Chapter

# Health Effects of Plant Foods and the Possibility of Reducing Health Risk

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## Abstract

Many nutritional studies point to an inverse relationship between diet with predominant consumption of plant foods and the incidence of diseases of civilization. The health benefits of plant foods come from a sufficient intake of protective nutrients, which are key food commodities of the vegetarian diet. These include vegetables, fruits, whole grains, legumes and oilseeds, including various types of nuts. The nutritional and non-nutritional components of plant foods reduce the risk of chronic diseases by various mechanisms, so a well-planned vegetarian diet is nutritionally adequate, fully healthy and able to provide health benefits in the prevention of many diseases. The data we find agree that the benefits far outweigh the potential risks.

**Keywords:** antioxidants, oxidants, intestinal microbiota, benefits and risks of alternative eating

## 1. Introduction

Despite the fact that vegetarianism has been known for several millennia, this diet still has many of its adherents as well as despisers. We have been dealing with the issue of health effects of plant foods since 2000 in partial epidemiological studies, so we decided to summarize our findings on how vegetarianism affects the population of Slovakia.

Plants are the basic components of the food chain, in which they provide all the essential mineral and organic nutrients for humans either directly or through animal production. A comprehensive, varied diet ensures nutritional quality (intake of all nutrients) and, despite the concentrations of nutrients in the consumed food mixture corresponding to the recommended nutritional doses, also the nutritional quantity. Both of these criteria for optimal diet are essential for the development and maintenance of human health [1]. Vitamin B12, vitamin D and n-3 fatty acids are lacking in plant food sources. In particular, the content of methionine and lysine (also other essential amino acids), iodine and carnitine is significantly reduced compared to animal food [2, 3]. The absorption of iron, calcium, zinc, copper, manganese and selenium can be inhibited by plant food components [4, 5]. These facts may be associated with many health risks in the population with exclusive or dominant consumption of plant foods [6, 7].

To prevent these risks, it is necessary to consume food or pharmaceutical supplements to compensate for deficient nutrients.

Saturated fats (animal source) cause hypercholesterolemia, polyunsaturated fats (vegetable oils) can lower blood cholesterol levels. Monounsaturated fatty acids (oleic acid - olive oil, rapeseed oil, sesame oil, hazelnuts, almonds) also have a hypolipidemic effect and, in addition, have a saving (non-reducing) effect on HDL-cholesterol [8]. Compared to nonvegetarians, vegetarians in the Oxford vegetarian study had a 24% reduction in mortality from ischemic heart disease (DRR/death rate ratio/ = 0.76) [9]. When the meat-consuming group was divided into regular consumers of red meat (at least once a week) and semi-vegetarians (consumption of fish and white meat less than once a week), the DRR for semi-vegetarians was 0.78 and for vegetarians 0.66. Consumption of cheese, eggs, total fat, and cholesterol has been associated with mortality from ischemic heart disease [10]. Compared to subjects who ate relatively few of the commodities and nutrients listed, the DRR in those with the highest consumption of cheese was 2.47, eggs 2.68, animal fat 3.29, and cholesterol 3.53. Analysis of 5 prospective studies also expressed mortality from cerebrovascular disease - DRR in vegetarians 0.93 [11]. The results of a study of more than 34,000 Adventists on Day 7 in California showed a significant association between beef consumption and fatal coronary heart disease. In men eating beef more than 3 times a week, the relative risk (RR) was 2.31 compared to vegetarians. Furthermore, this study found a significant protective association between nut consumption and fatal and non-fatal ischemic heart disease for both sexes (RR = 0.5 for subjects consuming nuts more than 5 times per week compared to less than 1 time/week) and a reduced risk of ischemic heart disease in subjects who prefer wholemeal bread versus white [12].

Fiber consumption can also reduce the risk of cardiovascular disease [13]. An analysis of 10 prospective studies in the US and Europe of more than 336,000 subjects showed that an increase in fiber consumption every 10 g/day was associated with a 14% reduction (RR = 0.86) in all coronary cases and a 27% reduction (RR = 0.73) in risk coronary death [14]. Other plant food components (saponins in legumes, plant proteins, antioxidant nutrients - vitamin C, vitamin E, β-carotene, selenium, polyphenols and flavonoids) are added to the beneficial effect of fiber [15]. By evaluating current knowledge about the protective effects of plant food ingredients, scientists agree on the need to consume a variety of plant foods [7]. Vegetarian studies have confirmed that subjects with low or no consumption of animal fats and with a dominant consumption of plant foods have low values of atherosclerosis risk factors and higher values of parameters with antisclerotic properties compared to the general population [14].

Cardiovascular disease can be positively influenced by the consumption of plant proteins. Vegetable proteins versus hen egg reference protein or other animal proteins have a reduced content of some essential amino acids and an increased content of some non-essential amino acids [15]. Experimental studies have shown that cholesterol-free diets containing milk protein, casein or other animal proteins, induced an increase in plasma concentrations of total and LDL-cholesterol, while plant proteins did not have such an adverse effect [16]. By selectively increasing amino acid intake, hypercholesterolemia was found to be primarily due to essential amino acids. Higher intake of lysine and methionine (from animal proteins) adversely affects the metabolism of phospholipids in the liver. Higher amounts of methyl groups from methionine may lead to increased secretion of apoB lipoproteins. These data suggest that lower methionine and lysine intake in subjects with exclusive or predominant consumption of plant proteins represents a protective effect against cardiovascular risk. In the general population, the ratio of animal to vegetable protein consumption is about 60:40, more preferably 50:50.

*DOI: http://dx.doi.org/10.5772/intechopen.94096*

## 2. Characteristics of the monitored group of probands with different eating habits

Our research included a randomly selected adult population from Bratislava and its surroundings. A database of volunteer addresses from previous solved research projects monitoring the health and nutritional status of the population was used. The number of probands in both research groups (non-vegetarian group/general population /and alternative diet group with predominance of plant food consumption/vegetarians)) as well as other characteristics of the groups are given in **Table 1**. Examinations were performed in the spring. The intake of vitamins, minerals and trace substances was only in its natural form (no supplementation). Another limitation of random selection was the requirement that probands be subjectively healthy, i. zn. without cardiovascular disease, cancer, diabetes, kidney disease, digestive tract and thyroid disease.

Monitoring of health and nutritional status was performed by somatometric, dietary, biochemical and microbiological examination.

### 2.1 Somatometric examination

Somatometric examination was performed by measuring body height and weight. The body mass index (BMI = weight/height$^2$) was calculated to assess overweight and obesity. A calipometer, Omron and InBody 230 were used for further somatometric measurements the type of obesity was determined after recalculation. We used Omron to measure bioimpedance in the upper half of the body, but the results can be influenced by the type of obesity and data entry. InBody 230 was performed by bioimpedance measurement using DSM-BIA (segment multi-frequency bioelectric impedance) technology, ie measurement of the upper and lower body, so the results are not affected by the type of obesity.

At first glance, most vegetarians are recognizable. They have a slim figure, pale skin and are anemic. These facts were also confirmed in our study (**Table 2**). Subcutaneous fat on the abdominal algae measured with a caliper, as well as visceral fat, have been significantly reduced by vegetarians compared to non-vegetarians. Also, the percentage of fat measured using the InBody 230 and Omron BF-306, both instruments using the bioimpedance method, confirmed a significantly reduced percentage of fat in vegetarians.

| | Vegetarians | Non-vegetarians |
|---|---|---|
| n | 920 | 958 |
| age range (y) | 20–60 | 20–60 |
| average age (y) | 40,41 ± 0,96 | 40,50 ± 1,13 |
| BMI (kg/m2) | 21,46 ± 0,35*** | 25,60 ± 0,47 |
| time vegetarianism (y) | 18,61 ± 0,63 | |
| smokers | 0 | 0 |
| Systolic blood pressure (mmHg) | 120,57 ± 1,44*** | 143,59 ± 1,70 |
| Diastolic blood pressure (mmHg) | 72,46 ± 0,95*** | 88,27 ± 0,94 |
| Pulse rate (1 minute) | 70,57 ± 1,33 | 77,01 ± 1,25 |

**Table 1.**
*Characteristics of the monitored group.*

| | Vegetarians | Non-vegetarians |
|---|---|---|
| Belly (mm) on caliper | 24,40 ± 1,13*** | 30,16 ± 1,01 |
| Visceral fat (cm2) | 70,06 ± 4,94*** | 100,56 ± 4,13 |
| % fat on InBody230 | 23,16 ± 0,69* | 25,93 ± 0,88 |
| % fat on Omron BF-306 | 27,00 ± 0,67* | 28,27 ± 0,86 |

**Table 2.**
*Subcutaneous and visceral fat.*

### 2.2 Dietary examination

The nutritional regime was determined on the basis of a questionnaire on the frequency of use of selected commodities. The questionnaire focused on the amount and frequency of consumption of 144 individual foods, food groups and recipes. Groups and recipes include soups, soups, sauces, pickled vegetables, fruits and jams. Probands responded to the consumption of 4 categories: almost never, x times a month, x times a week, x times a day. The data after dispensing into individual foods as well as after the exact expression of the amounts of consumption and conversion to daily intake were processed using the Nutrition program, which is a food data bank of the Food Research Institute in Bratislava. The calculation revealed the loss of vitamins during technological food processing. The output of the evaluation of the nutritional regime was the average daily energy intake and selected nutrients.

### 2.3 Biochemical examination

Collection of biological material - blood, urine and stool.

Blood was collected in the morning on an empty stomach after standard food intake in the previous days to examine the monitored parameters. Blood collection for plasma was performed in commercial syringes with EDTA (ethylene diamine tetraacetate), which in addition to its anticoagulant properties is also an inhibitor of free radical reactions. Morning urine and feces were also collected and processed and stored at −80° C on the day of collection. Average sample 24-h. urine volume for the determination of iodine probandi brought in the morning on the day of examination.

Total cholesterol, HDL-cholesterol, triacylglycerols, glucose and iron were determined by standard laboratory methods with a Vitros 250 automated analyzer (Johnson & Johnson, USA). The LDL-cholesterol content was determined by calculation from the Friedewald formula: LDL-cholesterol = total cholesterol - triacylglycerols/2,2 - HDL-cholesterol (6). The condition of the calculation was the value of triacylglycerols <4.5 mmol/l. The atherogenic index was calculated from the ratio of total and HDL-cholesterol. Vitamin C, E, A and β-carotene, malondialdehyde were measured by HPLC [17–20]. Protein carbonyls, conjugated dienes were determined spectrophotometrically [21]. The alkaline comet assay was used to detect DNA breaks, oxidized purines and oxidized pyrimidines in isolated lymphocytes [22–24]. Determination of 25-hydroxyvitamin D and other hydroxylated metabolites of vitamin D (both its forms - ergocalciferol D2 and cholecalciferol D3) in serum was performed by the classical equilibrium RIA method. Plasma N-carboxymethyllysine and plasma fluorescent AGEs were determined by competitive ELISA [25]. The iodine value was determined in the 24-h samples. Urine by modification of the Sandell-Kolthoff reaction [26].

### 2.4 Microbiological examination

The qualitative and quantitative representation of the microflora was evaluated by classical microbiological methods by culturing on selective diagnostic nutrient media according to Mitsuok et al. [27]. In probands for which we did not notice differences in the qualitative representation by culture methods, we determined bacterial profiles using molecular biology methods. The presence of potentially mutagenic substances was determined by Ames plate incorporation assay using S. typhimurium TA98 (shift mutations) and TA100 (point mutations) bacterial cultures. Sterol analysis by gas chromatography was performed using a GC17-QP5000, with a split/splitless injector (Shimadzu, Kyoto, Japan).

### 2.5 Statistical processing of results

Commercial programs - Excel 2000 and Statgraphics for Windows, version 1.4 and PASW Statistics 18 were used for computer processing of the obtained data. The comparison was supplemented by determining the percentage occurrence of risk values of monitored parameters as well as the occurrence of protective values of antioxidant vitamins in each evaluated group. This partially eliminated the fact that malnutrition probands could also be included in the traditional diet (this is a current sample of our population, whose malnutrition ultimately reflects a high incidence of morbidity and mortality from the two main diseases - cardiovascular and cancer), while in vegetarians, incorrect vegetarianism is less common (the risks are given by the one-sidedness of the diets resulting from the type of alternative diet).

## 3. Health risks of dominant consumption of vegetable food

### 3.1 Vitamin B12

Vitamin B12 is absent from plant foods; bacteria in the lower part of the small intestine are its only source in subjects with exclusive consumption of plant foods, therefore vitamin B12 deficiency is one of the risk factors for alternative diets [28]. Vitamin deficiency can have many adverse health consequences: folate "flap" in the methylation cycle, deterioration of DNA biosynthesis, pernicious anemia, increased atherogenic homocysteine in the blood, and neural tube defects [29]. Consumption of dairy products and eggs in lacto-ovo-vegetarians and, in addition, intake of white meat in semi-vegetarians provides a better ability to meet the body's vitamin B12 needs [28, 30]. In the monitored groups of volunteers, we found significantly reduced concentrations of vitamin B12 in the group of vegans (VV) and lacto-ovo-vegetarians (V-LO) and insignificantly lower serum concentration in semivegetarians (VS) compared to the traditional diet of the general population (NV). Deficiency values occurred in 67% V-V, 32% V-LO, 7% V-S, but no non-vegetarian. From a global perspective, vitamin B12 deficiency prevention requires monitoring of serum vitamin B12 levels and strict vitamin B12 fortified food or vitamin B12 supplements, especially in strict vegetarians but also in V-LO. One of the many functions of vitamin B12 is its involvement in the metabolism of homocysteine (HCy), which has atherogenic properties. HCy is a sulfur amino acid that is metabolized in two ways by B-group vitamins - remethylation (requires vitamin B9 and B12), which converts HCy back to methionine, and transsulfurization (requires vitamin B6), which converts HCy to cysteine and taurine [31]. The first of the pathways dominates with lower methionine intake, which occurs in V-LO and V-V diets, because plant proteins contain less of this amino acid. The results shows a higher incidence of mild hyperhomocysteinemia in lacto-ovo-vegetarians and vegans, in which remethylation predominates, but in vitamin B12 deficiency the remethylation cycle is inhibited and HCy is not degraded to methionine.

### 3.2 Vitamin D

Subjects with limited animal food intake may be at greater risk of vitamin D deficiency compared to non-vegetarians because the food that provides the highest amounts of vitamin D is of all animal origin [32]. The authors of Crowe et al. [33] reported that plasma concentrations of 25 (OH) vitamin D reflect the rate of elimination of consumption of animal products. Meat consumers had the highest average vitamin D intake (3.1 μg / day) and a mean plasma concentration of 25 (OH) D (77.0 nmol / l), vegans had the lowest average intake and plasma concentration (0.7 μg /day) and 55.8 nmol/l). Our results show that under conditions of the same and low intensity of sunlight (spring - April) a significantly reduced plasma concentration of vitamin D was found in V-LO, while in V-S (white meat consumers) this concentration was balanced with non-vegetarians. A higher incidence of deficit values was found in V-LO versus NV and V-S (67% versus 46% and 50%). It should be noted that vitamin D concentrations are low in all three groups examined (the lower limit of recommended values is 30 ng/ml), suggesting the need for supplementation or pharmaceuticals in the winter and early spring months.

### 3.3 Iron

Decreased utilization of minerals and trace elements from food has been observed in people with a dominant consumption of plant foods due to the high content of phytic acid in plant food (whole grains, legumes) as well as fiber (whole grains, legumes, seeds, nuts) [34]. Phytic acid and fiber form undesirable insoluble complexes with some minerals and trace elements, which cannot be used by the organism. These food commodities are significantly more consumed by vegetarians versus non-vegetarians. We observed significantly reduced serum iron concentrations in the V-LO group, hyposiderinemia occurred in 44% versus 20% in NV and 30% in V-S).

In addition to known iron deficiency disorders and diseases, the latest and lesser-known finding is that iron deficiency adversely affects the biosynthesis of long-chain n-3 fatty acids.

### 3.4 Iodine

The iodine content of food of plant origin is lower compared to food of animal origin due to the low iodine content of the soil. On the other hand, regular consumption of animal products (eggs, cheese, milk, meat, fish and poultry) can make a significant contribution to overall income. The literature review indicates that in vegans and vegetarians not consuming iodine supplements and seafood, iodine consumption is inadequate [35]. A wide range of mental, psychomotor and growth abnormalities cause iodine deficiency [36]. Determination of iodine is a more exact criterion than iodine intake, and urinary iodine excretion expresses the degree of saturation in the body. In group V-V we measured urinary iodine excretion 78 μg /l, in V-LO 172 μg /l and in group NV 216 μg /l. A clinically significant deficit (less than 50 μg /l) was reported in 27% V-V, 10% V-LO, but no non-vegetarian. The authors Leung et al. (14) measured median urinary iodine concentrations of 78.5 μg /l in vegans and 147 μg/l in vegetarians.

### 3.5 AGEs - advanced glycation endproducts

These products (AGEs - advanced glycation endproducts) are formed by the non-enzymatic reaction of an aldehyde or keto group of reducing sugars with a free amino group of amino acids, proteins, nucleic acids, phospholipids and other macromolecules; the reaction is called the Maillard reaction or glycation [37]. AGEs negatively affect the functional properties of proteins, lipids and DNA [38]. These chemical modifications accumulate in the body with age and may contribute to the pathophysiological processes associated with aging and to the complications of diabetes, atherosclerosis and chronic renal failure [39, 40]. AGEs are produced from monosaccharides (glucose, fructose), but also from dicarbonyl intermediates of the Maillard reaction, sugar autoxidation, and other metabolic pathways [41, 42]. The reactivity of monosaccharides in AGE formation is given by the ratio between the occurrence of the acyclic and cyclic forms. Only open chain sugar can enter the glycation [43]. Fructose is more reactive because it has a higher proportion of the acyclic form [44]. The mentioned processes of the Maillard reaction in the organism and exogenous AGE from food (especially culinary and technologically modified) are the main sources of intracellular and plasma AGEs [45]. CML ($N^{\varepsilon}$-carboxymethyllysine) values and fluorescence AGE values are significantly higher in vegetarians. CML, a major product of oxidative modification of glycated proteins, represents a common marker of oxidative stress and long-term protein damage in the aging process, atherosclerosis, and diabetes [46]. Vegetarians consume less protein and carbohydrates, and lysine intake is significantly reduced. They prefer the use of lower temperatures and shorter periods in food preparation. Some dairy products, specially cooked and with added sugar and stabilizers, have a higher CML content [47]. V-LO consumes significantly smaller amounts of dairy products (220–17 g / day versus 469–41 g/day NV, traditional nutrition) [48]. By excluding all of the above options for increasing plasma AGE values in vegetarians, we focused on monosaccharides. Fluorescence as an index of advanced glycation has been shown to increase linearly for human serum albumin incubated with glucose and exponentially using fructose [44]. The fructose-induced AGE fluorescence was higher than the glucose-induced AGE fluorescence due to the higher content of the more reactive acyclic form of fructose versus glucose.

### 3.6 Amino acids

The body needs to take in all the essential amino acids and in the optimal amount. Only under these conditions can amino acids from food be adequately used for protein synthesis [48]. The limiting amino acid in the protein mixture consumed (which is the lowest) is crucial for the productive utilization of all essential amino acids for anabolic processes by initiating peptide chain synthesis. Additional amino acids are incorporated into the peptide chain depending on the availability of an amount of limiting amino acid. The major limiting amino acids in plant proteins are methionine, lysine and tryptophan. The content of tryptophan is approximately the same in plant and animal proteins. Significantly reduced methionine and lysine intake in subjects with dominant or exclusive consumption of plant proteins may indicate a reduced rate of proteosynthesis, which is expressed by deficient plasma protein concentrations. Hypoproteinemia has been reported in 20% of adult vegans. The incidence of hypoproteinemia in children was higher - 33% in vegans and 11% in vegetarians. In our earlier experimental study using labeled amino acids, we found that protein synthesis was significantly reduced in young animals during the period of growth fed with plant protein (wheat gluten) compared to animal protein (milk casein). Caso et al. [49] also used the isotope technique and two diets with the same energy and protein content but with different protein sources. They reported that albumin synthesis in healthy volunteers was significantly reduced after eating plant food (67% plant protein, 33% animal protein) compared to eating 74% animal protein and 26% plant protein).

### 3.7 Microflora of the gastrointestinal tract

The microflora of the large intestine is an important indicator of human health. Currently, many studies point to the association of various diseases with the quantitative or qualitative representation of individual microorganisms in the gastrointestinal tract. This complex community of microorganisms can "communicate" with cells of the gastrointestinal immune system and thus coordinate the immune response to harmful pathogens. The microflora is also involved in the production of various substances such as certain vitamins or enzymes that help the proper digestion of food, but also acids, bacteriocins and the like that inhibit the growth and adhesion of harmful microorganisms to the surface of the intestinal epithelium. To ensure digestion and immune function of the intestine, it is necessary to properly represent the intestinal microflora, which is influenced by many factors, but to a large extent by the composition of the diet.

The gastrointestinal microflora consists of a very complex community of microorganisms consisting of more than 400 different bacterial species. The number of bacteria increases from 103/ ml in the stomach, 104–106/ ml in the small intestine to more than 1012/ml in the large intestine [50]. The majority of intestinal bacteria are coliform bacteria, streptococci, lactobacilli and strictly anaerobic bacteria of the genera Bacteroides, Bifidobacterium, Fusobacterium and Clostridium. Quantitatively, the microflora of the intestinal tract represents huge numbers of microorganisms approaching a trillion bacterial cells [51]. This huge proportion of bacteria makes up about half the weight of stool [52].

Up to 25–70% of diseases can be prevented by eating optimal food, its specific and balanced components. I. Mečnikov was already thinking about the connection: diet - microbes - health - disease. The bacterial flora can affect colorectal carcinogenesis by producing enzymes that transform procarcinogens into active carcinogens. These include β-glucuronidase, glycosidase, nitroreductase. The main effector mechanisms by which the microflora affects the development of cancer include: activation of procarcinogens, fermentation leading to the formation of short-chain fatty acids, formation of diacylglycerol, synthesis of pentanes and adsorption of hydrophobic molecules [53].

In our study, we divided the quantitative and qualitative representation of the microflora according to age categories and eating habits, while the numbers of microorganisms were expressed as logarithmic values. In the age category 21–30 years we found the largest differences in the total number of clostridia, in non-vegetarians this number was almost 2 orders of magnitude higher than in vegetarians, as evidenced by several studies [54]. In the case of lecithinase-positive clostridia, which play an important role in certain diseases such as e.g. We have not seen a significant difference in colon cancer in terms of eating habits. The number of spores was slightly higher in vegetarians and we assume that they enter the digestive tract mainly through plant food.

In non-vegetarians, we observed a slightly higher number of probiotic bacteria (genus Lactobacillus, Bifidobacterium), which may be due to higher intake of sour milk products in this category of people compared to vegetarians who consume these products to a lesser extent, or not at all. At the same time, we observed an increased number of staphylococci in these people (by about 2 rows), but the *Staphylococcus aureus* strain predominated in people eating plant foods. Occurrence of this strain in the gastrointestinal tract is rare, and it can enter the GIT from contaminated food or from the upper respiratory tract and cause various disease states [55]. In vegetarians, we also observed a slightly higher number (about half an order) of bacteria of the genus Enterococcus spp. and Listeria spp.

The values in the quantitative representation of individual microorganisms of the population of people aged 31–40 were mostly balanced. We observed slight differences especially in the case of eukaryotes (yeast, fibrous fungi) and clostridia, which were increased in non-vegetarians and, conversely, the numbers of listeria, spores and veilonel were higher in vegetarians. By comparing the representation of the intestinal microflora of vegetarians and non-vegetarians in the age category 41–50, we found a difference in the number of total anaerobes, which was 0.3 logarithmically higher in non-vegetarians. In contrast, the total number of aerobic microorganisms was higher in vegetarians. The number of representatives of Bacteroides spp., Veillonella spp., LP-clostridia was comparable in both groups. In vegetarians, we observed a slight increase in Enterococcus spp., Which was approximately 0.5 logarithmically higher compared to nonvegetarians. There was a small difference in the number of bacteria of the family Enterobacteriaceae, with higher values belonged to vegetarians. At the same time, we observed an increased number of Clostridium spp. about 0.5 logarithmic order. We registered the largest difference in the coagulase-positive species *Staphylococcus aureus*. The value of *S. aureus* in vegetarians reached 1.61 ± 0.38 CTU / g stool, which is significantly higher compared to the number of 0.34 ± 0.24 CTU/g stool in non-vegetarians. By comparing the group of people aged 51–60 years, we did not notice a significant difference in the amount of total anaerobes and aerobes. However, we found a significant difference in the number of LP-clostridia by a logarithmic order of magnitude higher in nonvegetarians. The production of α-toxin by LP-clostridia causes cell endothelial damage, resulting in changes that in some cases can lead to colon cancer [56]. In the age group, we registered an increased number of coagulase-positive *S. aureus*, especially in non-vegetarians.

In the etiology of colon cancer, not only genetic predisposition plays an important role, but also some microorganisms, whose quantitative and qualitative representation in the colon is largely influenced by the diet consumed. For example, some bacteria of the genus Bacteroides are able to transform certain substances into mutagens [57]. Another factor influencing this disease is the intake of carcinogenic substances that may arise during food processing [58]. Therefore, another goal was to monitor for the presence of potentially mutagenic substances in the colon. In the group of people aged 21–30 years, we recorded the presence of potentially mutagenic agents in approximately 20% of probands. In the group of non-vegetarians, we captured a slightly higher number of people (25%) compared to vegetarians (21.87%), but we did not register a significant difference between the two groups. In the age category of 31–40 years, we found a much more significant difference in the percentage of probands with detected potential mutagenic substances in the stool. In this case, it is surprising that this percentage was almost five times lower for non-vegetarians (3.84%) compared to vegetarians (19.44%). We assumed that the situation would be the opposite, as vegetarians have a higher dietary fiber intake. This is made up of important oligosaccharides that serve as prebiotics for probiotic bacteria to help prevent colon carcinogenesis. Fiber also increases the peristalsis of the large intestine, is indigestible by the human body, which mechanically "cleanses" the intestinal epithelium and removes carcinogens from the body [59]. On the other hand, people consuming a mixed traditional diet receive more frequently roasted, grilled or otherwise cooked foods in which the presence of mutagenic substances has been demonstrated [60]. However, this trend was confirmed in the category of probands aged 41–50 years, where we determined the presence of potentially mutagenic substances, especially in non-vegetarians. The percentage of the population in the age category 51–60 was relatively balanced in both groups, but at the same time almost half lower compared to the young (21–30 years) generation of probands.

Diet is one of the important indicators affecting the composition of the intestinal microflora. It is important to monitor and at the same time modulate the composition of food intake in the prevention or treatment of certain diseases. From our results of intestinal microflora values, it is clear that a randomly selected population consumes a varied diet, i. the intake of animal food is supplemented by a plant-based diet. The result of such a diet is a balanced intestinal microflora. Under certain circumstances and on the basis of certain measured parameters, it can be assessed that the conventional diet is "more advantageous" than a predominantly plant-based diet. Increased intake of beneficial bacteria in the form of probiotic products could have a positive effect on health in old age.

## 4. Health benefits of dominant consumption of vegetable food

### 4.1 Lipid parameters

Epidemiological studies document that the consumption of animal fats that contain cholesterol and saturated fatty acids causes hypercholesterolemia, while unsaturated fatty acids (plant sources) have a cholesterol-lowering effect [61]. Also, consuming a high-fiber diet prevents the risk of cardiovascular disease [7]. The hypocholesterolemic effect of fiber is explained by the binding to bile acids and the increase in fecal sterol excretion. Fermentation of soluble fiber produces short chain fatty acids that inhibit cholesterol synthesis in the liver. Whole grains, legumes, fruits, vegetables and various types of nuts are very good sources of fiber [62]. The analysis of standardized and validated dietary questionnaires on the frequency of food consumption in our vegetarians versus non-vegetarians significantly reduced the daily intake of total fats, saturated fatty acids and cholesterol and on the other hand significantly increased daily intake of vegetable fats, unsaturated fatty acids, linoleic acid and α-linolenic acid. We have found that vegetarians consume significantly more fiber, whole grains, legumes, fruits, vegetables, nuts, vitamin E, vitamin C, β-carotene and selenium. The consequence of this diet is favorable values of markers of cardiovascular risk. Total cholesterol, LDL-cholesterol and triacylglycerol levels are significantly reduced in vegetarians versus non-vegetarians and the incidence of risk-related values is low (8.2% versus 43.3% total cholesterol; 2.5% versus 28.7% LDL-cholesterol; 10.8% versus 25.6% triacylglycerols). HDL-cholesterol levels are significantly elevated in vegetarians.

### 4.2 Non-lipid parameters

C-reactive protein (high sensitivity, hsCRP), a marker of inflammation, can predict the risk of myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death in healthy subjects, as well as death and recurrent events in patients with acute or persistent coronary heart disease [63]. Prospective clinical studies demonstrate that atherosclerosis is not a simple disease caused only by lipid imbalance, but is an inflammatory process with highly specific cellular and molecular responses. HsCRP expresses additional prognostic value to cholesterol levels, Framingham risk score, metabolic syndrome, blood pressure with or without subclinical atherosclerosis. Vegetarians have significantly reduced hsCRP levels. No vegetarian was found to be associated with a higher risk versus 11.6%

of non-vegetarians. Favorable values of the inflammatory marker in vegetarians are explained and proven by the consumption of fruits and vegetables, which are rich sources of salicylates and other anti-inflammatory components [64]. We also measured favorable values of another non-lipid marker of cardiovascular risk in vegetarians, namely values of insulin resistance. Complex carbohydrates with a low postprandial glycemic effect, called the glycemic index, and a high fiber content are absorbed slowly and thus have a beneficial effect on glucose, insulin, insulin resistance, and blood lipids [65]. The vegetarian group versus non-vegetarian group had an IR/HOMA/value significantly reduced and very low (0.97) with no incidence of risk values versus 7.9% in the non-vegetarian group. Compared to non-vegetarians, vegetarians consume significantly more wholegrain products, legumes, barley and oat products, as well as fruits and vegetables, which contain complex carbohydrates and fiber.

### 4.3 Vegetable proteins

Consumption of plant proteins can also reduce the risk of cardiovascular disease. Vegetable proteins versus hen egg reference protein or other animal proteins have a reduced content of some essential amino acids and an increased content of some non-essential amino acids [66]. Consumption of essential amino acids methionine and lysine is significantly reduced in vegetarians compared to the traditional diet, intake of non-essential amino acids arginine, glycine and serine is significantly higher, when evaluating questionnaires in both types of subjects with the same protein intake corresponding to OVD, consumption is significantly increased in vegetarians alanine. Essential amino acids are relatively more effective at releasing insulin, while non-essential amino acids arginine and pyruvate precursors are effective at secreting glucagon. Glucagon increases (and inhibits) c-AMP-dependent mechanisms that suppress fat and cholesterol synthesis enzymes and, conversely, increase LDL receptor activity in the liver [67]. The effect of a chronic increase in glucagon activity by regular, sufficient consumption of plant proteins means a reduction in de novo lipogenesis, a decrease in fat stores, a reduction in cholesterol and LDL-cholesterol synthesis, and a reduction in triacylglycerol synthesis [68]. These literature data suggest that the favorable lipid profile and low IR (HOMA) value in vegetarians may also be due to higher intake of the non-essential amino acids arginine, glycine, alanine and serine from plant protein consumption.

### 4.4 Antioxidant vitamins; oxidative damage products of lipids, proteins, DNA

Oxidative stress can lead to cell dysfunction and eventually cell death. It is defined as an imbalance between pro-oxidants or free radicals on the one hand and antioxidant systems on the other. The harmful effects of oxygen occur through the production of free radicals that are toxic to cells (superoxide anion, hydroxyl radical, peroxyl radical, hydrogen peroxide, hydroperoxides and peroxynitrite anion) [69]. Subjects with a dominant consumption of plant foods have significantly increased plasma concentrations of antioxidant vitamins C, E, β-carotene, values for lipids standardized required E as well as the value of the ratio of large C and E and these average values of antioxidants are above the threshold. The incidence of protective (above-threshold) values is high in vegetarians - 92% versus 42% for vitamin C, 67% versus 33% for vitamin E, 100% versus 79% for vitamin C/vitamin E, 87% versus 50% for vitamin E /cholesterol, 96% versus 62% for vitamin E/triacylglycerols and 67% versus 17% for β-carotene. The results document the better antioxidant status of vegetarians as a consequence of regular and sufficient consumption of protective food and are consistent with the results of other authors [70]. Due to more effective antioxidant protection, we measured significantly reduced plasma values of lipid peroxidation (conjugated fatty acid dienes as the first product of the process), insignificantly reduced values of protein damage and significantly reduced values of oxidized purines and oxidized pyrimidines in lymphocytes in vegetarians. The values of oxidative damage of DNA, lipids and proteins were significantly lower in persons with above-threshold values of antioxidant vitamins compared to sub-threshold, deficient values.

## 5. Conclusion

Epidemiological data clearly suggest that high and most important regular consumption of fruits, vegetables, dark or whole grains, cereal germ, vegetable oils and oilseeds rich in minerals and trace substances, mono- and polyunsaturated fatty acids, antioxidant vitamins, fiber, complex carbohydrates, flavonoids and nutrients together with a healthy lifestyle protect against degenerative diseases. Our results indicate that vegetarian nutrition can represent effective disease prevention. Optimal traditional nutrition with sufficient consumption of protective food commodities can also ensure beneficial effects on health.

## Acknowledgment

This study was supported by research agency VEGA under the contract 1/0096/17 and APVV-16-171.

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