Original article

Cardioprotective dietary pattern of Siberian population

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Abstract: *Background* — Analysis of eating habits can help identify cardioprotective dietary patterns. It is necessary to qualitatively study the diet of modern inhabitants of Siberia and identify food stereotypes that contribute to effective cardiac prophylaxis.
Objective — to study the diet of the inhabitants of Siberia and to identify the features of cardioprotective nutrition.
Methods — A clinical and epidemiological prospective group study of the population permanently residing in Kemerovo Oblast (administrative entity of the Russian Federation) was carried out. The baseline study included 1,124 women (70.3%) and 476 men (29.7%). To identify latent factors (stereotypes of eating behavior), we used factor analysis (method of principal components).
Results — Adherence to the fruit-and-vegetable stereotype was associated with an increased risk of obesity according to body mass index (BMI) (OR=1.57, CI: 1.27-1.96), waist circumference (WC) (OR=1.43, CI: 1.1-1.9), and presence of diabetes mellitus (DM) (OR=1.27, CI: 1.2-2.2). Adherence to the protein-and-carbohydrate dietary pattern was connected to a reduced risk of detecting obesity in terms of BMI criteria (OR=0.75, CI: 0.6-0.95, p=0.015), WC (OR=0.52, CI:0.41-0.66), DM (OR=0.66, CI:0.47-0.93), hypercholesterolemia (OR=0.78, CI:0.62-0.98) and hypertriglyceridemia (OR=0.66, CI:0.52-0.83). Prospective observation demonstrated the variability of stereotypes: after three years, the following five stereotypes were identified: vegetable, protein-and-carbohydrate, fruit, dairy, and mixed.
Conclusion — In contrast to the protein-and-carbohydrate diet, the fruit-and-vegetable stereotype of nutrition was associated with the development of obesity and DM. Considering the obtained results, it is necessary to study the qualitative characteristics of each stereotype (the content of macro- and microelements, kcal) and the motor activity of the respondents.

Keywords: nutrition; factor analysis, stereotypes of eating behavior, cardiac prophylaxis.

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Introduction

An important strategy for the prevention of cardiovascular disease (CVD) worldwide is associated with changing dietary habits [1, 2]. Many studies related to this topic were based on the association of individual macronutrients, micronutrients, or foods with CVD risk [3]. However, the human diet consists of complex combinations of foods rather than individual nutrients. In this regard, the analysis of eating behavior via identifying dietary patterns would accurately reflect the dietary preferences of the entire population, which could help identify cardioprotective measures [4].

The World Health Organization (WHO) has released General Dietary Guidelines that recommend eating fresh fruit and vegetables, saturated fatty acids, fast-digesting carbohydrates, and red meat. Furthermore, the Mediterranean diet is associated with lower cardiovascular risk, lower mortality, and incidence of CVD [5, 6]. However, evidence suggests that people who do not live near the Mediterranean Basin benefit from a change in the traditional Mediterranean diet [7, 8]. Geographical remoteness, ethnic and religious restrictions, along with established food traditions, reduce the effectiveness of the diet; therefore, it cannot be recommended for everyone [9]. An example of such restrictions are the inhabitants of Siberia: a sharply continental climate and temperature changes limit year-round access of the residents to fresh vegetables and fruit; remoteness from the sea limits their access to fresh fish, and ethnic diversity, as well as established food traditions, contribute to the consumption of large amounts of canned vegetables, fruits, and fatty meats [10].

Therefore, there is a necessity to study the diet of the inhabitants of Siberia and to identify the features of cardioprotective nutrition.

Material and Methods

Material

A prospective epidemiological study of the population in Kemerovo Oblast (administrative entity of the Russian Federation located in the south of Western Siberia, part of the Siberian Federal District) was conducted at the Federal State Budgetary Institution, *Research Institute for Complex Problems of Cardiovascular Disease*, via an epidemiological survey. Respondents were enrolled using the Kish selection grid in three consecutive stages: selection of municipal research institutes of preventive medicine, medical facilities, and households. Households were randomly selected using the Microsoft Excel random number function. All people 35 to 70 years of age were invited to participate in the study. All participants consented to publication prior to their inclusion in the study.

The baseline study included 1,124 women (70.3%) and 476 men (29.7%) (*Table 1*). Their mean ages were 54.9±9.75 years and 52.6±10.0 years, respectively, $p<0.001$. Considering age difference, to identify gender specifics all participants were distributed among three age groups: 35-49 years, 50-59 years, and 60-70 years.

The follow-up period lasted three years from the moment of the respondent's first face-to-face visit (during the baseline study). Contact with participants was re-established (via phone call or home visit), and participants who agreed to continue participating in the study were invited to the research institute for a personal examination. In connection with the mass outbreak of coronavirus infection COVID-19, confirmed on December 31, 2019, in the Chinese city of Wuhan, and the subsequent declaration of a global pandemic by WHO on March 1, 2020, a high alert regime was introduced in Kemerovo Oblast; that is, all personal visits were limited. It was not possible to follow all participants in compliance with the study design. Enrolment was considered complete when 60.0% of the respondents of each age group from the original study were included (with a follow-up period of no more than three years).

Of the 807 participants who met the above criteria (the response was 84.1%), 731 people visited the research institute in person and underwent a complete medical examination, 44 died, 32 changed their place of residence, 157 people refused to continue participating in the study. In the group of participants aged 35-49 years, 5 people died, 18 people changed their place of residence; in the group of participants aged 50-59 years, 6 people died, 8 people changed their place of residence; in the group of participants aged 60-70 years, 33 people died, 6 people changed their place of residence.

To analyze the association of dietary habits with CVD risk factors in a healthy population, respondents with normal blood pressure, lipids, glucose, body mass index (BMI) and waist circumference (WC), as well as without chronic diseases, were included. Healthy individuals accounted for 5.2% of the total number of those examined: they were mostly women (71.1%) living in urban areas (84.3%).

Table 1. Age- and gender-related characteristics of participants

	Gender	*Age groups, years*	*Total, n (%)*
Baseline	Male, n (%)	35-49	178 (11.1)
		50-59	158 (9.9)
		60-70	140 (8.7)
	Female, n (%)	35-49	331 (20.7)
		50-59	351 (21.9)
		60-70	442 (27.6)
Total, n (%)			1600 (100)
Follow-up	Male, n (%)	35-49	71 (9.7)
		50-59	81 (11.1)
		60-70	71 (9.7)
	Female, n (%)	35-49	127 (17.4)
		50-59	181 (24.8)
		60-70	200 (27.3)
Total, n (%)			731 (100)

Research methods

The Food Frequency Questionnaire (FFQ) was used to assess habitual food intake. When processing the data, the consumed foods were classified into several categories: dairy products; fruit; vegetables; eggs/meat; potatoes, grain; soups; beverages; sweets; chips / crackers; nuts; sauces; vegetable oil.

Blood pressure was measured twice, and the mean arterial pressure was also measured. Arterial hypertension (AH) was diagnosed according to the following criteria: systolic blood pressure (SBP) greater than or equal to 140 mmHg, diastolic blood pressure (DBP) greater than or equal to 90 mmHg. The hypertension group included participants with diagnosed AH, blood pressure ≥ 140/90 mmHg, or receiving antihypertensive treatment.

Medical scales were used to measure body weight. The measurement accuracy was up to 0.1 kg. A stadiometer was used to measure body length. Its measurement accuracy was up to 0.5 cm. BMI was determined by the formula: BMI=m/h2, where m is body weight in kilograms; h2 is the height in meters squared. According to the WHO classification (1999), body weight was considered healthy with a BMI of 24.9 kg/m2, and overweight was at the level of 25 kg/m2 or more. A measuring tape was used to measure waist circumference. Values were determined to the nearest 0.1 cm after normal exhalation.

Blood for biochemical studies was taken from the cubital vein in the morning, on an empty stomach. Lipids (total cholesterol, TC; high-density lipoproteins, HDL; triglycerides, TG; low-density lipoproteins, LDL) were determined using Thermo Fisher Scientific testing systems (Finland). The increase in lipid levels was assessed in accordance with the 2012 guidelines, *Diagnosis and Correction of Lipid Metabolism Disorders for the Prevention and Treatment of Atherosclerosis*, 5th edition. During the follow-up study, HbA1c was measured in all patients with diabetes mellitus (DM) using standard measuring kits (Human, Germany). The DM group of patients included individuals diagnosed with DM, receiving hypoglycemic drugs, or being on diet therapy, with fasting glycemia ≥7.0 mmol/L.

Statistical data processing

Statistical processing of the results was carried out using the Statistica v. 6.0 software. The normality of the sample distribution was determined via the Kolmogorov-Smirnov test. In our results, quantitative variables are presented as median (Me); interquartile range (25%; 75%) was used as a measure of variance; percentages were employed to describe categorical features. To assess the differences between the comparison groups, the Mann-Whitney test and Kruskal-Wallis U test were used; Pearson's chi-squared test was performed to compare categorical variables (Yates' correction was used for small groups). Principal component analysis was conducted to identify latent variables (dietary patterns). At the beginning of the study, a correlation matrix was obtained showing the frequencies of consuming different foods. According to the Kaiser's criterion, two risk factors were identified (four in a prospective study), the eigenvalues of which were greater than 1. A baseline correlation matrix of factor loadings of the food consumption frequencies on selected factors was created. Then varimax rotation was performed, and the final matrix of factor loadings was developed. The identified latent factors were formed at factor loadings of the food consumption frequency >0.50.

Table 2. Dietary patterns (DP) of the population in a large industrial region depending on gender and age, %

Age groups, years	*Gender*	*Fruit-and-vegetable DP*	*p*	*Protein-and-carbohydrate DP*	*p*	*Mixed DP*	*p*
35-49	M	21.3	0.129	45.5	0.078	33.1	0.667
	F	27.5		37.5		35.0	
50-59	M	27.2	0.030	35.4	0.597	37.3	0.084
	F	37.0		33.0		29.6	
60-70	M	27.9	0.014	43.6	<0.001	28.6	0.202
	F	39.4		26.2		34.4	

M, male; F, female.

The effect of several predictors was assessed using linear regression analysis. The relationship between dietary pattern and cardiovascular risk factors was assessed using logistic regression analysis. To account for the effect of age, the variable 'age' was introduced into the regression equation. The coding of variables for regression analysis was as follows: 'gender': 0 – women, 1 – men. At the same time, risk factors were considered as independent variables and were coded as 1, and the absence of a risk factor was coded as 0. The presence and magnitude of association were measured using odds ratio (OR) and 95% confidence interval (CI). A p value ≤ 0.05 was considered statistically significant.

Results

Principal component analysis identified three types of eating behavior. The first dietary pattern focused on the consumption of vegetables (both heat-treated and unprocessed, in season and throughout the year) and fruit (throughout the year) and was named *fruit-and-vegetable*. The second type was characterized by the consumption of non-dietary meat, along with complex carbohydrates and sweets (*protein-and-carbohydrate* dietary pattern). The third category included individuals not assigned to the previous groups (*mixed* diet).

Most study participants aged 35-49 years adhered to the protein-and-carbohydrate regimen (40.3% vs. 25.3% and 34.4% of those who adhered to the fruit-and-vegetable and mixed regimens, respectively, p<0.001). There were no statistically significant differences between the middle and older age groups. E.g., 34.0% of participants 50-59 years of age preferred fruit and vegetables, 33.8% consumed mainly protein foods and carbohydrate foods, and 32.0% adhered to a mixed regimen. In respondents aged 60-70 years, adherence was 36.6%, 30.4% and 33.0%, respectively.

Gender differences were noted in the middle and older age groups (*Table 2*). Women 50-59 and 60-70 years old adhered to the fruit-and-vegetable diet more often than men (p=0.030): 10% and 11.5%, respectively, while older men were twice as likely to adhere to a protein-and-carbohydrate diet (p<0.001). With increasing age, the number of women exercising the fruit-and-vegetable eating behavior increased (from 27.5% in the age group of 35-49 years to 39.4% in the age group of 60-70 years, p=0.001), while adherence to the protein-and-carbohydrate regimen declined by 11.3% (p=0.001). There were no statistically significant differences in adherence of aging men to different dietary patterns.

The highest prevalence of obesity was observed in study participants who observed the fruit-and-vegetable diet, whereas the lowest prevalence was associated with the protein-and-carbohydrate dietary pattern. However, statistically significant differences were found only when focusing on WC (*Figure 1*). In men, the prevalence values of AH (p=0.034), hypertriglyceridemia (p=0.007), and DM (p=0.005) were higher in those who adhered to a fruit-and-vegetable diet. The prevalence of conventional risk factors in men following the protein-and-carbohydrate regimen ranged from 10.1% (DM) to 55.7% (AH).

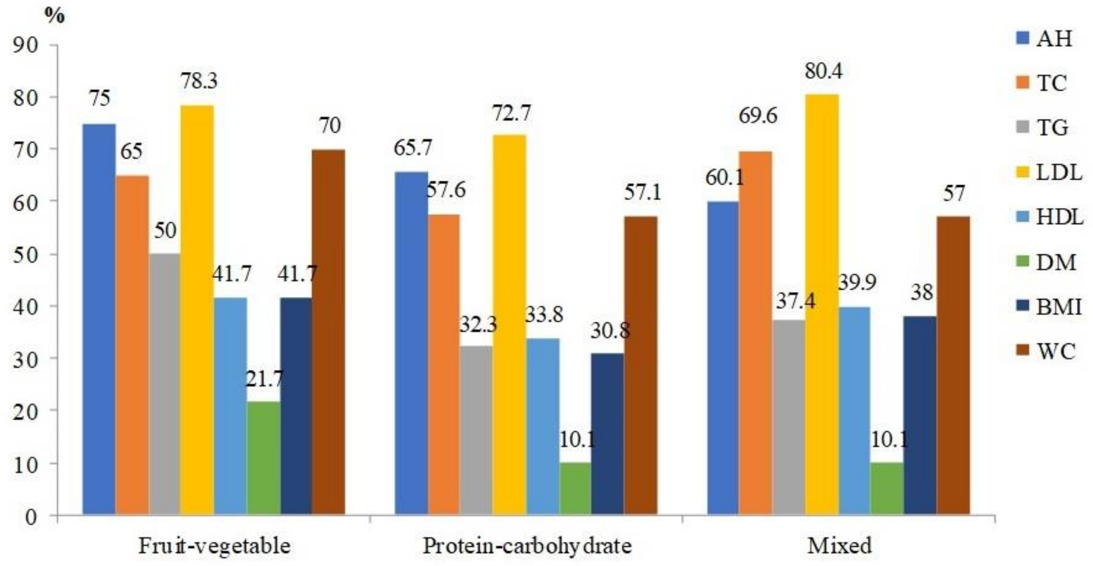

Figure 1. Prevalence of conventional risk factors in men adhering to different dietary patterns.

AH, arterial hypertension; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoproteins; HDL, high-density lipoproteins; DM, diabetes mellitus; BMI, body mass index; WC, waist circumference.

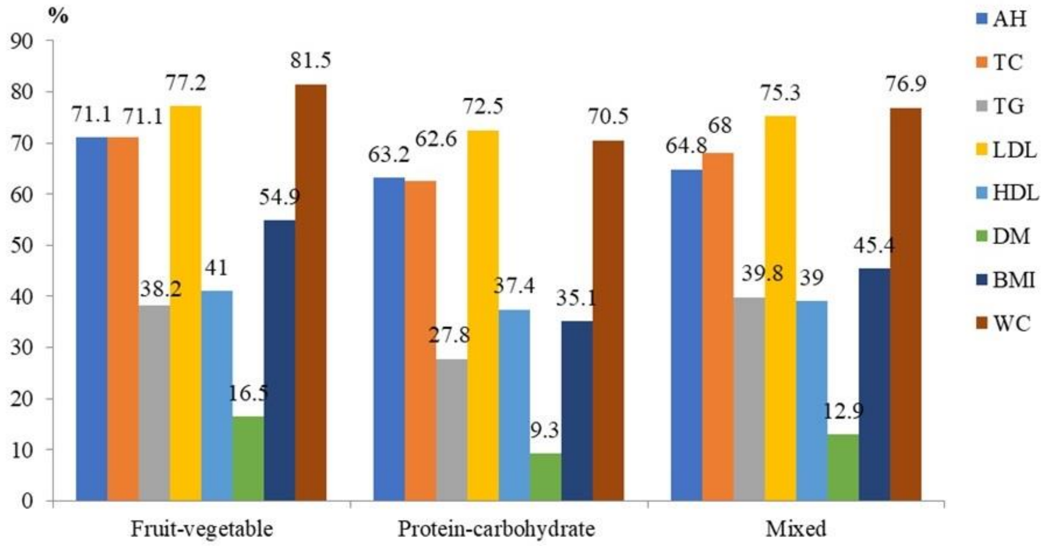

Figure 2. Prevalence of conventional factors among women adhering to dietary patterns

AH, arterial hypertension; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoproteins; HDL, high-density lipoproteins; DM, diabetes mellitus; BMI, body mass index; WC, waist circumference.

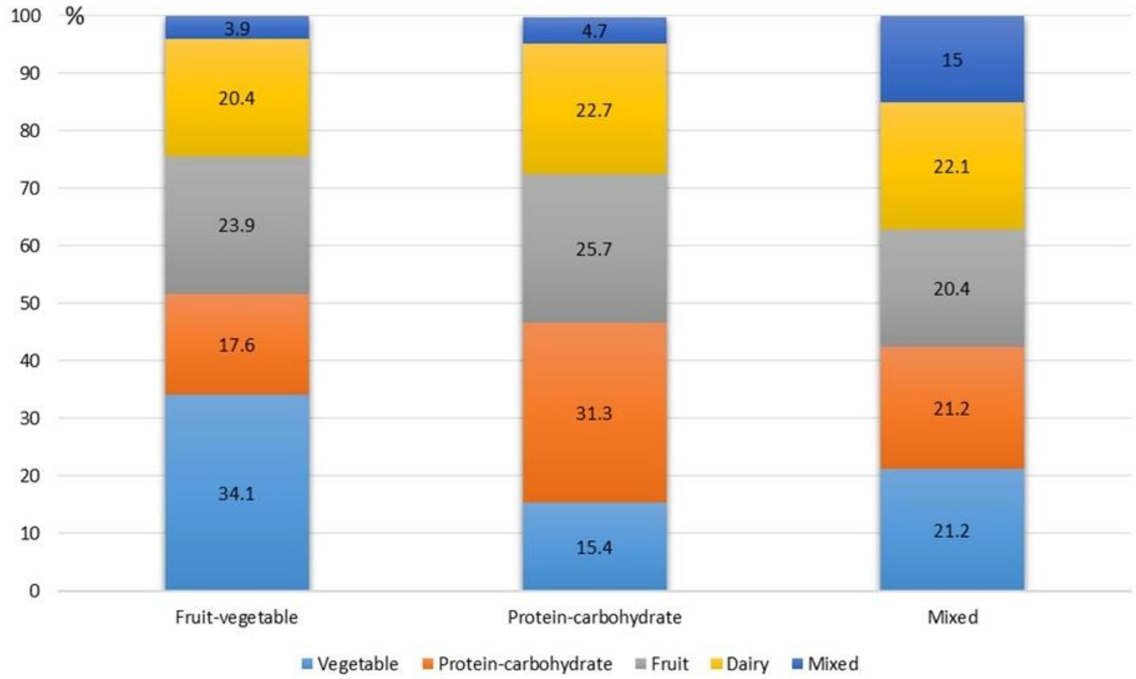

Figure 3. Variability of the diet over a three-year monitoring of the population in a large industrial region of Siberia.

In women, the highest prevalence of obesity in terms of BMI ($p<0.001$) and WC ($p=0.002$) was observed in people who adhered to a fruit-and-vegetable diet (*Figure 2*). In women and men, the highest prevalence of AH ($p=0.049$), hypercholesterolemia ($p=0.044$) and DM ($p=0.014$) was also observed in people who followed a fruit-and-vegetable diet (*Figure 3*). Hypertriglyceridemia ($p=0.001$) was more common in individuals observing the mixed and fruit-and-vegetable regimens. The prevalence of the above risk factors in women who adhere to the protein-and-carbohydrate regimen ranged from 9.3% (DM) to 62.6% (hypercholesterolemia).

Gender-based differences were noted in men aged 35-49 years adhering to mixed models, when analyzing the prevalence of obesity according based on WC / TC: it was 1.4 times higher vs. women of the same age group (*Table 3*). According to BMI and WC values, among those who adhered to the fruit-and-vegetable diet, obesity was more often detected in women in the age group of 50-59 years. Among those who observed the protein-and-carbohydrate dietary pattern, statistically significant differences in the prevalence of obesity (based on BMI and WC values) were noted in the older age group. For instance, the prevalence of obesity based on these criteria was twice as high in women as in men. Among the participants who adhered to the mixed diet, obesity according to BMI values was more often detected in women aged 50-59 years and in men 50-70 years of age. Furthermore, the prevalence values of hypertriglyceridemia and DM were higher in young men who observed a fruit-and-vegetable diet vs. women of the same age group. In men adhering to this dietary pattern, higher level of TG was observed nearly twice as often as in women (p=0.009), and DM was diagnosed in 7.9% of men vs. 0% in women. Hypercholesterolemia in people aged 50-59 years adhering to the fruit-and-vegetable regimen was 1.2 times more common in women than in men.

Similar trends were established in study participants observing the protein-and-carbohydrate dietary regimen: young men were almost twice as likely as women to have elevated TG levels, while women aged 50–59 years were 1.4 times more likely to have hypercholesterolemia than men of the same age group.

Among 35-49-year-old participants who adhered to the mixed pattern, hypercholesterolemia, hypertriglyceridemia, and high LDL levels were observed more often in men than in women.

\Logistic regression analysis showed that adherence to the fruit-and-vegetable regimen was associated with an increased risk of obesity according to BMI (OR=1.57, CI:1.27-1.96, p<0.001), WC (OR=1.43, CI: 1.1-1.9, p=0.006) and DM (OR=1.27, CI: 1.2-2.2, p=0.046).

Compliance with the protein-and-carbohydrate dietary pattern was associated with a reduced risk of obesity according to BMI (OR=0.75, CI:0.6-0.95, p=0.015), WC (OR=0.52, CI:0.41-0.66, p<0.001), DM (OR=0.66, CI:0.47-0.93, p=0.018), hypercholesterolemia (OR=0.78, CI: 0.62-0.98, p=0.032) and hypertriglyceridemia (OR=0.66, CI: 0.52-0.83, p<0.001).

At a repeated face-to-face visit (after 35.6±2.9 months) among healthy individuals adhering to a fruit-and-vegetable dietary pattern, 37.5% had no risk factors, 4.2% developed hypercholesterolemia, 4.2% had hypertriglyceridemia and overall obesity, 12.5% exhibited high LDL levels and 16.7% had abdominal obesity. Among healthy individuals who adhered to the protein-and-carbohydrate dietary regimen, 71.4% remained healthy, 8.6% developed hypercholesterolemia, 2.8% had hypertension, 2.8% experienced abdominal obesity, and 14.3% exhibited a high level of LDL. Among healthy individuals who adhered to the mixed diet, 58.3% remained healthy, 8.3% developed hypercholesterolemia, 12.5% had high LDL levels, and 12.5% had abdominal obesity. Logistic regression analysis demonstrated that adherence to the fruit-and-vegetable regimen was associated with an increased risk of obesity in terms of BMI (OR=1.57, CI: 1.27–1.96, p<0.001), WC (OR=1.43, CI:1.1-1.9, p=0.006) and DM (OR=1.27, CI:1.2-2.2, p=0.046). Compliance with the protein-and-carbohydrate dietary pattern was associated with a reduced risk of obesity, according to the values of BMI (OR=0.75, CI:0.6-0.95, p=0.015), WC (OR=0.52, CI:0.41-0 .66, p<0.001), DM (OR=0.66, CI:0.47-0.93, p=0.018), hypercholesterolemia (OR=0.78, CI:0.62-0.98, p=0.032) and hypertriglyceridemia (OR =0.66, CI: 0.52-0.83, p<0.001).

During the follow-up period, the eating habits of the population changed (*Figure 3*). We identified the following five major dietary patterns: vegetable, protein-and-carbohydrate, fruit, dairy, and mixed. Dairy consumption has been identified as a fundamentally new eating behavior. In addition, vegetable oil and low-fat meat foods have been added to the list of basic food products of a protein-and-carbohydrate dietary pattern. It should be noted that in the baseline study, two new independent dietary patterns were discovered: vegetable diet and fruit diet. As can be seen from the *Figure 3*, most respondents who adhered to this model (34.1%) preferred to eat vegetables, and 23.9% preferred fruit. Overall, 20.4% changed their preference to eat a dairy diet, and 17.6% changed their preference to adhere to a protein-and-carbohydrate dietary pattern. While 31.3% of the respondents maintained a protein-and-carbohydrate diet, 25.7% switched to a fruit pattern, 22.7% to a dairy pattern, and 15.4% to a vegetable diet. Participants who initially adhered to a mixed diet changed their eating habits in much the same way over the follow-up period, with only 15.0% continuing to follow a mixed dietary pattern.

Table 3. Prevalence of major risk factors for CVD in individuals adhering to different dietary patterns depending on their gender and age, %

Dietary patterns (DP)	*Age groups, years*	*Gender*	*AH*	*p*	*TC*	*p*	*TG*	*p*	*HDL*	*p*	*LDL*	*p*	*DM*	*p*	*BMI*	*p*	*WC*	*p*
Fruit-and-vegetable DP	35-49	M	60.5	0.067	57.9	0.323	44.7	0.009	31.6	0.463	71.0	0.055	7.9	0.007	39.5	0.731	68.4	0.333
		F	42.9		48.3		22.0		25.3		52.7		0.0		36.3		59.3	
	50-59	M	76.7	0.302	67.4	0.028	48.8	0.051	39.5	0.690	76.7	0.188	23.3	0.237	34.9	0.025	62.8	0.041
		F	68.5		83.1		32.3		36.1		85.4		15.4		54.6		78.5	
	60-70	M	87.2	0.897	69.2	0.531	56.4	0.552	53.8	0.912	87.2	0.610	33.3	0.343	51.3	0.111	79.5	0.001
		F	87.9		74.1		51.1		52.9		83.9		25.9		64.9		95.4	
Protein-and-carbohydrate DP	35-49	M	49.4	0.350	42.0	0.726	22.2	0.022	33.3	0.244	60.5	0.255	4.9	0.536	24.7	0.290	44.4	0.264
		F	42.7		39.5		10.5		25.8		52.4		3.2		18.5		52.4	
	50-59	M	69.6	0.669	55.4	0.006	30.4	0.980	32.1	0.396	75.0	0.098	7.1	0.610	41.1	0.537	62.5	0.231
		F	66.4		75.9		30.2		38.8		85.3		9.5		36.2		71.5	
	60-70	M	83.6	0.776	80.3	0.357	47.5	0.650	36.1	0.120	86.9	0.324	19.7	0.484	29.5	0.005	68.8	0.001
		F	81.9		74.1		44.0		48.3		81.0		15.5		51.7		88.8	
Mixed DP	35-49	M	44.1	0.142	52.5	0.008	30.5	0.044	40.7	0.131	71.2	0.001	6.8	0.318	42.4	0.108	57.6	0.926
		F	32.8		31.9		17.2		29.3		45.7		3.4		30.2		56.9	
	50-59	M	64.4	0.615	74.6	0.355	3.09	0.678	40.7	0.601	79.7	0.249	8.5	0.067	35.6	0.044	57.6	0.002
		F	68.3		80.8		42.3		36.5		86.5		19.2		51.9		79.8	
	60-70	M	77.5	0.142	87.5	0.912	45.0	0.247	37.5	0.234	95.0	0.335	17.5	0.794	35.0	0.047	55.0	<0.001
		F	86.8		86.8		55.3		48.0		90.1		15.8		52.6		90.1	

AH, arterial hypertension; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoproteins; HDL, high-density lipoproteins; DM, diabetes mellitus; BMI, body mass index; WC, waist circumference; M, male; F, female.

Discussion

Factor analysis revealed the following three models of nutrition in the subjects: fruit-and-vegetable, protein-and-carbohydrate, and mixed. However, follow-up observation demonstrated the variability of patterns over time: after three years, we identified the following five major patterns of eating behavior: vegetable pattern, protein-and-carbohydrate pattern, fruit diet, dairy dietary pattern, and mixed behavior. Most participants aged 35-49 adhered to the protein-and-carbohydrate dietary pattern (40.3%). There were no statistically significant differences between the middle and older age groups. With increasing age, the number of women adhering to the fruit-and-vegetable diet increased, while their adherence to the protein-and-carbohydrate diet declined by 11.3%. There were no similar statistically significant differences in men. Most participants diagnosed with obesity, AH, and DM followed a fruit-and-vegetable diet. Moreover, many men with hypercholesterolemia and women with hypertriglyceridemia adhered to this pattern. Preference for a mixed pattern at a young age contributed to a higher prevalence of hypercholesterolemia, hypertriglyceridemia, and high LDL in men vs. women. Regression analysis demonstrated that adherence to the fruit-and-vegetable dietary pattern was associated with an increased risk of developing DM, and adherence to the protein-and-carbohydrate regimen was linked with a reduced risk of developing DM, hypercholesterolemia, and hypertriglyceridemia. Thus, various dietary patterns exhibited age- and gender-specific features of the prevalence of obesity and conventional CVD risk factors. This finding implies the need for men and women to follow different diets at different ages. Furthermore, our study demonstrated paradoxical patterns in the relationship between the fruit-and-vegetable model and CVD risk factors, as well as the cardioprotective effect of the protein-and-carbohydrate model, which somehow contradicted traditional ideas about ‘proper’ nutrition. According to the published sources, the consumption of vegetables and fruit is the main aspect of any diet aimed at preventing both CVD and associated risk factors [11, 12, 13], while the consumption of fatty meats and sweets, according to all known recommendations, should be limited or eliminated [14, 15]. Individuals without risk factors were selected from the sample and followed to explain this phenomenon. However, we discovered that adherence to the fruit-and-vegetable dietary pattern was still associated with an increased risk of obesity and DM, while adherence to the protein-and-carbohydrate regimen was associated with a reduced risk of obesity, DM, hypercholesterolemia, and hypertriglyceridemia.

Conclusion

We identified the three most common dietary patterns among the Siberian population as fruit-and-vegetable, protein-and-carbohydrate, and mixed. Even though recommended by the international guidelines, the fruit-and-vegetable diet was associated with the development of obesity and diabetes, unlike the protein-and-carbohydrate regimen. Based on our results, we propose to further study the qualitative characteristics of dietary patterns (macro- and microelements, kcal) depending on the physical activity of the respondents.

Ethical approval

The study protocol was approved by the Institutional Review Board at the Research Institute for Complex Problems of Cardiovascular Disease (Protocol No.12 of 10 July 2015). The study was carried out in accordance with the ethical principles of the Declaration of Helsinki.

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Conflict of interest

The authors declare no conflicts of interest.

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