40	1.87	6.16
	rs3211830	T	Lutein + zeaxanthin	2.80	1.59	4.94
			Lycopene (M)	2.49	1.76	4.55
BCMO1	rs6564863	T	α-carotene (F)	2.19	1.28	3.75

M – male group, F – female group.

4. CONCLUSIONS

1. Mean concentrations of carotenoids were assessed in the blood serum of the study individuals (μmol/l): lutein and zeaxanthin 0.28 ± 0.13, β-cryptoxanthin 0.15 ± 0.17, α-carotene 0.15 ± 0.12, β-carotene 0.52 ± 0.37, lycopene 0.56 ± 0.3, total 1.60 ± 0.72. Both the total concentrations of carotenoids as well as the concentrations of individual carotenoids in the Lithuanian population were among the lowest as compared with the results of analogous studies carried out in other European countries.

2. Gender, age, season and smoking habits were the most important demographic and life-style factors having a significant influence on the total levels of blood serum carotenoids and the concentrations of the majority of individual carotenoids:
   a) mean serum levels of carotenoids in females were significantly ($p = 0.0015$) higher than in males;
   b) the lowest mean values of total and indivudual carotenoids were reported in the group of individuals aged, above 65 years.
   c) the lowest concentration of carotenoids was reported in spring (1.31 ± 0.64 μmol/l) and the highest in autumn (1.76 ± 0.85 μmol/l);
   d) lower mean levels of α-, β-carotene and β-cryptoxanthin were found in the group of obese individuals, while lower mean levels of β-carotene and lycopene were characteristic of individuals involved in very high physical activities;
   e) serum concentrations of all carotenes, except xanthophylls, were statistically significantly different ($p < 0.05$) between the groups of smokers and non-smokers;
   f) mean blood serum concentrations of α-carotene, lycopene and β-cryptoxanthin were statistically significantly different ($p < 0.002$) among the groups of individuals with different habits of alcohol use.

3. Mean lycopene intake was calculated, its main food sources and correlations with lycopene serum concentration were established:
   a) the main sources of lycopene are tomatoes and their products (78.8%), watermelons (11.9%), pink grapefruits (9.3%);
   b) one individual consumes 5.05 (±7.48) mg of lycopene per day on average (i.e. 0.08 milligrams per one kilogram of body mass).
   c) gender, seasonal changes and age have a statistically significant influence on the lycopene consumption differences in the Lithuanian population;
   d) a statistically significant but weak correlation between the consumption of lycopene with food and blood lycopene levels was discovered ($r = 0.361$, $p < 0.01$).

4. Genome markers associated with the blood serum concentration of carotenoids were established:

    a) eleven SNPs (rs4545029, rs1334511, rs1534314, rs3212160, rs1049654, rs3211885, rs3211908, rs3173798, rs3211931, rs3211956, rs3211830) were detected in region 21.1 of chromosome 7, that are statistically significantly associated with reduced levels of lutein and zeaxanthin; four SNPs (rs1334511, rs1534314, rs3211908, rs3211830) associated with the lower blood serum lycopene concentration in the group of males;
    b) SNP rs6564863 of the gene *BCMO1* detected in the 16q11.2 region is associated with a lower serum α-carotene concentration in the group of females.

5. Correlations of blood serum levels of carotenoids and lycopene intake with cardiovascular morbidity and blood biochemical risk factors of CVD were established:

    a) lower mean blood serum concentration of lycopene and the total concentration of carotenoids were associated with an increased cardiovascular risk;
    b) statistically significant difference was not discovered when the lycopene intake was compared among the groups of the study individuals with and without CVD ($p = 0.393$);
    c) a higher lycopene intake correlated with higher blood concentrations of HDL-C ($r = 0.079$; $p < 0001$);
    d) a higher mean concentration of individual and total carotenoids statistically significantly correlated with increased concentrations of HDL-C and a lower concentration of the high sensitivity CRP;
    e) positive correlations were reported between the concentrations of Apo A-I and α-carotene, β-carotene and the total concentration of carotenoids.

REFERENCES

1. Ashor AW, Siervo M, Lara J, Oggioni C, Mathers JC. Antioxidant vitamin supplementation reduces arterial stiffness in adults: a systematic review and meta-analysis of randomized controlled trials. J Nutr 2014; 144(10): 1594–602.
2. Banys V, Kučinskienė ZA, Andrejevaitė V, Kaminskas A, Jablonskienė V, Vitkus D. Ciklofilino A ir tradicinių aterosklerozės žymenų reikšmė vertinant širdies ir kraujagyslių ligas. Laboratorinė medicina 2014; 16(1): 3–13.
3. Banys V. Trombocitų hiperfunkcijos vaidmuo aterosklerozės ir metabolinio sindromo patogenezėje. Laboratorinė medicina 2012; 14(3): 136–46.
4. Borel P, de Edelenyi FS, Vincent-Baudry S, Malezet-Desmoulin C, Margotat A, Lyan B, et al. Genetic variants in BCMO1 and CD36 are associated with plasma lutein concentrations and macular pigment optical density in humans. Ann Med 2010; 43: 47–59.
5. Borel P, de Edelenyi FS, Vincent-Baudry S, Malezet-Desmoulin C, Margotat A, Lyan B, et al. Genetic variants in BCMO1 and CD36 are associated with plasma lutein concentrations and macular pigment optical density in humans. Ann Med 2011; 43(1): 47–59.
6. Borel P, Desmarchelier C, Nowicki M, Bott R, Morange S, Lesavre N. Interindividual variability of lutein bioavailability in healthy men: characterization, genetic variants involved, and relation with fasting plasma lutein concentration. Am J Clin Nutr 2014; 100(1): 168–75.
7. Coj, A. Molytė, Z. A. Kučinskienė, J. Arasimavičius, V. Kučinskas. Aterosklerozės žymenų tyrimai lietuvių šeimose. Laboratorinė medicina 2013; 15(3): 119–25.
8. Craft NE, Brown ED, Smith JC Jr. Effects of storage and handling conditions on concentrations of individual carotenoids, retinol, and tocopherol in plasma. Clin Chem 1988; 34(1): 44–8.
9. Cruickshank TM, Reyes AR, Ziman MR. A systematic review and meta-analysis of strength training in individuals with multiple sclerosis or Parkinson disease. Medicine (Baltimore) 2015; 94(4): e411.
10. Didžiapetrienė J, Uknevičiūtė G, Bublevič J, Uleckienė S, Kazbarienė B, Stukas R. Antioksidantų vartojimo galimybės onkologijoje. Sveikatos mokslai 2011; 21(7): 154–9.
11. Domarkienė I. Lietuvos populiacijos genetinės struktūros tyrimas remiantis vieno nukleotido polimorfizmų asociacijos su liga analize. Daktaro diseratcija. Vilnius, 2015.
12. Domarkienė I, Pranculis A, Germanas S, Jakaitienė A, Vitkus D, Dženkevičiūtė V, Kučinskienė Z, Kučinskas V. RTN4 and FBXL17 genes are associated with coronary heart disease in genome-wide association analysis of Lithuanian families. Balkan Journal of Molecular Genetics 2013; 16(2): 17–22.

13. Domarkienė S, Tamošiūnas A, Rėklaitienė R, Šidlauskienė D, Jurėnienė K, Margevičienė L, Buivydaitė K, Kazlauskaitė M. Trends in main cardiovascular risk factors among middle-aged Kaunas population between 1983 and 2002. Medicina 2003; 12: 1193–9.
14. Ferrucci L, Perry JR, Matteini A, Perola M, Tanaka T, Silander K, et al. Common variation in the beta-carotene 15,15'-monooxygenase 1 gene affects circulating levels of carotenoids: a genome-wide association study. Am J Hum Genet 2009; 84: 123–33.
15. Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients 2014; 6(2): 466–88.
16. Frohlich K, Karin Kaufmann K, Roland Bitsch R, Bohm V. Effects of ingestion of tomatoes, tomato juice and tomato pureé on contents of lycopene isomers, tocopherols and ascorbic acid in human plasma as well as on lycopene isomer pattern. British Journal of Nutrition 2006; 95: 734–41.
17. Grabauskas V, Klumbienė J, Petkevičienė J, Petrauskienė A, Tamošiūnas A, Kriaučionienė V, Ramazauskienė V. Lėtinių neinfekcinių ligų rizikos veiksnių paplitimas tarp Lietuvos kaimiškųjų rajonų gyventojų: CINDI programos tyrimas, 2007 m. Medicina (Kaunas) 2008; 44(8): 633–9.
18. Hart DJ, Scott KJ. Development and evaluation of an HPLC method for the analysis of carotenoids in foods and measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. Food Chem 1995; 54: 101–11.
19. Hirvonen T, Virtamo J, Korhonen P, Albanes D, Pietinen P. Intake of flavonoids, carotenoids, Vitamins C and E, and risk of stroke in male smokers. Stroke 2000; 31(10): 2301–6.
20. Jakaitienė A., Kučinskas V. Lietuvių populiacijos struktūros įvertinimas pagal etnolingvistines grupes. Lietuvos statistikos darbai 2013; 52 (1): 34–8.
21. Khachik F, Carvalho L, Bernstein PS, Muir GJ, Zhao DY, Katz NB. Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. Exp Biol Med (Maywood) 2002; 227(10): 845–51.
22. Klumbienė J, Petkevičienė J, Tamošiūnas A, Plieskienė A, Misevičienė I, Milašauskienė Ž. Lėtinių neinfekcinių ligų rizikos veiksnių paplitimo pokyčiai 1987–1999 metais, Medicina 2002; 38: 77–85.
23. Kristenson M, Zieden B, Kucinskiene Z, et al. Antioxidant state and mortality from coronary heart disease in Lithuanian and Swedish men: concomitant cross-sectional study of men aged 50. Br Med J 1997; 314: 629–33.
24. Kučinskienė Z. Laisvaradikalinė oksidacija, antioksidacinė gynyba ir ligos. Laboratorinė medicina 2001; 1(9): 31–7.

25. Laucevicius A, Kasiulevicius V, Jatuzis D, Petrulioniene Z, Ryliskyte L, Rinkuniene E, et al. Lithuanian High Cardiovascular Risk (LitHiR) primary prevention programme – rationale and design. Seminars in Cardiovascular Medicine 2012; 18: 3.
26. Laucevičius A, Rinkūnienė E, Skorniakov V, Petrulionienė Z, Kasiulevičius V, Jatužis D, et al. High-risk profile in a region with extremely elevated cardiovascular mortality. Hellenic J Cardiol 2013; 54(6): 441–7.
27. Laucevičius A, Rinkūnienė E, Skujaitė A, Petrulionienė Ž, Puronaitė R, et al. Prevalence of cardiovascular risk factors in Lithuanian middle-aged individuals participating in the primary prevention program, analysis of the period 2009–2012. Blood Press 2015; 24(1): 41–7.
28. Lobo V, Patil A, Phatak A, and Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Can J Cardiol 2015; 31(5): 631–41.
29. Mažeikienė A, Kučinskienė ZA, Noreika R. Likopeno suvartojimas, jo koncentracija kraujo plazmoje ir ryšys su 61–62 metų vyrų aterosklerozės rizikos veiksniais. Laboratorinė medicina 2008; 10(3): 139–45.
30. Montezano AC, Dulak-Lis M, Tsiropoulou S, Harvey A, Briones AM, Touyz RM. Oxidative stress and human hypertension: vascular mechanisms, biomarkers, and novel therapies. Pharmacogn Rev 2010; 4(8): 118–26.
31. Namitha KK, Negi PS. Chemistry and biotechnology of carotenoids. Crit Rev Food Sci Nutr 2010; 50: 728–60.
32. O'Neill ME, Carroll Y, Corridan B, et al. A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. British J of Nutr 2001; 85: 499–507.
33. Olmedilla, B, Granado F, Blanco I, Rojas-Hidalgo E. Seasonal and sex-related variations in six serum carotenoids, retinol, and alpha-tocopherol, Am J Clin Nutr 1994; 60: 106–10.
34. Olmedilla B, Granadoa F, Rojas-Hidalgoa E, Blancoa I. A rapid separation of ten carotenoids, three retinoids, alpha-tocopherol and d-alpha-tocopherol acetate by high performance liquid chromatography and its application to serum and vegetable samples J Liq Chromatogr 1990; 13(8): 1455–83.
35. Rinkūnienė E, Laucevičius A, Petrulionienė Ž, Badarienė J. Lietuvos didelės kardiovaskulinės rizikos pacientų atrankos ir prevencinių priemonių (LitHir) programos rezultatai: dislipidemijos paplitimas tarp vidutinio amžiaus suaugusių Lietuvos gyventojų. Medicinos teorija ir praktika 2012; 18 (4.2): 636–42.
36. Rinkūnienė E, Petrulionienė Ž, Zdanevičiūtė I, Dženkevičiūtė V. Mirtingumo nuo širdies ir kraujagyslių ligų tendencijos Lietuvoje ir Europos šalyse. Medicinos teorija ir praktika 2013; 19(2): 130–6.

SANTRAUKA

Žmogaus organizme vykstanti įvairių medžiagų oksidacija – tai nuolat vykstantis procesas. Dėl įvairių tiek endogeninių procesų, tiek egzogeninių priežasčių oksidacijos metu laisvųjų radikalų susidarymas intensyvėja, jie sąveikauja su ląstelių biomolekulėmis ir, dažniausiai, jas pažeidžia. Nustatyta daugiau nei šimtas ligų, tarp jų ir širdies bei kraujagyslių ligos, kurių patogenezėje dalyvauja laisvieji radikalai ir kiti reaktyvūs deguonies dariniai. Tačiau organizmas geba apsisaugoti nuo žalingo minėtų darinių poveikio – juos neutralizuoja įvairios fermentinės ir nefermentinės antioksidacinės sistemos. Ypatingą vaidmenį šalinant reaktyviųjų deguonies darinių kenksmingumą ir tokiu būdu slopinant oksidacinį stresą atlieka antioksidantai karotenoidai – natūralūs augaliniai pigmentai. Tačiau tyrimai, patvirtinantys karotenoidų biologinį aktyvumą, dažniausiai atliekami *in vitro* bei pasitelkiant gyvūnų modelius. Todėl mūsų supratimas apie šių junginių metabolizmą ir biologinį poveikį žmogaus organizmui yra labai ribotas. Tikėtina, jog karotenoidų rezorbcija, jų koncentracija kraujyje, bei pasiskirstymas audiniuose yra veikiami ne tik mitybos ar kitų fiziologinių veiksnių, bet ir polimorfizmų genuose, koduojančiuose lipoproteinų receptorius, lipidų pernašoje bei karotenoidų metabolizme dalyvaujančius baltymus, fermentus. Pasaulyje atlikta vos keletas tyrimų, nustačiusių su karotenoidų kiekiu žmogaus organizme asocijuotas genetines sritis ir genų variantus, kurių koduojami baltymai dalyvauja karotenoidų metabolizme ir yra siejami su individualaus atsako į suvartojimą įvairove.

Mūsų tyrimo metu vertintos kraujo serumo karotenoidų koncentracijų sąsajos ne tik su demografiniais, gyvensenos, mitybos ar kitais epidemiologiniais veiksniais, bet ir su genotipo nulemtais požymiais. Mitybos, gyvensenos, demografiniai veiksniai ir ligų anamnezės duomenys tirti naudojant validuotą klausimyną. Likopeno suvartojimas tirtas 72-valandų anketinės apklausos būdu. Serumo karotenoidų (liuteinas, zeaksantinas, kantaksnatinas, β-kriptoksantinas, α-karotenas, (E)-β-karotenas, (9Z)-β-karotenas, (E)-likopenas ir (Z)-likopenas) koncentracija – tirta taikant didelio efektyvumo skysčių chromatografijos metodą. Genotipavimas buvo atliktas *Illumina HiScan®SQ* platforma naudojant *Illumina HumanOmniExpress-12 v1.0* arba *v1.1* ~770 000 VNP lustus. Tiriamieji buvo genotipuoti pagal 91 VNP 9 genuose kandidatuose: *APOE, BCMO1, NPC1L1, CD36, GSTP1, ABCG5, FABP1, FABP2* ir *LIPC.*

STUDY APPROBATION

The study results have been published in peer-reviewed scientific journals and presented in scientific conferences in Lithuania and in foreign countries.

Publications (3)

1. Asta Mažeikienė. Structure, metabolism, mechanisms of action and benecifial health effects of carotenoids. Laboratorinė medicina ISSN 1392-6470; 2013, 15 vol., Nr. 2(58), p. 88–98.
2. Mažeikienė A., Jakaitienė A., Karčiauskaitė D., Kučinskienė Z.A., Abaravičius J.A., Kaminskas A., Kučinskas V. Dietary lycopene and cardiovascular health in ethnic Lithuanians. Acta Medica Lituanica 2015, Vol. 22, No. 4, p. 179–190.
3. Asta Mažeikienė, Žydrūnas Daunoravičius, Zita Aušrelė Kučinskienė. Detection of carotenoid concentrations and their association with lifestyle and demographic factors in ethnic lithuanians. Laboratorinė medicina ISSN 1392-6470; 2015, 17 vol., Nr. 3(67), p. 95–102.

Oral presentations (5)

1. Mažeikienė A., Kučinskienė Z. A. Comparison of the nutritional habits and biochemical parameters among members of the families with or without anamnestic data of common noninfectious diseases. Oral presentation for PhD students, diploma students and other members of the community of Institute of Nutrition of Friedrich-Schiller-University, 13 06 2012 Jena, Germany.
2. Asta Mažeikienė. Carotenoids: prevalence, biological importance and methods of analysis. Lycopene and cardiovascular diseases. Scientific-practical conference "Scientific work at VU MF FBMLM Department: Present and Future Prospects"; 06 12 2012 Vilnius, Lithuania.
3. Asta Mažeikienė. Why lycopene is beneficial against cardiovascular diseases? 6th Baltic Atherosclerosis Congress; 11–12 10 2013, Riga, Latvija.
4. Asta Mažeikienė. Investigation of blood serum carotenoids in the ethiopathogenesis of chronic noninfectious diseases. 12th Baltic Congress in Laboratory Medicine (BALM); 18–20 09 2014, Riga, Latvija.
5. Asta Mažeikienė. Carotenoids: from Nutrition to Genetics. Conference LITGEN: Genetic diversity of the population of Lithuania and changes of its genetic structure related with evolution and common diseases; 06 03 2015 Vilnius, Lithuania.

Poster presentations (11)

1. Mažeikienė A., Jakaitienė A., Arasimavičius J., Domarkienė I., Uktverytė I., Ambrozaitytė L., Kučinskienė Z. A., Kučinskas V. Association of serum carotenoid concentrations with single nucleotide polymorphisms in *APOE, BCMO1, HL* and *FABP* genes. June 29 – July 4, 2014. 17th International Carotenoid Symposium, Park City, Uhta, JAV. Carotenoid Science. Volume 18, June 2014, p. 129. ISSN 1880-5671.
2. Burokienė N., Mažeikienė A., Karčiauskaitė D., Miklošiūtė V., Daunoravičius Ž., Kučinskienė Z. A. Serum malondialdehyde concentration and its relation to cardiovascular risk markers and morbidity in Lithuanian population. 17th International Symposium on Atherosclerosis; 23–26 May 2015; Netherlands, Amsterdam http://www.isa-2015.com/wp-content/uploads/2015/05/ISA-2015-abstracts-501-750.pdf
3. Mažeikienė A., Kučinskienė Z. A., Kučinskas V. Serum carotenoid concentrations in ethnic Lithuanians. Abstract book of the international conference "Evolutionary medicine: perspectives in understanding health and disease". 27th–30th of May, Vilnius, Lithuania, 2014, p. 76.
4. Burokienė N., Mažeikienė A., Karčiauskaitė D., Kučinskienė Z. A. Impact of risk factors on cardiovascular disease in general Lithuanian population. 2–5 d. July 2–5, 2014; 19th *WONCA* Europe Conference; Lisbon, Portugal.. Abstract book, p. 563.
5. Karčiauskaitė D., Mažeikienė A., Burokienė N., Kučinskienė Z. A. Impact of psychosocial stress and other risk factors on cardiovascular morbidity in Lithuanian population. Atherosclerosis. ISSN 0021-9150 2014, vol. 235, iss. 2, p. e116.
6. Mažeikienė A., Kučinskienė Z.A., Kučinskas V. Association between serum lycopene concentration and cardiovascular morbidity. 6th Baltic Atherosclerosis Congress, October 11–12, 2013; Riga Latvia; Abstract book, p. 13.
7. Mažeikienė A., Jakaitienė A., Kučinskienė Z. A., V. Kučinskas V. Investigation of lycopene intake in the different Lithuanian ethnolinguistic groups. IUNS 20th International Congress of Nutrition, 2013 Granada, Spain, September 15–20. Ann Nutr Metab 63(suppl 1) 1–1960 (2013), p. 1225. e-ISBN 978–3–318–02516–3 print ISSN 0250–6807; online e-ISSN 1421–9697.
8. Mažeikienė A., Burokienė N., Karčiauskaitė D., Kučinskienė Z. A. Relation between family history of common chronic diseases, nutritional habits and blood biochemical parameters. 20th IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine (EuroMedLab); 45th Congress of the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC) Milan, 19–23 May 2013. Biochimica Clinica,

Special Supplement (SS); 2013 Abstracts volume (Vol. 37), ISSN 0393-0564. S593.

9. Karčiauskaitė D., Mažeikienė A., Burokienė N., Kučinskienė Z. A. Impact of anxiety and depression on biochemical blood parameters in general Lithuanian population. 81st Congress of the European Atherosclerosis Society in Lyon, France, June 2–5, 2013. http://w3.kenesgroup.com/apps/eas2013/abstracts/Index.htm
10. Uktverytė I., Meškienė R., Ambrozaitytė L., Domarkienė I., Pranculis A., Burokienė N., Coj A., Mažeikienė A., Kasiulevičius V., Kučinskienė Z. A., Kučinskas V. LITGEN – revealing genetic structure of the population of Lithuania. European Human Genetics Conference; June 8–11, 2013; Paris, France. European Journal of Human Genetics. Vol. 21 Suppl. 2, June 2013, p. 394.
11. Mažeikienė A., Burokienė N., Kučinskienė Z. A. Nutritional habits and prevalence of the multiple cardiovascular risk factors among children and young adults. Abstract book of the international conference "Evolutionary Medicine: New Solutions for the Old Problems", 12th–15th June, Vilnius, Lithuania, 2012, p. 61.

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