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FUNCTIONAL FOOD IN PREVENTION OF CARDIOVASCULAR DISEASES AND OBESITY

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Abstract: The term "functional food" refers to modified food products that claim to provide an additional function besides basic nutrition needs. The consumption of functional food is known to exert a positive impact on health and to prevent the occurrence of pathological conditions, such as cardiovascular diseases, some types of cancer, and obesity. Functional food products should resemble conventional food in terms of appearance and taste. The goal is usually achieved by adding active ingredients to the traditional food products (e.g., phytosterols/stanols are added to margarine, dairy, and cereal products), removing or limiting the concentration of potentially harmful agents, or by agricultural and genetic modifications of already existing edible plants and animals (e.g., feeding hens on algae or fish in order to obtain n-3 PUFAs-enriched eggs, and inducing genetic and/or nutritional changes during animal production to obtain meat with lower cholesterol levels). Well-designed intervention trials are scarce in this field, and more effort should be directed toward conclusively proving the role of functional food in disease prevention and health improvement among the population. These associated benefits and the advances in the food processing industry should stimulate the development of products that would match the requirements of a healthy diet, simultaneously reducing the risk of chronic diseases. The aim of the present review was to present the examples of functional foods that are essential for the prevention of obesity and cardiovascular disease, and thereby report on their putative mechanisms of action, health-promoting effects, and limitations by conducting various intervention studies.

Keywords: functional food, cardiovascular disease, obesity, non-nutrient food components

Functional food – an overview

The concept of functional food was first introduced in Japan in the late 1980s and was defined by the Ministry of Health and Social Welfare as the "Food for Specified Health Uses" (FOSHU). Currently, it has been propagated in Western Europe and the United States, and growing interest of the society in health-promoting and disease-preventing characteristics of this kind of food has boosted its production and research in this field. This category of foods includes products that have been enriched with physiologically active compounds or from which potentially harmful ingredients have been separated out. According to the FOSHU definition, functional foods should resemble conventional food products consumed on a regular basis, as it is designed for people whose main intention is to incorporate them in their everyday lifestyle so as to protect themselves from diseases (1). Additionally, according to the Concluding Document FUFOSE (Functional Food Science in Europe) issued by the European Commission in 1999, alimentary products may be considered as functional food when they have an added positive health benefit and exert a positive effect on the human body beyond basic nutritional value. Functional foods, enriched in active ingredients, form a link between food and drug; however, they are not available in the form of tablets or capsules. Hence, popular "food supplements" cannot be considered as functional foods, although both categories of products may be targeted to populations and individuals suffering from diverse nutritional deficiencies and/or at risk of a particular disease (2).

The composition of functional foods introduced to the commercial market should consider the dietary habits of the population, and the products need to be certified regarding their safety and health beneficial properties by performing laboratory tests and human intervention studies (3).

Various innovative agricultural and biotechnological procedures have been applied to produce

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functional foods possessing the desired properties of a particular raw material or its specific variant. The modification cannot, however, alter the taste and/or flavor of the product which often depends on the food matrix. Water or lipid solubility of an active substance must also be taken into consideration. It is worth mentioning that modern food technology often implements some traditional procedures or national recipes, e.g., fermentation of Chinese food products, to add these unique flavors to contemporary diets (4). Technological modifications may include: addition of biologically active substances, e.g., probiotics, prebiotics, vitamins, minerals, and plant extracts at various stages of food processing; eliminating anti-nutritional compounds which can increase the bioavailability of nutrients and/or drugs; and production of the preparations with reduced calorie load by lowering the content of saturated fatty acids (SFAs) (5). Bioactive components used for functional food preparation are either synthetic or extracted from natural sources and then condensed, and hence their concentration in a particular product might reach harmful levels. Moreover, they might interfere with the functions of other bioactive substances in diet or drugs. The effectiveness and safety of functional foods must be confirmed by the producer, which is not required in the case of regular, conventional alimentary products. To meet these high standards, producers should conduct a chemical and biological survey of the tested food. Particular attention is paid to the antioxidant activity of raw materials due to their role in the prevention of, e.g., obesity, cardiovascular disease (CVD), some forms of cancer, and diabetes. Results of the *in vitro* and *in vivo* experimental systems applying isolated compounds revealed the ability of naturally occurring antioxidants to neutralize reactive oxygen species (ROS) and promote health (5-7). These goals are achieved through the reduction of inflammatory reactions, protection of endogenous macromolecules against oxidative damage, and enhancement of the *in vivo* defense systems by providing a suitable pool of low molecular weight antioxidants such as vitamins C and E, selenium, and antioxidant phytochemicals along with food. These molecules reduce the harmful effects of free radical reactions *via* interference with ROS formation (3, 8-11). Several classification systems have been proposed to classify functional food products based on the techniques applied to producie the modified compound, e.g., fortification, enrichment, alteration, and improvement (1).

Food fortification has a long history of practical application for treating the deficiencies related to iron, iodine, and vitamins (A, D, B). Salt fortification with iodine has been known since the 1920s, and due to its effectiveness in improving the thyroid function, it has since been introduced in numerous countries all over the world (12). Cereal products have been fortified with selected B-complex vitamins (niacin, riboflavin, thiamine, and folic acid). Vitamin A is commonly added to margarine and vitamin D fortified milk products are prepared, which effectively reduced the risk of rickets in children and osteoporosis in the elderly. Fortification of wheat flour with folic acid reduced the rate of neural tube pathologies and decreased the homocysteine concentration in plasma (13). The latter has been considered to be a measure to prevent atherosclerosis (14). For a long time, foods formulated for infants have been fortified with iron to reduce the frequency of anemia resulting from iron deficiency (15). Moreover, biofortification of foods with iron represents an interesting and novel technology that involves increasing the content and bioavailability of iron in crops *via* undertaking plant breeding and/or agronomic practices such as genetic modifications (16). The technique of genetic manipulation has been used to produce certain varieties of sweet potatoes and carrots with increased beta-carotene content or maize varieties with decreased phytic acid, which subsequently enhances the availability of zinc and iron (17). Although the results of trials with biofortified foods obtained through genetic modifications of plants are very promising, still much uncertainty exists concerning the safety of their consumption and impact on the environment. Owing to these reasons and social pressure, some countries have banned the culture and processing of genetically modified crops, suggesting a limitation in the production and consumption of these foods in the developing countries (18, 19). Production of fortified meat represents a new approach to the technology of functional food manufacture. Meat and related products are an essential source of various nutrients that are crucial for proper growth and development; however, high SFAs and cholesterol levels in these products have been linked to a greater risk of chronic diseases, such as, obesity, CVDs, and type 2 diabetes (20). Additionally, frequent consumption of traditionally grilled or smoked meat, containing polycyclic aromatic hydrocarbons, leads to the induction of cytochrome P450 isoforms which are responsible for metabolic activation of food and environmental carcinogens and alteration of drug metabolism (20-22). An interesting approach toward reduction of risk associated with the consumption of traditionally processed meat products has been pro-

posed at the Gdańsk University of Technology, Poland, where the so-called *Brassica* sausages were invented, based on the popular belief that the combination of meat and cabbage has a positive impact on health. The product has been in the market since 2008 and is accepted well by the local consumers (23). Phytochemicals in white cabbage (*Brassica oleracea* var. *capitata* f. *alba*) exhibit preventive activity against certain types of cancer, mainly colon and breast tumors (24, 25). The most active chemicals are glucosinolates and indoles, which are known to effectively neutralize mutagenic heterocyclic aromatic amines formed in the meat during thermal processing and to affect estrogen levels in the blood and breast tissue through redirection of estrogen metabolic pathway to produce less oncogenic derivatives. Besides cabbage, other members of the family *Brassicaceae*, such as cauliflower, broccoli, kale, and Brussels sprouts, are also a rich source of glucosinolates and indole alkaloids (26). In contrast to most vegetables that lose their nutritional value on cooking, the beneficial potential of cabbage is known to increase during the heating process because it releases antioxidant substances gradually (27). Therefore, the addition of cabbage extract to meat products can not only enhance the uptake of bioactive health-promoting substances but can also reduce a load of mutagenic and carcinogenic compounds in the meal. Moreover, the combination of meat and cabbage gives a salty taste to these products, and hence it is possible to reduce the salt content, one of the hypertension risk factors, by almost 30% while *Brassica* products are additionally enriched with other valuable ingredients like vitamins, micronutrients, low-processed diets, and phytosterols which affect the level of cholesterol. However, *Brassica* sausages, due to their high cost, have not yet been subjected to clinical trials.

Functional food may also be classified according to the special target to which it has been directed: consumers at risk of CVDs, cancers, osteoporosis, and other disorders. Further proposed types of food may meet the requirements of specific population groups, infants, young adolescents, women, or the elderly. Additionally, a special group of products may be used at the time of menopause, for prostate overgrowth, or in lactation mothers (28).

Factors contributing to the functional food market are presented in Figure 1. The responsibility of the food industry to fulfill the consumers' needs, preferences, and requirements, initiates the research and development of special-purpose products, by improving the existing brands and conducting clinical trials to test their efficacy and safety. Thus obtained functional foods, after assessing for their quality and safety, are added to the list of products that positively influence the health of the community and the individual consumer (29, 30).

In the next section, we describe the applications of functional foods in the prevention of obesity and CVDs by providing examples. CVD by itself and the complications associated with it represent a

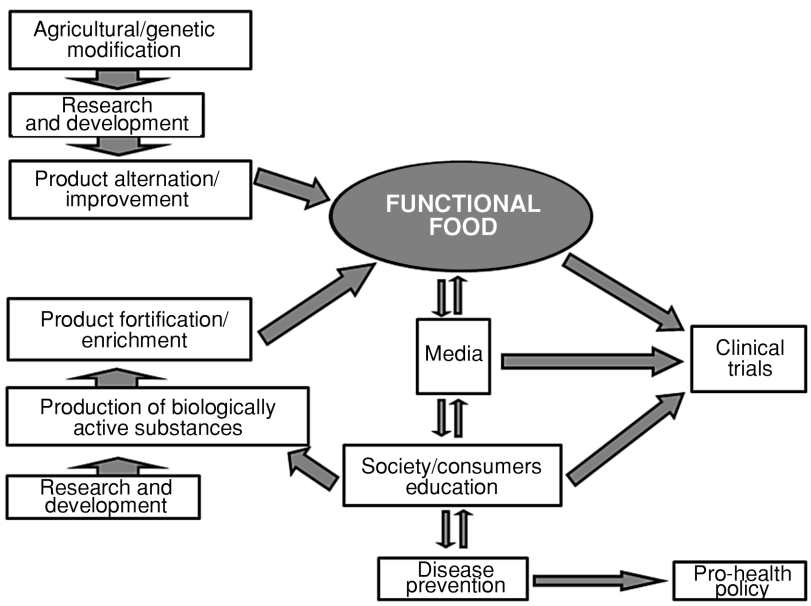

Figure 1. Factors shaping the functional food market.

serious public health concern, as it is considered to be the major cause of mortality in human beings. Since the conductance of Framingham's study in the late forties of the 20th century and other epidemiological surveys, the risk of CVD has been associated with factors such as lifestyle, namely diet, physical inactivity, obesity, and tobacco smoking, as well as with other coexisting conditions (e.g., hypertension, etc.). When the alimentary products were deeply analyzed to determine their role in the prevention of obesity and/or CVDs, the results often revealed other beneficial activities, such as their activity against cancer or type 2 diabetes, thus contributing to the overall term of "healthy diet" (31, 32). This has stimulated numerous studies on the diet-dependent pathomechanisms of the disease and possible preventive measures; however, some of them do not meet the criteria of Evidence-Based Medicine and represent a popular belief only. From the broad collection of the published reports, we have selected human intervention trials and highlighted the methodology applied in the study (randomized, placebo-controlled, crossover, double-blinded, or other). However, these experimental models have not been standardized with respect to sample size, control groups, protocols of intervention or exposure, factors analyzed at baseline, and defined outcomes. This lack of integration among the human trials may be the cause of discrepancies in the interpretation of results.

Functional food in the prevention of obesity and cardiovascular disease

Instant coffee enriched with chlorogenic acid

Instant coffee enriched with chlorogenic acid is available in the market and is dedicated to overweight and obese people. Chlorogenic acid is abundantly present in green coffee beans and has been extensively studied for its antioxidant activity and capability to prevent type 2 diabetes. The latter function is supposedly achieved through various mechanisms: delay of glucose absorption in the intestine, stimulation of incretin hormone glucagon-like peptide-1, decrease in hepatic glucose output due to inhibition of glucose-6-phosphatase, and change in mineral composition that improves glucose tolerance (33). This particular phytochemical has been found to reduce weight, which was proved in a randomized, placebo-controlled study conducted in Norway. Twelve volunteers of normal weight (BMI < 25.0 kg/m^2) and 15 slightly to moderately overweight individuals (BMI 27.5–32.0 kg/m^2) consumed chlorogenic acid-enriched instant coffee for 12 weeks, whereas the matched controls received normal instant coffee as placebo. The tested group lost 5.4 ± 0.6 kg at the end of the experiment, which was a statistically significant loss in comparison to the result shown by the control group (34). Though the use of green coffee extract as weight loss medication provided promising results in this study and other intervention trials, the product has faced serious criticism with regard to methodological limitations of these studies, including lack of safety assessment and estimation of its effects on weight following discontinuation of the product consumption (35).

β-glucan products in weight control and CVD prevention

Consumption of bran for many years has been known to effectively control weight. This excellent source of indigestible insoluble fiber is often consumed as an additive to cereal meals or in the form of whole-grain flour products. Foods prepared with whole grains and bran contain less starch, have a reduced calorie load, and contain more micronutrients of important health value, and the role of these foods in the prevention of obesity, type 2 diabetes, and CVDs has been appreciated. Notwithstanding, the exact mechanism(s) underlying their action and factor(s) responsible for their actions remain unclear (36). Analysis of observational studies reported by Cho et al. shows that the consumption of whole-grain alone is not sufficient to achieve CVD reduction if the whole grain does not include added bran (37). Currently, the attention is being focused on the soluble fiber, β-glucan, obtained from oat, barley, and bran of other grains. β-glucan is a carbohydrate polymer with the unique combinations of glucose moieties at positions C1-C3 and C1-C4. The polymer with C1-C4 combination only is a water-insoluble fiber similar to cellulose present in plant cell walls. In the β-glucan molecule, 30% of C1-C3 connections are sufficient to impart chain mobility and water solubility to the compound. Hydrothermal processing of grain results in the formation of a product having high β-glucan content. In this procedure, β-glucans absorb a large volume of water eventually forming gums of considerable viscosity, which is important to exert its action; in the stomach and small intestine, β-glucans form a mucoid protective layer, thus delaying starch hydrolysis and glucose absorption. β-glucans are also responsible for the prolonged feeling of stomach fullness. Moreover, β-glucan-derived mucus protects the intestinal mucosa against irritation and bacterial infections (38-40).

The effect of β-glucan (5 or 10 g), obtained from oat or barley, was tested for five weeks in a

group of 100 consumers having elevated cholesterol levels in a placebo-controlled, double-blind study. A designated dose of β-glucan was taken up in the beverage (500 mL/day). Consumption of 5 g of oat β-glucan was sufficient to improve lipid (reduction in total cholesterol level by 7.4%) and glucose metabolism levels, whereas 10 g of β-glucan from oats did not affect the serum lipid concentration significantly in comparison with control. Moreover, no significant effects were observed in individuals consuming barley β-glucan (41). In a similarly designed trial, a dose of 5 g of oat β-glucan added to industrially prepared fruit drink reduced total cholesterol by 4.8% and low-density lipoprotein (LDL)-cholesterol by 7.7% in a group of hypercholesterolemic volunteers. However, no significant differences in the level of serum high-density lipoprotein (HDL) cholesterol or triglycerides were found when compared to the control group. Although the authors conclude that the reduction in cholesterol absorption contributes to the observed hypocholesterolemic effect of β-glucan consumption, no decrease in lipid-soluble antioxidants was demonstrated. (42). Data from other studies showed that oat bran intake resulted in an increase in the synthesis of bile acids, which might contribute to the stated cholesterol-reducing effect (43). A controlled, randomized crossover study included 30 volunteers suffering from mild hypercholesterolemia and the participants were asked to consume daily 3 g of high-molecular-weight 3 or 5 g of low-molecular-weight barley β-glucan or a control diet for five weeks, in the form of crepes, tortillas, porridge, and chips. Results of the trial indicate that increased bile acid synthesis, rather than inhibition of cholesterol absorption or synthesis, may be responsible for the cholesterol-lowering effect of barley β-glucan. This effect probably depends on the high molecular viscosity of barley β-glucan (44). Although results of the intervention trials with β-glucan products convincingly indicate the health benefits in hypercholesterolemic subjects, most reports are based on observational studies in populations consuming whole grain and bran foods, and great diversity in the final outcomes might result from the problem of inconsistent definitions of whole grains in the published studies (45).

Red yeast rice products modulate the cholesterol metabolism

Many longitudinal studies conducted recently revealed that LDL cholesterol contributes to the development of CVD, whereas HDL cholesterol exhibits a preventive action. Since a reduction in the total and LDL plasma cholesterol levels has been considered to be an important factor for CVD prevention, various approaches have been recommended to meet this requirement. Besides the consumption of meat and related products and/or modified eggs with decreased cholesterol content, inhibition of cholesterol synthesis pathway or inhibition of intestinal cholesterol uptake may help to reach this goal. A decrease in endogenous cholesterol synthesis may be achieved through the consumption of red yeast rice which is a traditional Chinese culinary product, popular also in other Asian countries. Red yeast rice is a product of rice fermentation and contains mold culture (*Monascus purpureus*). The red yeast rice contains a molecule called monacolin K which is responsible for the inhibition of HMG-CoA reductase, a crucial enzyme in cholesterol synthesis. The mold was originally used in the production of lovastatin, the first statin introduced to the pharmaceutical market, and the drug appeared chemically identical to monacolin K. Human intervention studies revealed that consumption of commercial preparation of red yeast rice causes a mean reduction in total and LDL cholesterol and triglyceride and a mean rise in HDL cholesterol levels (46). In a double-blind trial involving 4870 subjects in China, with a history of high plasma cholesterol level and myocardial infarction, daily intake of 1.2 g of a commercial red yeast rice preparation reduced the prevalence of serious coronary events, in comparison to results obtained from placebo controls (5.7% vs. 10.4%) (47). The beneficial effect of red yeast rice was also observed in diabetics and the elderly (48). Besides lipid-lowering potential, the red yeast rice also protects the functions of the endothelium in patients with CVD (49). Monacolin K in red yeast rice and statins present a similar mode of action and similar side effects as well. A thorough analysis of the natural product and its efficacy revealed great variability in monacolin K concentrations in various batches of the fermented rice, presence of other types of monacolins, and other chemicals of unknown but potential toxicity. That is why red yeast rice is currently not recommended as a cholesterol-lowering dietary regime, due to the lack of assurance regarding its efficacy, safety, and proper standardization protocol (50).

Carotenoids and lycopene products

Carotenoids such as lycopene, beta-carotene, lutein, and zeaxanthin are common ingredients of several edible plants (e.g., tomatoes, pumpkins, and carrots). Among them, lycopene was the most intensively studied. Lycopene is a strong antioxidant mainly associated with the prevention of prostate

cancer; however, as it has been found to inhibit cholesterol synthesis through attenuation of HMG-CoA reductase, resembling the action of statins, it has been used as an agent to reduce the CVD risk (51). In a large cohort study (1212 men, aged 61–80 years), a significant inverse correlation between various plasma carotenoids and atherosclerosis has been observed in carotid arteries (52). Human intervention studies assessing anti-CVD effects, especially, tomatoes as a source of lycopene and other carotenoids are numerous, and despite the fact that the bioavailability of these phytochemicals is enhanced by heating and homogenization, several trials are based on the consumption of raw tomatoes (53,54). The most common tomato products tested in human intervention trials for their potential to reduce various CVD risks are tomato soups, sauces, pastes, encapsulated lycopene, but studies based on products that meet the criteria of functional food items are limited (55).

Human intervention trial conducted by Bohn et al. (2013) is an exception. In this protocol, nine healthy men and nine women (aged 21–40 years) consumed a soy germ-fortified juice daily (300 mL containing 66 mg of isoflavones and 22 mg of lycopene) for eight weeks. This intervention trial was designed to assess the cancer prevention effects, but it also provided data on the improvement of lipid metabolism. There was a significant increase in plasma HDL cholesterol levels (47.3 ± 15.8 mg/dl at baseline vs. 51.7 ± 14.8 mg/dl at week 8), and the ratio of plasma total cholesterol/HDL cholesterol was markedly reduced (4.25 ± 1.59 mg/dl at baseline to 3.63 ± 1.16 mg/dl at week 8). Additionally, fractions of LDL and VLDL cholesterol appeared to be more resistant to Cu(II)-dependent oxidation (56). In a randomized, double-blinded, placebo-controlled crossover study, 50 healthy men and 40 women (aged 45–70 years) consumed a tomato extract-enriched orange juice (6 or 18 g of extract in 200 mL of juice, equivalent to two or six fresh tomatoes, respectively). Placebo drink did not contain tomato extract. The blood was collected at baseline and 3 h after ingestion of a tested drink, and hemostatic functions were measured. In subjects consuming tomato-enriched juices, significant reductions in *ex vivo* platelet aggregation induced by ADP or collagen were noticed, and the range of this reduction significantly correlated to plasma homocysteine or C-reactive protein levels (57).

Products used in studies conducted by Bohm (2013) and O'Kennedy (2006) were prepared specifically for the trial purpose, although the authors suggest that these products were produced on a large scale and used in cancer and CVD prevention studies and/or daily consumption with a regular diet. Recently, the Polish market of functional foods introduced an original product: a fruit juice enriched in lycopene isolated from tomatoes. This is a relatively recent achievement and no data on intervention trial has been published to date.

Phytosterol and stanol products

Discovery of plant-derived cholesterol-related agents (phytosterols and stanols), which are capable of reducing cholesterol uptake in the intestine and lead to decreased plasma LDL cholesterol levels, propelled research in food technology to obtain phytosterol/stanol-enriched dietary products, such as margarine, dairy, and cereal products (58). In a nine-week, balanced, double-blinded crossover trial, table spreads fortified with either soybean oil (non-esterified plant sterols) or a shea nut oil concentrate (esterified plant sterols) were tested in 77 healthy adult volunteers. Each participant consumed approx. 25 g/d of tested or control spread for three weeks. The product containing soybean and phytosterols significantly reduced total and LDL cholesterol levels in plasma (by about 4% and 6%, respectively) when compared to the effect shown by control spread, which did not alter the plasma HDL cholesterol levels and exerted a limited effect on plasma carotenoid levels. The spread fortified with shea nut phytosterols did not influence the mean plasma lipid levels compared with control spread. The authors suggest that the observed reductions in plasma total and LDL cholesterol upon soybean oil sterols consumption could contribute to a reduction in CVD risk by about 15% at age 40 and 6% at age 70 years (59). In a randomized, double-blind, placebo-controlled balanced experiment, 95 normocholesterolemic or mildly hypercholesterolemic participants consumed 30 g/d of fortified spread containing soybean, rice bran oil, or shea nut esterified phytosterols or non-fortified margarine in four consecutive periods of 3.5 weeks. Within 2.5 weeks, margarine enriched with soybean oil sterols (esters of sitosterol, campesterol, and stigmasterol) lowered blood total and LDL cholesterol levels by 8% and 13%, respectively, when compared to the effect exerted by spread not enriched in sterols. The rice bran oil and shea nut oil sterol-fortified margarine did not lower cholesterol levels in plasma. No influence of the sterol-fortified margarine on plasma HDL cholesterol was observed. However, a decrease in plasma carotenoid (α- and β-carotene and lycopene) levels suggests further studies to elucidate and exclude this effect (60). Moreover, it was found

plant sterols and stanols displayed more beneficial effects on LDL cholesterol level when compared to the effects shown by statin medication (61). Doubling the statin dose caused an additional decrease in plasma LDL cholesterol by only 7%, but adding phytosterols to the daily diet was found to be more effective – additional LDL reduction by 16-20% was observed (62-65). However, phytosterol intake did not change the plasma HDL cholesterol level (64). Although initial studies investigating the impact of phytosterol on LDL cholesterol provided promising results, recently published guidelines recommend more attention with this class of food supplements. Epidemiological surveys revealed that increased plasma phytosterol concentrations correlate with probable coronary events in groups of patients at high coronary risk (66).

Polyunsaturated fatty acid (PUFA)-enriched products

Polyunsaturated fatty acids (PUFAs) are a class of lipid compounds that are found to be essential for CVD prevention, and they are the constituents of dietary phospholipids. Phospholipids are mainly consumed with eggs and fish, and this has led to the concept of manipulating the egg content through modifying the diet of laying hens, by enriching the diet with fish oil, microalgae, or flaxseed pomace. The product thus obtained meets the criteria of functional food, and consumption of these eggs provides a recommended daily intake of n-3 PUFAs (between 1000 and 2000 mg/day) without the need for additional supplements and change of eating habits. Moreover, additives added to the hens feed enrich eggs with other micronutrients, e.g., lipid-soluble vitamin E, carotenoids, lutein, and selenium (67). Such a composition of additives possessing antioxidant potential protects PUFAs from oxidative damage during egg storage and culinary processing. The main health benefits resulting from consumption of n-3 PUFAs-enriched eggs is an increase in the plasma level of these PUFAs and a decrease in plasma triglycerides, blood pressure (systolic and diastolic), and platelet aggregation ability (68). Some intervention trials also report a decrease in plasma cholesterol levels following the consumption of n-3 PUFAs-enriched eggs. In the same study, however, in 2 of the 25 participants, an elevation of total and LDL cholesterol was observed after consumption of n-3 PUFAs-enriched eggs, which might result from the individual metabolic response to the diet (69). To decrease cholesterol level in egg yolk, the hens were treated with atorvastatin or garlic paste, the former resulting in an undesired pharmacological modulation of the product (70, 71). The flaxseed-enriched diet of hens produces eggs rich in α-linolenic acid (ALA) which is the least desired n-3 PUFA in humans, whereas hens fed on algae and fish produce eggs enriched with the most required eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This modification, however, brings about a change in the taste of eggs which is not acceptable to the consumers.

N-3 PUFA products

Dietary products fortified with n-3 PUFAs at the stage of industrial processing were tested in 16 male volunteers who took part in the intervention study and consumed diverse foods containing n-3 PUFAs either naturally (fresh fish, canned fish, flaxseed meal, and canola oil) or fortified with fish oil (margarine, milk, sausage, luncheon meat, and dip). A significant decrease in total n-6 PUFAs (by 15%) and a marked increase in the proportion of ALA (by 56%), EPA (by 174%), and DHA (by 80%) was observed in the plasma of participants. In addition, a significantly increased level of n-3 PUFAs was demonstrated in mononuclear and platelet membranes accompanied by a decrease in the content of n-6 PUFAs. The authors of the study then suggest that consumption of foods fortified with n-3 PUFAs would provide the beneficial proportion of phospholipids in individuals who do not include fish into their diet, preventing the occurrence of obesity, atherosclerosis, hypertension, or type 2 diabetes (72).

Conjugated linoleic acid (CLA) products

Beneficial effects associated with the replacement of saturated fats with PUFAs have driven attention to conjugated linoleic acids (CLAs), which are present in animal fodder along with, linoleic acids, and other PUFAs. CLAs are formed naturally in the gastrointestinal tract of ruminants and occur as a mixture of 28 positional and geometric isomers; the greatest biological activity is exhibited by trans-10, cis-12 and cis-9, trans-11 octadecadienoic acids. However, they differ in the particular biological functions (73). One of the suggested mechanisms behind the beneficial activity of CLAs in CVD prevention is probably related to an increase in the muscle mass and the subsequent enhancement of metabolic rate and weight loss which in turn is associated with the diminished activity of carnitine palmitoyl-transferase-1 (74, 75). The trans-10, cis-12 isomer is found to be the main form responsible for anti-diabetic, anti-carcinogenic, and anti-obese actions, while the cis-9, trans-11 isomer mostly

exhibits anti-carcinogenic activity. Furthermore, CLAs were shown to modulate mutagenesis and immune functions, prevent atherosclerosis and reduce hypertension. The anti-obesity activity of trans-10, cis-12 CLAs can be attributed to decreased adipogenesis and lipogenesis; increased lipolysis, fatty acid oxidation, and adipocyte apoptosis; as well as effects on inflammation, browning of adipose tissue, and energy metabolism. The mechanisms underlying these activities are complex and still unclear, also the link between CLA-dependent body mass reduction and modulation of inflammation and thermogenesis is not completely understood (75). Much data with regard to the impact of CLAs on lipid metabolism have been obtained from animal studies, and several reports describe the effects of consumption of alimentary products naturally enriched in CLAs. Modification of ruminants' diet may result in a fourfold increase in the concentration of CLAs and n-3 fatty acids and a decrease in the content of SFAs by approx. 23% in milk, so thus resulting in dairy functional foods might represent particular nutritional value in modulating lipid metabolism and CVD prevention (76, 77). In a study reported by Pintus et al., mildly hypercholesterolemic volunteers consumed 45 or 90 g of modified or control sheep cheese daily for three weeks. Modified cheese was obtained from the milk of sheep fed on the extruded linseed, and the alimentary products of the two groups of animals did not differ markedly in total fat, protein, or sugar content, whereas the modified cheese contained less SFAs compared to the control cheese (45.9% vs. 59.3%). Significant differences were observed in the proportion of unsaturated fatty acids: n-3 PUFA and ALA (2.1% in the modified vs. 0.6% in the control cheese), total CLAs (2.8% vs. 1%), and cis-9, trans-11 CLA (2.5% vs. 0.8%). Daily consumption of 45 g of modified cheese did not cause a change in the lipid metabolism of participants, whereas the dosage of 90 g/d significantly reduced total cholesterol and LDL cholesterol levels and increased HDL cholesterol and plasma CLA levels, with no impact on body mass. Consumption of the control cheese (90 g/d) also increased HDL cholesterol in comparison to the baseline values. In volunteers consuming 90 g/d of modified cheese, a significant reduction in plasma fatty acids and lipid hydroperoxides was found (78). However, other studies reported data on the susceptibility of CLAs to peroxidation (79). A similar study based on the consumption of CLA-enriched cheese showed a significant reduction in inflammatory markers (IL-6, IL-8, and TNF-α) and platelet aggregation (80). Although Pintus and coworkers did not show any effect of the consumption of 90 g/d of the CLA-enriched cheese on body mass reduction, the study reported by Lopez-Plaza et al. presented data on weight loss and waist circumference reduction after 24 weeks of consumption of skimmed milk enriched in a mixture of cis-9, trans-11 and trans-10, cis-12 CLA isomers (78, 81). The presented examples demonstrating the possible effects of CLAs in CVD prevention are yet unconvincing, because some reports show no anti-obese and adverse effects of CLAs on plasma lipid and C-reactive protein concentration, pro-coagulant activity, and induction of lipid peroxidation and oxidative stress, which contribute to insulin resistance in individuals susceptible to type 2 diabetes (81-83). Intake of trans fatty acids augments the LDL/HDL cholesterol ratio and thus increases cardiovascular risk (84). The opposite results may be the consequence of using a mixture of isomers and probably the effects are CLA isomer-specific (85). These reports suggest the need for the conductance of further intervention studies in overweight/obese and lean individuals by adding relatively pure preparations of single isomers to alimentary products. Moreover, the combined effect of food matrix composition, food dosage, study duration, and participants' characteristics on assumed endpoints should be taken into consideration.

Meat modifications

The proportion of n-3/n-6 PUFAs can be modulated to obtain meat-based functional food products. Other alterations include a reduction of fat content and cholesterol levels, and enrichment of sausages with probiotic bacteria, minerals, and various antioxidants. These goals may be attained by adopting various genetic and/or nutritional animal production practices. Nutritional modifications can be achieved by enriching the fodder of farm animals with bioactive components that are then recovered in the carcasses. Moreover, technological processing allows complementing processed meat products with pro-health ingredients. Finally, the content of the bioactive substances in the products may be a result of culinary preparation and storage (86). Nutritional modification of pork and the resulting modulation in the phospholipid content of humans consuming the modified meat was described by Coates and coworkers. Pigs were fed for six weeks on diet supplemented with 15% tuna fishmeal product, which resulted in no change in the sensory profiles of pork. Control animals consumed standard fodder. Control pork and n-3-enriched meat were trimmed of fat and processed into various foods

which were used in a double-blind, placebo-controlled intervention trial. Participants (16 males and 13 females, aged 18-65 years) were randomized to consume either control pork or n-3-enriched meat (1000 g/week) for 12 weeks and instructed to limit all fish and seafood consumption to no more than one serving per two weeks. Over the period of trial, in the group consuming n-3-enriched meat, the DHA level in erythrocyte membranes rose by 15% and fell by 5% in controls; however, no significant changes in EPA was found in either group. The serum triglyceride and thromboxane (TXB_2) production were decreased in the n-3 group when compared with the control group. As the n-3-PUFA content in erythrocytes is considered to be an independent negative risk factor for CVD and lowering of TXB_2 decreases the ability of platelets to aggregate, the authors suggest that consumption of n-3-enriched meat might impact profitably on CVD risk factors in individuals who avoid eating fish directly (87). Similarly, the chicken feed was modified to contain 2% rapeseed oil and 2% linseed oil (RLO), and control chickens were given a feed enriched with 4% soybean oil (SO) rich in n-6 fatty acids. In a double-blind dietary intervention study, volunteers (11 males and 35 females, aged 20–29 years) were randomized to consume 160 g of meat of either soybean-fed chicken (SO group) or rapeseed/linseed-fed chicken (RLO group) daily for four weeks. The sera of RLO subjects contained significantly higher levels of ALA and EPA and saturated 14:0 (myristic) and 15 : 0 (pentadecylic) acids after intervention when compared to the participants who consumed SO-fed chicken. Moreover, a decrease in the ratio of arachidonic acid to EPA was demonstrated in RLO individuals. The volunteers having low levels of EPA and DHA in serum phospholipids (less than 4.6%) showed an increase in the amount of these n-3 PUFAs after four weeks of intervention with RLO chicken meat. In both groups of participants, there were no significant differences in cholesterol (total, LDL, and HDL), triglycerides, and C-reactive protein concentrations as well as in body weight or blood pressure. The individuals with EPA+DHA levels higher than 4.6% of total fatty acids in serum phospholipids have been associated with a 70% lower CVD risk when compared to those with lower concentrations of these n-3 PUFAs, thus authors of the study postulate that a simple change in chicken feed can have beneficial effects in consumers (88). Cattle fed with grain-based diets produced beef with the ratio of monounsaturated fatty acids (MUFAs) to SFAs equal to 1.10, whereas animals grazed on native pasture gave meat with a lower proportion of MUFAs and SFAs (0.70). Meat from both groups of animals was used in a crossover dietary intervention study including 27 normocholesterolemic men. Participants consumed five 114 g patties made of ground beef per week for five weeks. The washout period between treatments lasted four weeks. In each portion, there was 24% total fat, but low-MUFA patties contained more SFA, less MUFA, more trans-fatty acids (18 : 1), and more ALA than did the high-MUFA patties. After both interventions, plasma insulin and diameters of HDL2- and HDL3-cholesterol particles were decreased, and plasma stearic (18 : 0) and arachidonic (20 : 4, n-6) fatty acids were increased in comparison to baseline values. High-MUFA intervention only caused a rise in plasma HDL cholesterol and fall in plasma LDL : HDL cholesterol ratio relative to baseline values, whereas there were no changes in triglyceride, total cholesterol, and LDL cholesterol levels following both treatments. Moreover, among individuals consuming a high-MUFA diet, plasma triglycerides were positively correlated with plasma insulin level and inversely correlated with HDL cholesterol concentration. The authors conclude that a high-MUFA diet offers a beneficial rise in HDL cholesterol; however, at present, it is not clear if the decreased diameter of HDL2 and HDL3 particles indicates elevated or diminished risk for CVD (89).

Berry fruits and related products

Edible berries deserve special interest because besides having high polyphenol (mostly anthocyanins and ellagitannins) content, they are also rich in fiber with low-calorie content. Moreover, they also contain vitamins E and C, selenium, and carotenoids (90). The correlation between the intake of berries and CVD risk has been analyzed in several observational studies where consumption of whole fruits and related products has been considered; for example, in Kuopio Ischemic Heart Disease Risk Factor Study, an adverse relationship between consumption of berries and mortality was noticed in men (91). In numerous human intervention trials, berries were consumed as whole fruits or juices or even encapsulated extracts, and only a few studies used products satisfying the criteria of functional food definition (90). In the study performed by Kay and Holub (2002), eight healthy male subjects (aged 38–54 years) underwent a single-blinded crossover trial. Participants consumed a high-fat meal and a control supplement, and after one week they took up the same high-fat meal supplemented with 100 g of freeze-dried wild blueberry (*Vaccinium angustifolium*) powder. The measured

outcome was serum antioxidant status assayed as oxygen radical absorbance capacity and the total antioxidant status. The wild-blueberry treatment caused a significant rise in serum antioxidant status, which is supposed to reduce the risk of chronic degenerative diseases (92). This impact on serum antioxidant status was associated with the absorption of blueberry anthocyanins from high-fat meals in their intact glycosylated and possibly acylated forms (93).

Selenium and CVD

Selenium is a microelement essential for the proper functioning of the immune and antioxidant defense systems, is a component of selenoproteins, particularly glutathione peroxidase, is capable of reducing hydrogen peroxide, induces oxidative modifications of lipids, inhibits platelet aggregation, and reduces inflammation. Selenium is supposed to protect cardiovascular health, owing to its antioxidant activity (94). However, results reported by the studies investigating the relationship between Selenium and CVD risk factors are inconsistent or even conflicting. Inconsistencies might be partly explained by the variability with regard to selenium status and selenium intake in various regions and population subgroups (95).

In a cross-sectional analysis comprising 5452 adult men and women, selenium in serum was measured by atomic absorption spectrometry and related to lipid metabolism parameters. Higher serum selenium was associated with elevated serum concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and apolipoproteins B and A-I (96).

The meta-analysis of randomized controlled trials on the effects of supplementation of selenium as a single ingredient on changes in CVD risk factors, CVD mortality, and type 2 diabetes showed that there is no sufficient evidence supporting the role of selenium supplementation in the primary prevention of CVD (97). However, in a randomized, placebo-controlled, parallel-group intervention trial conducted for six months in 501 volunteers (aged 60–74 years) with low plasma selenium status (approx. 88.8 ± 19.2 ng/g) and supplemented with selenium as high-selenium yeast (100 or 200 or 300 µg/day), a significant decrease in total and non-HDL cholesterol concentrations was observed in subjects ingesting 100 and 200 µg doses. The proportion of the total to HDL cholesterol decreased with increasing selenium doses (98). These results suggest that the association between selenium and cardio-metabolic outcomes resembles a U-shape with inverse effects likely to occur either below or above optimal concentrations of selenoproteins (97).

Discrepancies between results of studies on selenium vs. CVD in humans and evidence from animal studies in which selenium prevented experimental atherosclerosis should prompt future studies in this field. It was hypothesized that the association between selenium and lipid metabolism depends on the genetic polymorphism of genes controlling lipid transport and that selenium and lipids share common metabolic pathways (99).

Perspectives

Rapid advances in various scientific fields, including animal and plant biochemistry, molecular biology, and biotechnology, have brought numerous spectacular applications in food science. Much effort has been laid on studies that aim to determine the health impact of natural products that might fulfill the standards of functional food. Edible mushrooms form a group of natural foods identified with great potential to prevent various diseases. They are neither plants nor animals; however, for a long time mushrooms have been considered as important alimentary products, which are collected from the natural environment or cultivated. Mushrooms contain glycoproteins and polysaccharides, mainly of β-glucan type, terpenoids, sesquiterpenes, polyphenols, alkaloids, lactones, sterols, microelements, chelating agents, nucleotide analogs, and vitamins. Besides having nutritional value, mushrooms contain a variety of compounds that are known to perform diverse functions; hence, they are also used in drug formulations as they exhibit anti-cancer, anti-viral, immunopotentiating, hypocholesterolemic, and hepatoprotective activities, especially in Asian countries and Russia (Siberia). In addition, mushrooms can be used for the dietetic prevention of obesity and atherosclerosis owing to their low fat and high fiber content (100, 101). Mushrooms easily absorb soil components which might pose a toxicological risk when collected from a polluted environment. However, if cultivated on suitable growth substrates under controlled conditions, they may be enriched with health-promoting compounds (102, 103). Hence, mushrooms modulated in this way might fulfill the standards of functional food. Several mushroom species, e.g., *Lentinus edodes* (shiitake), *Schizophyllum commune, Inonotus obliquus,* and *Ganoderma lucidum* (reishi) have been for long appreciated for their anti-oncogenic, immunostimulatory, and chemopreventive potential (104). *Ganoderma lucidum* has been supposed to attenuate the cardiovascular risk; however, results

from a prospective, double-blind, randomized, placebo-controlled trial conducted for 16 weeks in 54 participants suffering from type 2 diabetes mellitus and metabolic syndrome (aged 60.2 ± 10 years) and consuming *G. lucidum* extract and spores (encapsulated, 2980 mg daily) and in 30 placebo participants (aged 57.1 ± 8.3 years) did not support the use of reishi mushroom for CVD risk diminishment, at least in this group of patients. Although the applied alimentary product did not meet the standards of functional food, the cited trial is worth mentioning for the study design (105).

The use of algae as additives to food for laying hens, applied in order to enrich eggs in the most required PUFAs (EPA and DHA), has been already discussed in the section *Polyunsaturated fatty acid (PUFA)-enriched products*. This modification changes the taste of eggs which is not acceptable to consumers; however, n-3 PUFA-enriched eggs obtained through such a method have become a source of phospholipid–protein complex which was isolated, purified, and successfully marketed as a dietary supplement. Its application for one month in patients with metabolic syndrome brought a significant decrease in systolic blood pressure, improvement of endothelial functions, and reduction of waist-to-hip ratio, but the product is no longer a functional food (67). Several algae species, *Spirulina platensis, Spirulina maxima, Spirulina fusiformis,* and *Chlorella vulgaris*, have been currently used in human food technology as meal additives and/or dietary supplements. They are considered to be a potent source of carbohydrates, proteins, enzymes, fiber, PUFAs, antioxidants, and carotenoid pigments (106, 107). Particularly, *Spirulina* species have been intensively investigated for their hypolipidemic, antioxidant, and anti-inflammatory properties; however, in the described human intervention trials, *Spirulina* has been administered in the encapsulated form at the doses of 2.0–4.2 g for 3–4 weeks. The results of this trial confirmed an increased HDL level and decreased the low-density lipoprotein (LDL) level among healthy male volunteers and patients with coronary artery disease (108-110).

Other kinds of marine products, e.g., alga *Ulva intestinalis* and sea cucumber *Acaudina molpadioidea,* have been tested for the ability of their peptides to inhibit angiotensin-converting enzyme (ACE) and reduce hypertension (111, 112). Inhibition of ACE by plant- and animal-derived peptides is not a novel finding, and a large selection of natural ACE inhibitory sources was thoroughly reviewed (113). These peptides are promising components of functional food products and further studies are necessary to prove the *in vivo* efficacy, in comparison to drugs effects, of these peptides when combined with diet on ACE inhibition in the treatment of CVDs, as their low concentration in food may be the activity limiting factor (114).

A deeper understanding of the impact caused by diverse nutritional components on health has led to the development of new food processing technologies. They include specific manipulations at the level of animal and plant production and contribution from the field of genetics, namely recombinant genetic technologies, which have resulted in the production of numerous genetically modified products; however, these are often not accepted by the regulatory authorities and societies. In the recent past, development of new technologies like "clustered regularly interspaced short palindromic repeats" (CRISPR) and "CRISPR-associated sequences" (Cas) have contributed to the emergence of gene editing methods to improve the expression of genes associated with a selected metabolic pathway(s) through the precise modification of target genes, which in turn helps in acquiring the desired trait (87). CRISPR-based genome modifications have already been applied in diverse fields of food science, e.g., breeding of tomatoes with enhanced lycopene content, which is in accordance with the standard definition given for functional foods (111). Legal aspects of innovations in the food industry, health claims, and safety assessment are the set of problems that present a challenge to breeders, food producers, and nutrition scientists, and solving them would accelerate the acceptance of functional food on a larger scale. Directing the research toward using genetically modified organisms as a source of functional food seems inevitable. However, citizens in many countries are still very skeptical about this approach and would prefer traditional agricultural methods of crossbreeding and selection of plants and animals with required characteristics. An example of functional food thus obtained is broccoli with increased content of glucoraphanin, an ingredient that shows protective activity against colon and breast tumors (24, 25, 115).

Finally, the response of consumers to the designated food product has recently been a matter of analysis. Nutrigenomics (personalized nutrition) aims to provide individual dietary recommendations basing on the human genome data; nonetheless, research in this area is still at a preliminary stage (116). Concerted action of personalized nutrition and personalized therapy (precision medicine) may

bring real value to the prevention and treatment of cardiovascular, cancer, and other degenerative diseases.

CONCLUSIONS

Despite possible benefits to the public health, highlighted in the current review, functional food seems to be a scientific idea that is boosting research in the field of biological principles and the mechanism of their functioning. A combined effort between medical and nutrition scientists and educational policy analysts should aim to implement functional food as a constant element of a healthy lifestyle, along with physical activity and body mass reduction. The health-promoting effects resulting from enhancement of function and reduction of disease risk should be proved scientifically in well-designed trials that fulfill the Evidence-Based Medicine requirements and would provide conclusive results that can be further incorporated into clinical practice and community health care.

Special attention should be directed to the fact that the published studies have provided convincing and solid data supporting the beneficial effects of functional food products. Successful implementation of the functional food concept depends on cultural factors, health motivation of consumers, and their confidence in the food industry, product safety, and marketing strategies.

Conflict of interest

The authors declare no conflict of interest.

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