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SPECIAL ARTICLE

# Functional foods and cardiometabolic diseases☆

## International Task Force for Prevention of Cardiometabolic Diseases

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**Abstract** Mounting evidence supports the hypothesis that functional foods containing physiologically-active components may be healthful. Longitudinal cohort studies have shown that some food classes and dietary patterns are beneficial in primary prevention, and this has led to the identification of putative functional foods. This field, however, is at its very beginning, and additional research is necessary to substantiate the potential health benefit of foods for which the diet–health relationships are not yet scientifically validated. It appears essential, however, that before health claims are made for particular foods, in vivo randomized, double-blind, placebo-controlled trials of clinical end-points are necessary to establish clinical efficacy. Since there is need for research work aimed at devising personalized diet based on genetic make-up, it seems more than reasonable the latter be modeled, at present, on the Mediterranean diet, given the large body of evidence of its healthful effects.

The Mediterranean diet is a nutritional model whose origins go back to the traditional diet adopted in European countries bordering the Mediterranean sea, namely central and southern Italy, Greece and Spain; these populations have a lower incidence of cardiovascular diseases than the North American ones, whose diet is characterized by high intake of animal fat. The meeting

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in Naples and this document both aim to focus on the changes in time in these two different models of dietary habits and their fall out on public health.
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## An overview of nutritional genomics and of the gene–environment interaction[1]

### *The dawning of the brain versus brawn struggle*

To understand the emergence of nutritional genomics, one must inevitably start with the history of human nutrition. This is traditionally traced back to about 2.5 million years ago, when soon after the appearance of Lucy, the nutritional phenotypes of the human species are thought to have appeared, and much later, in the Neolithic, when nutritional phenotypes of human populations began to emerge. A major event that was not only to condition human diets but also to shape human destiny, and that eventually transformed hunter–gatherers into digital natives, was the emergence of a frame-shift mutation that "switched-off" the myosin heavy chain (MYH16) gene that was expressed in the masticatory muscles of monkeys. Using the coding sequence of the myosin rod domains as a molecular clock, Stedman et al. [1] dated, the loss of this protein isoform, which caused a reduction in the size of these muscles, to about 2.4 million years ago. This may be considered the first example of nutritional genomics. Indeed, it is a striking case of a mutation inducing a drastic change in nutrition and thus in lifestyle.

As shown in Fig. 1A, the loss of the MYH16 protein isoform and the consequent appearance of less powerful masticatory muscles together with other random mutations in early hominins that led to the upright position, prompted these populations to search for more tender food, which in turn resulted in migration to the savannah/woodland interface to gain access to the soft bone marrow of the carcasses of carnivore preys [1]. The new food provoked epi-mutations, which, probably with germinal mutations, promoted a hierarchy of organs (Fig. 1B). In fact, encephalization, namely, brain enlargement, resulted in improved structural, cognitive and problem-solving ability that are features typical of humans. Synergistically, evolution, based also on dietary changes, produced a reduction in gut length thereby releasing energy for brain development. Thus, a virtuous circle was set in motion between brain development and nutrition, thereby generating not only the *brain versus brawn struggle* but also the *brain versus gut struggle* (Fig. 1C) [2]. This scenario vividly evokes the challenging *nature versus nurture* dilemma raised by Sir Francis Galton in 1857 of which more below.

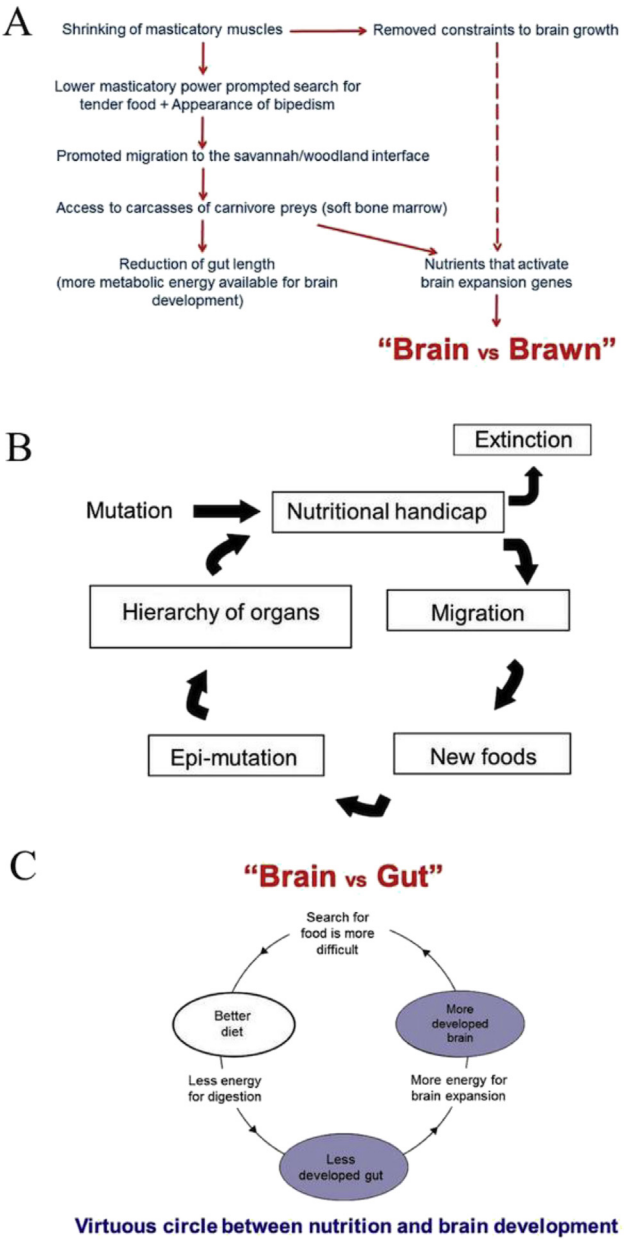


**Figure 1** (A) Consequences of myosine mutations in the masticatory muscles of early hominins that ultimately provoked, on one hand, the need to look for more tender food, and on the other hand, activation of brain expansion genes (*brain versus brawn*). (B) Interaction between nutritional handicap and migration that would provoke the hierarchy of organs in the human body during evolution. (C) The virtuous circle that allowed greater brain development because of better nutrition and a less developed gut. Source: Rotilio G. Roma: Carocci, 2012.

[1] The authors of this section are Salvatore F., Daniele A., Buono P.

### *Nutritional handicap induced by gene mutations and predictive medicine*

To take a leap forward in man's progress, the finding that all living beings store their genetic material in their DNA provided the basis for the concept of risk. Being the probability that an event will or will not happen, risk also indicates a predisposition in terms of developing a disease. Ultimately, it indicates a susceptibility to a disease when a gene variant (or a series of gene variants) are present in a genome. Jonsen and colleagues labeled subjects with a susceptibility to a disease as "unpatients" [3], a term that aptly describes their condition of being in a kind of limbo between health and disease. This applies also to subjects with a variant genotype that prevents them from metabolizing a given nutrient, which thus becomes toxic. In fact, such individuals are "healthy" until they encounter the offending food.

Diet exerts a strong effect on such types of diseases that are known as "single-gene autosomal recessive disorders", examples of which are phenylketonuria, galactosemia, and fructose intolerance [4–6]. These conditions can be managed by personalized nutrition strategies. Moreover, thanks to new genetic laboratory tools, they can be identified in newborn screening programs. Early detection and dietary or supplement-driven treatment are crucial, for example, in patients with phenylketonuria or other monogenic diseases, and also in patients with multigenic dietary diseases (e.g., celiac disease), in which patients are advised to avoid a given food or to adhere to a specific diet for their well-being [7–12].

More than 6000 human monogenic disorders have been identified, including more than 100 protein-based metabolic disorders. Some are rare and complex dietary diseases, namely, fatty acid oxidation disorder, organic acid metabolism disorders, urea cycle defects and glycogen storage disease. Patients may reduce their intake of the dietary substrates or metabolites that accumulate in such diseases, and nutrigenomics will improve their prevention and treatment by identifying specific mutations or haplotype combinations that modulate the dietary response in affected subjects [13]. In multifactorial diseases like cardiovascular diseases, obesity, type 2 diabetes mellitus, cancer etc., nutrigenomic studies revealed that most of them are amenable to dietary intervention that may modulate their onset and progression [14]. This scenario has contributed to the concept and practice of the molecular diagnosis of diseases, and to DNA tests that are predictive, or even diagnostic of a given condition.

Nutritional therapy is based on nutritional science and aims at promoting the health, performance and wellbeing of a person. This therapeutic approach uses a wide range of tools to identify and assess nutritional problems and understand how they may contribute to health concerns [15]. Nutritional therapy also addresses nutritional balance and aims at maintaining health. This approach enhances the health and wellbeing of patients with chronic conditions [16].

According to genomics, each subject is a unique entity and consequently requires a personalized nutrition and lifestyle plan rather than a "one size fits all" approach. In fact, nutritional and lifestyle approaches to healthcare improve the health of all the main tissues and organs of the body (skeletal, nervous, respiratory, cardiovascular, digestive, excretory, endocrine, immune, and intertegumentary tissue) as well as the brain. Typical priorities in nutritional therapies are to obtain optimum energy levels, a healthy blood sugar balance, emotional and psychological wellbeing, optimum gastrointestinal health and tolerance to a broad range of food groups.

Future advances in pre-emptive medicine based on genetics, molecular diagnostics, and tailored interventions will improve the prevention of various metabolic diseases. Molecular diagnostics can identify subjects who are more likely to respond positively to personalized nutrition. Prediction is a crucial factor in medicine because it allows one to prevent or even to cure a disease, whereas a biomarker of a disease represents a target that can be manipulated. A biomarker can also contribute to scientific knowledge in terms of a better understanding of a disease, and the way it starts and develops, and finally it can serve as a therapeutic target [17–19]. Thus, the biomarker of today is the theranostic of tomorrow – *theranostics* being the development of diagnostic tests directly linked to the application of specific therapies [20].

### *Nutrigenetics, nutrigenomics and personalized nutrition*

#### Nutrigenetics and nutrigenomics: the potential role of omics in targeted nutritional intervention

The concept that nutrients can interact and affect the molecular mechanisms governing an individual's physiology generated two new disciplines: nutrigenetics and nutrigenomics [21]. Following initial confusion about the demarcations of the two disciplines and their terminology, there is now general consensus as to the specificity and meaning of each of them.

*Nutrigenetics* aims at identifying the genetic susceptibility to the effect of nutrients based on the architecture of the genome of each individual or group of individuals that have the same DNA sequence at specific loci or in specific gene(s), or even in a single (or a few) nucleotide polymorphisms. Although still in its infancy, the discipline is already starting to provide personalized dietary advice to people based on their genetic make-up. Nutrigenetics examines the impact of genetic variants on tolerance and sometimes on nutritional requirements under physiological and pathological conditions.

*Nutrigenomics* studies the effects of food and food constituents on gene expression. This means it investigates molecular interactions between nutrients and factors that affect genome expression. The overall aims of nutrigenetics and nutrigenomics are to provide better nutritional advice to the public-at-large, to genetic subgroups, and to single individuals with the ultimate aim of personalizing nutrition for a population with a presumably similar genetic background and then homing down to the level of single individuals. The two disciplines also aim at optimizing health, and at preventing and treating diseases, thereby transforming the practice of nutrition and dietetics into an evidence-based science. In other words, nutrigenetics and nutrigenomics may transform empiricism into scientific knowledge.

Most of the studies related to nutrition conducted to-date explored how the interaction between genotype and environmental exposure, including nutrition, affects an individual [22]. Thus far, most nutritional interventions have been either generic or tailored to population level rather than to the individual. For example, overweight subjects are, in general, advised to consume fewer calories, and lactose-intolerant subjects are advised to avoid or limit their milk intake, whereas subjects carrying the TT allele in the methylentetrahydrofolate (MTHFR) gene are advised to increase their daily folate intake from 200 μg to 400–600 μg to maintain their homocysteine serum concentration under risk level [23].

Figure 2 shows the interactions between nutrition and genetic factors, in other words, it shows the interaction between nutrigenetics and nutrigenomics that leads to variations in gene expression and ultimately to phenotype.

## Epigenetics and nutrition

All complementary modifications that impact on gene expression and function without modifying the genetic code, are defined "epigenetic". Examples are histone modification and microRNAs, but the major epigenetic process is, classically, DNA methylation. This is a common more stable epigenetic marker transmitted through DNA replication and cell division. DNA methylation induces transcriptional silencing and can affect gene regulation in response to the environment including nutrition. Increasing evidence indicates that early life environmental factors, including nutrition, influence the future risk of developing a given disease. For example, a diet rich in fat increases the glucose level in fetal blood, whereas a protein-restricted diet reduces the risk of type2 diabetes. The epigenetic regulation of the methylation status of specific CpG loci in the peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1A) gene in endocrine pancreas β-cells was found to be associated with insulin secretion in patients with type 2 diabetes, whereas methylation in the fat mass and obesity-associated (FTO) gene in blood lymphocytes was associated with this disease. Furthermore, a genotype–epigenotype interaction was described between haplotype-specific methylation in FTO-associated type 2 diabetes and the obesity locus. Thus, epigenetic markers may be induced before the onset of symptoms and will be predictive of disease risk [see Ref. [24]].

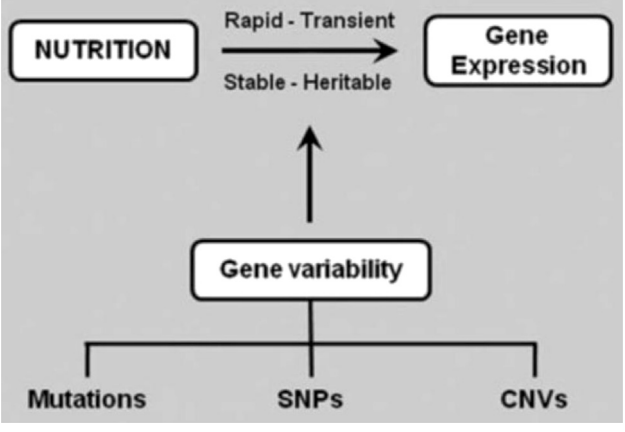


**Figure 2** Outline of the nutrition–gene interactions that result in the final phenotype. The latter depends on the gene expression specific to each individual, which in turn depends on both gene variability and nutrition, which in turn exert epigenetic effects that may modulate gene interactions. Modified from: Dauncey M. J., Proc Nutr Soc. 2012; 71:581–91.

## Role of genetic testing in personalized nutrition

*Omics* technology aims at increasing knowledge about the effects exerted by food on human metabolism, and at shedding light on the genetic determinants of these effects in individuals thereby opening the way to personalized nutrition. Personalized nutrition is designed to provide appropriate care to a given individual at the appropriate time thereby reducing the risk of disease and health care costs, as well as optimizing health and improving disease outcome.

Currently, personalized nutrition based on genetic information is considered premature. However, there has been a growth in the market for genetic analyses aimed at the general public. Most of the kits combine nutritional advice with nutritional supplements and biomarker assessment tests. Recently, the U.S. Government Accountability Office judged these tests misleading for consumers because they make predictions that are not adequately supported by scientific evidence. The European Council Working Party on Human Genetics also discussed the question of selling genetic tests to the public without reaching a decision.

Interestingly, also "systems biology" has been exploited in the field of personalized nutrition. In fact, mathematical modeling can be applied to dietary information and to an individual's metabolic profile to obtain a "nutrigenomic score", which may lead to system biomarkers that can be used to shed light on an individual's nutritional tendencies (Fig. 3). A fast emerging concept is that of the microbiota, the study of which could impinge on nutritional therapy [25].

To conclude this section, the interactions among genotype, dietary exposure and disease risk are a matter of intense research. The scientific evidence that a direct interaction between specific genotype and dietary

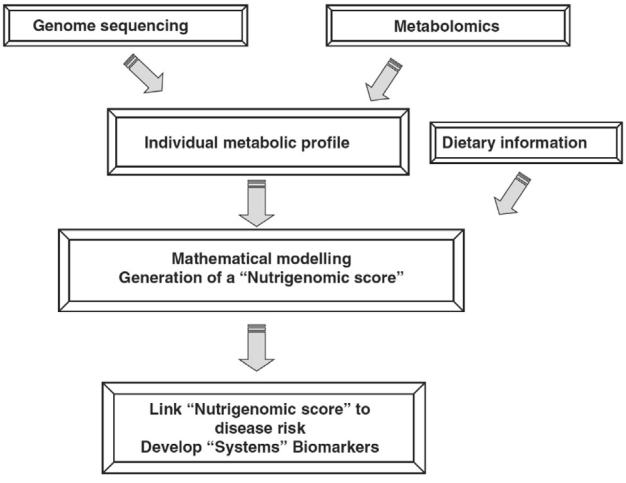


**Figure 3** The attempt to devise a "nutrigenomic score" for disease risk and to develop systems "biomarkers" based on multiple inter-related measurements. Source: Hesketh J, Eur J Clin Nutr. 2013; 67:430–5. Reproduced with permission from Nature Publishing Group.

components is involved in the development of polygenic diseases is, at present, fragmentary and does not yet support the widespread application of personalized nutrition.

### *Nutritional interventions and calorie restriction*

A less sophisticated aspect of nutrition is calorie restriction. Notwithstanding its lack of sophistication, calorie restriction is not only a major determinant of longevity but it can also reduce the incidence of neuro- and cardiodegenerative diseases, and even cancer [26]. This has been demonstrated in species ranging from yeast to mammals. In fact, calorie restriction reduced cardiovascular and cancer mortality by 50% in rhesus monkeys [27]. It also reduced age-associated brain atrophy in non-human primates. In man, recent experiments conducted in members of the Calorie Restriction Association demonstrated that this restriction dramatically modifies the transcriptional profile of skeletal muscle [28].

We now have preliminary data that the expression of several longevity-associated enzymes is several fold higher in human fibroblast cell lines incubated with sera from calorie-restricted individuals than in cells from individuals on a westernized diet. Moreover, we found a highly significant differential stress resistance to hydrogen peroxide in the presence of calorie-restricted sera [29].

### *Nature and nurture: the exposome*

The exposome may help to solve the challenging *nature versus nurture* dilemma in which *nature*, i.e., "genomics", or the innate quality of man, represents genetic features, whereas *nurture* refers to all the environmental factors that interact with genetic features. The latter interaction is crucial in understanding how human beings are and/or appear. The exposome is defined as "every exposure to which an individual is subjected from conception to death" or to a certain period during which the observation is made (Fig. 4). In essence, it is an environmental complement to each individual's genome in assessments of phenotype. And it is the measure of the effects of life-long environmental exposure on health. Exposome studies have received funding from the European Commission, and the exposome concept is well recognized by the U.S. National Institutes of Health. However, the major dilemma is how to handle the daunting amount of data that theoretically would need to be processed to evaluate a person's lifelong environmental exposure. The domains that should be evaluated to produce an exposome score come under three headings: general external factors, internal factors, and specific external factors. One possible approach to this problem is to evaluate exposure at key stages of life, for instance, infancy, puberty, adolescence, and mid-life. These evaluations can be combined to produce an exposome score; unfortunately, this is beyond our reach at present. Figure 4 shows how modern concepts can reconcile "nature" with "nurture".

In summary, the environment with its noxae, and an individual's lifestyle, namely, nutrition, physical activity etc., affect the individual's genotype, which, together with the effects evaluated by pharmacogenomics and nutrigenomics,

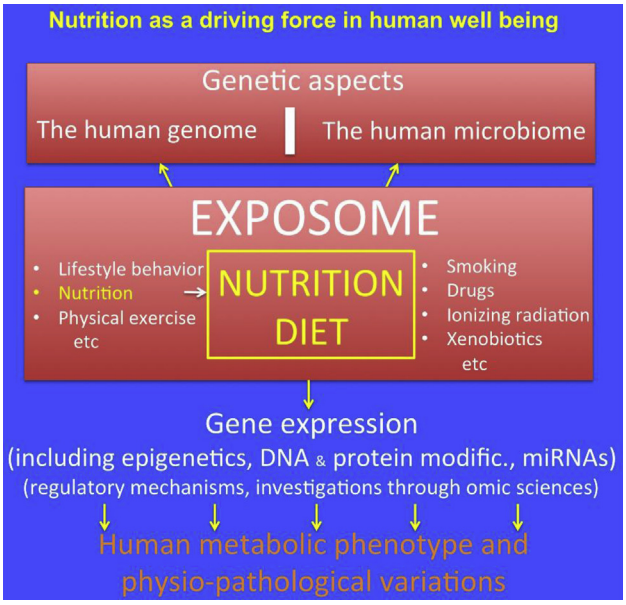


**Figure 4** Nutrition as a driving force in human well being. Note how the genetic aspects of the human genome and the human microbioma, by interacting with the exposome, in which nutrition plays a central role, influence each individual's gene expression thereby leading to his/her phenotype.

determines an individual's phenotype, thus opening the door to personalized medicine and personalized nutrition. Lastly, nutritional genomics holds great promise for disease identification and prediction as well as for disease prevention and treatment. For the present, one may apply the simple equation: Eat less (calorie restriction) + Eat better (nutrigenomics) = *Hic manebimus optime.*

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## Dietary supplements, seleniun and cardiometabolic health[2]

### *Introduction*

Use of dietary supplements has increased markedly in recent years, across several countries, because of the perception

[2] The authors of this section are Stranges S., Farinaro E, Salvatore V, Della Valle E.

that antioxidant vitamins and minerals may reduce the risk of cardiovascular disease (CVD), cancer and other chronic diseases [30]. However, there is inconclusive trial evidence for a role of dietary supplements in chronic disease prevention, at least among healthy individuals in the general population. Moreover, concern has been raised about possible adverse health effects for some of these supplements, especially when they are used at high doses [31]. For example, findings from randomized, controlled clinical trials showed increased all-cause mortality with high-dose β-carotene and vitamin E supplements, two antioxidant vitamins that were widely believed to be safe [32,33].

More specifically, selenium (Se) has been one of the most extensively investigated micronutrients in chronic disease prevention over the last two decades [34,35]. In fact, Se is a key component of a number of selenoproteins involved in essential enzymatic functions such as redox homeostasis, thyroid hormone metabolism, immunity and reproduction [36]. Because of the potential of these selenoproteins to protect against oxidative stress, significant expectations were raised for the prevention of several chronic diseases including cancer, cardiovascular disease (CVD), and type 2 diabetes [37], conditions commonly associated with oxidative stress. However, recent findings from observational studies and randomized clinical trials have raised concern that high Se exposure may lead to adverse cardio-metabolic effects, at least in well-nourished populations [31].

### *Selenium and type 2 diabetes*

Evidence from in vivo and in vitro studies suggests that Se could enhance insulin sensitivity by mediating insulin-like actions [38]. However, results from human studies on Se and diabetes are conflicting. Recent findings from observational studies and randomized clinical trials from the US, a Se-replete population, indicate that high Se status or Se supplementation may be associated with an increased risk of type 2 diabetes [39–41]. Specifically, in a *post-hoc* analysis of the Nutritional Prevention of Cancer (NPC) trial, in the eastern US, we found that supplementation with Se (200 μg/day as high-Se yeast), compared to placebo, increased the risk of type 2 diabetes [40], particularly among participants with high baseline plasma Se (hazard ratio of 2.70 in the highest tertile of plasma Se, i.e. $>$121.6 ng/ml). Moreover, results from the large Selenium and Vitamin E Cancer Prevention Trial (SELECT) in 35,533 North American men aged $\geq$ 50 y [41], showed a small, though non-significant, increase in the number of cases of adult-onset diabetes in subjects supplemented with Se alone (200 μg/day as selenomethionine). However, after a further 33 months of follow-up in the SELECT trial, the risk of diabetes further diminished: RR 1.04; 99%CI 0.91 to 1.18 [42]. Furthermore, a recent pooled longitudinal analysis from two US cohorts (i.e. Health Professionals Follow-up Study and Nurses' Health Study) showed inverse associations between toenail selenium and incident type-2 diabetes; diabetes risk reduced as quintile of toenail selenium increased [43].

In European populations, where Se status is generally lower than in the US, the evidence linking Se to glucose metabolism is conflicting. For example, in an early analysis of the EVA (Epidemiology of Vascular Ageing) study in France, plasma Se concentrations were positively, though non-significantly, associated with baseline glucose levels in women and with prevalent diabetes in men [44]. However, a later report from the same study showed that high plasma Se (1.19–1.97 μmol/L) was associated with a marginally significant reduced risk of hyperglycaemia (impaired fasting glucose or diabetes) in men over the 9-year follow-up [45]. Conversely, in a prospective study on a large sample of women from Northern Italy, an increased risk of type-2 diabetes was associated with higher dietary selenium intake; however it has to be admitted that dietary assessments of selenium intake are unreliable so not much weight can be attached to this result [46].

With regard to trial evidence, in the French SU.VI.MAX study, no effect of combined supplementation with antioxidants including Se (100 μg/day as high-Se yeast), was observed on fasting plasma glucose after 7.5 y of follow-up despite a positive association between glucose and Se concentrations at baseline in the whole population [47]. Recently, in the PRECISE Trial (PREvention of Cancer by Intervention with Selenium) among 501 elderly volunteers treated for 6 months with 100, 200 or 300 μg selenium/d as high-selenium yeast or placebo yeast, we found no effect of selenium supplementation on plasma adiponectin, as a surrogate marker of type-2 diabetes risk [48]. However, limitations of this trial were the short duration (6 months) and the limited age range (60–74 y) of the participants.

The explanation for these discrepant results is still unclear. Further research is needed to identify the optimal range of Se intake and status in order to minimize potential adverse effects on glucose metabolism while optimizing type 2 diabetes prevention [49].

### *Selenium and blood lipids*

A few studies from unrelated populations suggest that high Se exposure may be associated with adverse effects on lipid metabolism. Specifically, in the SU.VI.MAX trial, plasma concentrations of Se were positively associated with total cholesterol at baseline, while participants receiving long-term supplementation with Se (100 μg/day) and other nutrients had higher serum triglyceride concentrations than those on placebo [50]. Furthermore, compared to those on placebo, women who were supplemented had higher total cholesterol levels while men were more likely to be using lipid-lowering medication. Moreover, a cross-sectional analysis of serum Se and lipid levels in the NHANES III showed that high serum Se concentrations were associated with high total, LDL- and HDL-cholesterol, triglycerides, apo B, and apo A1 levels [51]. These findings were further corroborated by a recent analysis from the NHANES 2003–2004 showing positive associations between serum Se concentrations and blood lipids in a representative sample of the US population [52].

In agreement with these findings, our cross-sectional analysis from the 2000–2001 UK National Diet and Nutrition Survey (NDNS) indicated that high-normal Se status was associated with increased total and non-HDL cholesterol, but not with increased HDL, in a nationally representative sample of British adults [53]. In contrast to the US, a significant proportion of British adults are considered to have a sub-optimal intake of dietary Se. However, recent prospective findings from both the Young Finns Study and the Olivetti Heart study do not support the causality of the link between high selenium status and adverse blood lipid profiles [54,55]. Finally, in the UK-PRECISE trial the effect of a six-month supplementation with 100, 200 or 300 μg selenium/day as high-selenium yeast as compared to placebo were examined among 501 elderly volunteers with a mean plasma selenium concentration at baseline of 88.8 ng/g (equivalent to 91.2 μg/L). In this trial, supplementation at 100 and 200 μg selenium/day lowered total serum cholesterol and non-HDL cholesterol; the 300 μg/day dose had no significant effect on total or non-HDL cholesterol, but raised HDL-cholesterol significantly. In addition, the total–HDL cholesterol ratio decreased progressively with increasing selenium dose [56].

### *Selenium and cardiovascular disease*

A number of observational studies have examined the association between Se status and risk of coronary heart disease (CHD) across different populations [57]. Although some of the early studies suggest possible inverse associations, especially in populations with relatively low Se intakes, more recent observational evidence is suggestive of a possible U-shaped association between Se status and CHD risk, at least in well-nourished populations such as that of the US [34]. However, results from randomized trials of Se supplementation do not support a role for Se in cardiovascular prevention [35]. Specifically, in *post-hoc* analyses from the NPC trial [58], we found that Se supplementation (200 μg/d) was not significantly associated with any of the cardiovascular disease (CVD) endpoints after 7.6 years of follow-up [all CVD: hazard ratio (HR) = 1.03, 95% confidence interval (CI): 0.78, 1.37; myocardial infarction: HR = 0.94, 95% CI: 0.61, 1.44; stroke: HR = 1.02, 95% CI: 0.63, 1.65; all CVD mortality: HR = 1.22, 95% CI: 0.76, 1.95]. Few other randomized trials that examined the effect of Se in combination with other vitamins or minerals on CVD end points have also yielded null findings [57].

### *Potential mechanisms for adverse cardio-metabolic effects of high selenium exposure*

Evidence on potential mechanisms that may explain the detrimental effect of high Se exposure on cardio-metabolic health is sparse; therefore any such discussion is highly speculative. However, Se is known to be a trace mineral with a narrow therapeutic window and considerable inter-individual variability in terms of metabolic sensitivity and selenoprotein polymorphisms [36]. In animal models, the Se species (selenite and selenite), despite owning some insulin-like properties, have been shown to impair insulin responsiveness and induce a catabolic response in rat muscle with glycogen depletion and increased rates of glycolysis, and to reduce insulin release from pancreatic islets in mice [49]. Moreover, it has been shown that high-Se diets may stimulate the release of glucagon, promoting hyperglycaemia, or may induce over-expression of glutathione peroxidase (GPx) 1 and other antioxidant selenoproteins resulting in the development of insulin resistance and obesity [49]. In general, the toxic effects of Se which might explain an etiologic role in diabetes are the capacity of Se compounds to induce oxidative stress [49], a mechanism that is likely to play a key role in the etiology of this disease.

### *Public health perspective*

Potential adverse effect of dietary supplements such as Se on cardio-metabolic health warrants further consideration for several reasons. *Firstly*, in the UK and many other Western countries, use of Se enriched foods, fertilizers, and supplements has increased markedly in recent years [36,37] because of the perception that Se can potentially reduce the risk of cancer and other chronic diseases. Hence, from a public health perspective, it is essential to make sure that a higher Se status, which may result from the increasing use of these additional sources of Se, does not exacerbate the existing health, economic and social burdens associated with diabetes, and other cardio-metabolic disorders. *Secondly*, because of the current interest in Se for chronic disease prevention, it is important to understand the full range of effects of high Se exposure on cardio-metabolic endpoints, including diabetes, lipids, and CHD. *Thirdly*, dietary intakes of Se vary considerably between countries and regions largely due to the variability of the Se content of plant foods (and hence of animal forage) from one part of the world to another [36,37] and so may disease risk. Current recommendations for dietary Se intake (55–75 μg/day) are based on optimizing the activity of plasma GPx, which requires a plasma Se concentration of 92 μg/L [59]. In the US, the mean serum Se concentration among participants in the NHANES 2003–2004 was 137 μg/L, and most participants (99%) had serum Se above 95 μg/L [52]. It is therefore likely that the average NHANES participant would have had repleted selenoprotein status, including that of selenoprotein P, the carrier of Se in the plasma [59]. Health benefits of additional Se intake in such a population are therefore questionable and toxic effects, such as an increase in diabetes risk or adverse metabolic profiles, are possible. In Europe, Se status is generally lower than in the US with a large variability in dietary Se intakes by country, ranging from levels considered to be marginally adequate or adequate (Western and Central Europe: 30–90 μg/day) to low or deficient (Eastern European countries: 7–30 μg/day) [36,37].

### *Future directions*

Recent findings from observational studies and a few randomized clinical trials have suggested potential adverse cardio-metabolic effects associated with high doses of micronutrient supplements, such as selenium. However, this evidence has substantial limitations. Firstly, most of these studies were conducted in North American populations where Se status is well above the physiological range for optimal activity of plasma glutathione peroxidase and dietary Se intakes [59]. In those settings, increased Se intake might not be beneficial owing to high baseline levels. Secondly, most studies available were designed as cross-sectional surveys, and are therefore subject to reverse causation and incidence/prevalence biases. In general, there have been disappointing findings from several costly clinical trials of antioxidant supplements showing not only no health benefits but potential harms. The Selenium and Vitamin E Cancer Prevention Trial (SELECT), conducted among 35,533 North American men aged 50 and older, was prematurely stopped because of lack of efficacy of vitamin E and Se supplementation (200 μg/d) in cancer prevention and because of a small, though non-statistically significant increase in the number of cases of adult onset diabetes in participants taking only Se [41,42]. Thus, for ethical reasons it would be difficult to justify the initiation of additional RCTs of micronutrient supplements; moreover, it is highly unlikely that adequately powered trials of Se and cardiovascular outcomes will be initiated in the near future. Nevertheless, from a public health perspective, it is extremely important to understand the health effects of a nutrient as selenium that is frequently supplemented. Further research based on high-quality prospective studies across different countries with variability in Se intake and status may help define the full range of health effects, beneficial or detrimental, associated with different ranges of Se exposure, as well as the optimal ranges of Se intake and status for the general population (i.e., ranges that are likely to maximize the health benefits while avoiding potential toxic effects). However, at present the widespread use of dietary supplements such as selenium for cardio-metabolic disease prevention in the general population is not justified and should not be encouraged [31].

## Functional foods and nutraceuticals in primary prevention[3]

### *Introduction*

> "Let food be thy medicine and let thy medicine be food"
>
> Hippocrates of Kos
>
> Greek Physician
>
> 4th century BC

[3] The author of this section is Ferns G.

### *Diet and cardiovascular disease*

The relationship between dietary factors and cardiovascular disease (CVD) has been a major focus of health research for almost half a century. A small number of risk factors appear to account for a very large percentage of the attributable risk of CVD, and overall diet contributes substantially [60] to lifestyle associated risk factors [61]. Epidemiological and clinical studies indicate that the risk of CVD is reduced by a diet rich in fruits, vegetables, unrefined grains, fish and low-fat dairy products, and low in saturated fats and sodium [62] (Table 1). Other foods such as mono- and polyunsaturated fats, bran, nuts, plant sterols, and soy proteins have also been shown to have a favorable effect on lipid profile, blood pressure [63,64], endothelial and platelet function and the inflammatory response (Table 1). The potential benefits of entire diets, particularly the Mediterranean diet [65], and whole foods [66] have also been extensively studied. These studies have stimulated interest in the potential cardio-protective effects of individual food items, or components of these foods, such as antioxidants. However, it is often difficult to be sure whether any single dietary component is responsible for the cardio-protective effects, or whether this is due to the entire combination of nutrients and dietary habits. The conflicting results between the apparent protective effects of nutrients as part of normal dietary intake, and the lack of effectiveness in trials of single nutrient supplements, has led to a focus on the potential for whole foods, or modified diets (e.g. those rich in fruit and vegetables) as being protective against CVD [67]. Even this is not entirely straightforward; the effects of fruit and vegetables on cardiovascular risk are dose-dependent, and may be related to the type of fruit, or vegetable consumed [68].

### *Functional foods and nutraceuticals*

Functional foods are defined as foods that, in addition to supplying nutrients, offer potential health benefits that can enhance the well-being of individuals. They "… affect one or more target functions in the body, beyond their nutritional effects, to either improve health and/or reduce the risk of disease" [69], and should by several definitions, be consumed as part of a normal food pattern, "… and not as a pill, a capsule nor any form of dietary supplement …" [70]. Some examples of functional food are shown in Table 2. In Japan an approval process for functional foods (Foods for Specified Health Uses, FOSHU) was established in the 1980's [71]. The criteria for the FOSHU approval process are shown in Table 3.

The term nutraceutical was coined by DeFelice in the early 1980's [72], and it has subsequently been used interchangeably with functional food to designate foods for disease prevention and health promotion. DeFelice's original definition of nutraceuticals includes dietary supplements [73] and these are explicitly excluded in most definitions of nutraceuticals. An interest in nutraceuticals

**Table 1** Potential cardiovascular protective effects of functional foods.

| Protective mechanism | Functional food | Active component |
|---|---|---|
| Lipid lowering | Fruit and vegetables | Fibre |
| | Legumes | Fibre and phytochemicals |
| | Margarine | Phytosterols |
| | Nuts | Omega-3 fatty acids, fibre and polyphenols |
| | Oily fish | Omega-3 fatty acids |
| | Soy protein | Genistein and daidzein |
| BP lowering | Ginseng | Ginsenosides |
| | Grapes and red wines | Grape polyphenols |
| | Green and black teas | Polyphenols |
| | Legumes | Fibre |
| | Oily Fish | Omega-3 fatty acids |
| | Onion and garlic | Quercetin |
| | Whole grains | Fiber and phytochemicals |
| Antioxidant | Brazil nuts, grains and seeds | Vitamin E, selenium |
| | Grapes and red wines | Anthocyanins, catechins, cyanidins, flavonols, myricetin, resveratrol and quercetin |
| | Green leafy vegetables and fruits | Carotenoids, tocopherol, tocotrienols, vitamin C, flavonoids, indoles, lutein |
| | Soy proteins | Genistein and daidzein |
| | Tea (green and black) | Polyphenols |
| | Tomatoes | Lycopene |
| | Vegetable oils | Tocopherols |
| Anti-inflammatory | Fish | Omega-3 fatty acids |
| | Nuts, seeds, and oils | Vitamin E |
| | Legumes | Polyphenols |
| | Tea | Catechins |
| | Fruits and vegetables | Quercetin |
| | Grapes and red wines | Anthocyanins, catechins, cyanidins, flavonols, myricetin and quercetin |
| Endothelial function | Citrus fruits and vegetables | Vitamin C, polyphenols |
| | Chocolate (dark) | Flavonoid |
| | Fish | Omega-3 fatty acids |
| | Grapes and red wines | Anthocyanins, catechins, cyanidins, flavonols, myricetin and quercetin |
| | Nuts | Omega-3 fatty acids, polyphenols |
| Platelet aggregation | Grapes and red wines | Anthocyanins, catechins, cyanidins, flavonols, myricetin and quercetin |

for cardiovascular prevention was stimulated following the reports of a close association between the consumption of particular dietary factors, including those taken as supplements (e.g. vitamin E) with measures of status, and reduced cardiovascular event rate [74].

**Table 2** Practical examples of functional foods.

| Definition | Examples |
|---|---|
| A natural food, fruit or grain which may or may not be modified | e.g. Vitamin E-enriched vegetable oils, vitamin A-enriched, genetically engineered, "golden" rice |
| A food to which a component has been added | e.g. A spread with added phytosterols |
| A food from which a component has been removed or reduced | e.g. A yogurt with reduced fat |
| A food in which one, or several components, have been modified, replaced or enhanced to improve its health properties | e.g. A juice drink with enhanced antioxidant content, a yogurt with added prebiotic or probiotic |

### *Antioxidants, functional foods, nutraceuticals and cardiovascular disease*

The apparent conundrum of positive effects of whole diets and negative outcomes of most supplement trials has been particularly well debated in relationship to the role of antioxidants in CVD prevention. Oxidative stress is a disturbance in the pro-oxidant/antioxidant balance that favors oxidation that appears to be involved in the etiology of several chronic diseases including CVD [75]. Dietary nutrients, both water soluble (e.g. vitamin C) and lipid soluble (vitamins A and E), comprise an important component of the antioxidant defences. Observational, prospective cohort studies have suggested that a high dietary intake or supplementation of vitamin E is associated with a lower risk of CVD and mortality. In vitro and in vivo studies in experimental animal models [76] lent support to the potential benefits that vitamin E may have, but the evidence from clinical intervention trials is essentially negative [77]. The effects of antioxidant supplements (β-carotene, vitamin A and vitamin E) on all-cause mortality in primary and secondary prevention trials, may increase mortality, and the potential role of vitamin C and selenium on mortality needs further study [32,33]. Whilst one early study has shown a reduction in myocardial infarction and cardiac events [78], the majority of the other trials have shown no positive effect. Furthermore in at least five trials, antioxidant supplementation was associated with increased all-cause mortality and two have shown higher risk of fatal CHD (ATBC and CARET) [79,80]. Whilst these

**Table 3** Criteria for approval by the Japanese Ministry of Health, Labour and Welfare.

| FOSHU (Foods for Specified Health Uses) approval |
|---|
| Effectiveness in humans is clear |
| Absence of safety issue |
| Nutritionally appropriate ingredients (no excessive salt) |
| Compatibility with product specification by the time of consumption |
| Established quality control methods |

results do not invalidate the role of oxidative stress in CVD, they do indicate that high dose supplementation with antioxidant vitamins may not represent an optimal strategy to prevent CVD in individuals at high risk of CVD. Several factors may have contributed to confound the results of these clinical trials; these include the uncertainty about the dose and form of the antioxidant for optimum bioavailability; the composition of the mixture of antioxidant to be used, as antioxidants often work in synergy; and the stage of the disease at which they are used (primary or secondary prevention) [81]. The off-target effects of antioxidants may also be important, and for vitamin E several pleiotropic properties have been reported [82]. Furthermore, CVD is a complex, multi-factorial and multi-step disease, in which the off-target effects of antioxidants, and functional foods more generally, may also be important.

### *The complexity of defining a functional food and the benefits of its deconstructed products*

Health claims are often made for food in the absence of the definitive clinical trial data that have been advocated by DeFelice [72]. Whilst fruit juices contain antioxidants, they also contain sugars, or other factors, that appear to increase the risk of diabetes mellitus. It is therefore difficult to be sure about the overall cardiovascular benefits of fruit juice, and it is now recommended that fruit juice should only contribute 1 of the daily portions of fruit and vegetables. This report by Bassano and colleagues [83] illuminates a further important issue. The benefits of an entire functional food may not be mirrored by the effects of its constituent individual macro and micro-nutrients. Health claims have been made for these components in the absence of a formal clinical trial evaluation. Furthermore, within a particular category of food, e.g. fruit, vegetable, nut, or legume; there may great variations in biological activity. For example fruit juices vary substantially in their in vitro antioxidant potency [84] but their content of other potentially therapeutic constituents (e.g. fibre [85]) and the impact of conversion from food to juice on the process of digestion and glycaemic index, which may also be important properties in CVD prevention [86], are often disregarded. A further important complexity relates to the preparation of certain food products. Nuts are reported to have beneficial effects on CVD risk [87] and yet they, or their deconstructed products (oils, nut milk and nut butter), are consumed in several forms (e.g. salted, roasted, ground) that may to some degree offset their beneficial effects. It should also be remembered that the intake of functional foods may have an indirect effect on the quality of the diet, by replacing other foods and hence reducing the intake of dietary constituents that may increase CVD risk. It is clearly important that any health claim for a food is supported by a series of experimental data, including double blind, placebo-controlled clinical trials. The stages that may be used in the evaluation of a potential functional food, or nutraceutical are shown in Table 4.

**Table 4** Stages in the assessment of functional foods.

| Steps in process | Examples of investigation |
|---|---|
| Assessment of composition | Macronutrient (e.g. Fibre)<br>Micronutrient (e.g. Antioxidant) |
| Assessment of in vitro properties | Antioxidant properties<br>Effects on signaling |
| Bioavailability and pharmaco-kinetic studies | Absorption<br>Tissue distribution |
| In vivo studies in animal models | Pharmacokinetics<br>Toxicology<br>Intervention in disease models |
| Human studies | Bioavailability<br>Safety<br>Observational<br>Intervention [Un-blinded; placebo-controlled; double-blind, randomized, placebo controlled (surrogate end-point; clinical end point)] |

### *Conclusions*

There is evidence from longitudinal cohort studies that certain classes of food and dietary patterns are beneficial in primary prevention. The results of these studies have led to the identification of putative functional foods. The mechanisms by which these foods exert these effects are complex, but are probably related to the macro- and micronutrient content of the food, and the displacement of other constituents of the diet. The benefits observed may depend on the baseline risk factors, and state of existing disease, be dose dependent, and may be affected by the preparation of the food. The benefits of functional food have infrequently been reproduced by providing isolated components of foods as supplements. It appears essential that before health claims are made for particular foods, in vivo studies are undertaken that demonstrate efficient absorption and beneficial effects on the cardiovascular risk profile. Ultimately, randomized, double-blind, placebo-controlled trials of clinical end-points are necessary to establish clinical efficacy.

## Fish and fish oil[4]

### *Introduction*

Fish, especially fatty fish, is a rich source of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The intake of these fatty acids is inversely related to fatal coronary heart disease (CHD). This composite cause of death includes generally fatal myocardial

[4] The author of this section is Kromhout D.

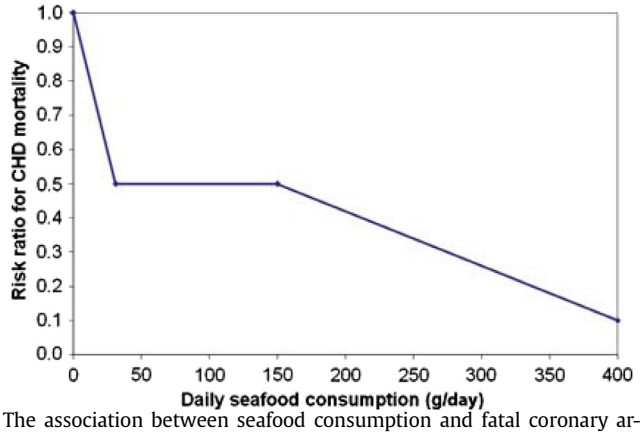
infarction (MI), cardiac arrest and sudden death. The GISSI-Prevenzione trial showed, in post–MI patients, that an additional amount of 900 mg/d EPA-DHA reduced CHD death by 35% and sudden death by 45% [88]. Basic research made clear that unstable vulnerable plaques are the main cause of fatal MI. Ventricular tachycardia and ventricular fibrillation may cause cardiac arrest and sudden death [89].

Several mechanisms of action may explain the protective effect of omega-3 fatty acids on fatal CHD [90]. EPA-DHA reduce serum triglyceride levels and remnant lipoproteins. They also reduce inflammation and improve endothelial function and vasodilatation. Together they promote plaque stabilization. Omega-3 fatty acids inhibit platelet aggregation and have a positive effect on blood rheology and mitochondrial function. Finally, EPA-DHA intake reduces the risk of severe arrhythmias and its sequelae, i.e., cardiac arrest and sudden death, through the enrichment of myocardial membranes with these fatty acids.

### *Fish consumption and fatal CHD*

In the 1970s epidemiological studies showed that the incidence of MI was 10 times lower among the Inuit compared to Danes [91]. The Inuit consumed approximately 400 g of seafood per day, with an average of 14 g per day of omega-3 fatty acids compared to 3 g per day among Danes [92]. The difference between the Inuit and the Danes in the intake of omega-3 fatty acids was reflected in the fatty acid composition of their platelets. Differences were also observed in hemostatic factors, bleeding time and high-density lipoprotein (HDL)-cholesterol levels [93]. To show that these associations are causal, the British physiologist Hugh Sinclair put himself in 1977 on an Inuit diet for 100 days [94]. His bleeding time rose from 3–5 to 50 min and substantial decreases were observed in blood platelets, erythrocytes, pack cell volume and haemoglobin. The triglyceride-rich very low-density lipoprotein fraction fell and the HDL fraction increased considerably. The results of this experiment are in accord with those found in the comparative studies among Inuit and Danes.

Based on the ecological studies among the Inuit and Danes and those comparing farmers and fishermen in Japan, Kromhout et al. hypothesized that fish consumption could reduce CHD mortality [95]. In 1985 in the Zutphen Study, a prospective cohort study in the Netherlands among originally 852 middle-aged men, these authors showed that eating fish once or twice a week was associated with a 50% lower risk of 20-year CHD mortality compared to those who did not eat fish. Kromhout deduced from these studies that two different mechanisms could be responsible for the association between fish consumption and CHD [96]. He hypothesized an acute effect on fatal CHD in cultures with a low level of fish consumption and a chronic effect in cultures with a high level of seafood consumption (Fig.).



The association between seafood consumption and fatal coronary artery disease. Reproduced with permission from reference [96].

The hypothesis that a low level of fish consumption may reduce fatal CHD was also tested in a small prospective cohort study of 272 elderly men and women in the Netherlands [97]. In this study 60% of the participants habitually consumed fish and 40% did not. In line with the results of the Zutphen Study, also this study showed that people who ate fish approximately once a week had a 50% lower risk of 17-year CHD mortality. The associations of fatty fish and lean fish intake with fatal CHD were studied in the Seven Countries Study cohorts from Finland, the Netherlands and Italy [98]. In this study 3000 men aged 50–69 were followed for 20 years. Men who consumed fatty fish (e.g. herring and mackerel) had a 34% lower risk of fatal CHD compared to those who did not eat fish. The intake of lean fish (e.g. plaice, codfish) was not associated with CHD mortality. Finally, we observed in the Dutch MORGEN project with 21,342 men and women aged 20–65 at entry that even a low level of fish consumption and a low intake of EPA-DHA was associated with a 50% lower risk of 10-year CHD mortality but not with non-fatal MI [99].

The most recent meta-analysis on fish consumption and CHD mortality included 17 cohorts with 315,812 participants and an average follow-up of 15.9 years [100]. Compared to people with the lowest fish consumption, a fish consumption of once a week was associated with a 16% (Relative Risk (RR) = 0.84; 95%CI 0.75–0.95) lower risk of fatal CHD. Similar RRs were obtained for those who ate 2–4 or at least 5 servings per week. For Western countries, the strongest associations were obtained in the Netherlands and the USA. This was possibly due to the low level of fish consumption in these countries and to the relatively large number of persons who did not eat fish. Therefore, in these countries the strongest contrast was observed between people who consumed fish once a week compared to non-fish eaters.

There is only one secondary prevention trial that tested the effect of an additional amount of fatty fish on fatal CHD. The diet and re-infarction trial (DART) was carried out in 2000 cardiac patients who were advised to eat oily fish at least twice a week and were followed for 2 years [101]. The results of the trial showed that an additional amount of two fatty fish dishes per week reduced fatal

CHD by 33%. The result of this trial and those of the prospective cohort studies provide convincing evidence that eating fatty fish at least once a week or lean fish twice a week reduces fatal CHD risk.

### *Fish consumption, omega-3 fatty acids, cardiac arrest and sudden death*

Less studies have been published on the relation between fish consumption and cardiac arrest or sudden death. A population-based case–control study from the USA showed that eating fatty fish once a week was inversely associated with cardiac arrest [odds ratio (OR) = 0.5; 95% CI 0.4–0.8) and even stronger with red blood cell membrane omega-3 fatty acids (OR = 0.3; 95% CI 0.2–0.6) [102]. Also in a nested case–control study carried out in the Physicians Health Study from the USA, the baseline blood level of omega-3 fatty acids was strongly inversely related to sudden death (RR = 0.19; 95% CI (0.05–0.71) [103].

The association of the long-term fish consumption and sudden cardiac death was investigated in the Zutphen Study [104]. Long-term fatty fish consumption was inversely associated with sudden coronary death in the subsequent 40 years, and men who consumed fatty fish had a 54% lower risk (RR = 0.46; 95%CI 0.27–0.78) than those who did not eat fatty fish. Lean fish consumption was not associated with sudden coronary death. The Physicians Health Study showed that men who consumed one fish meal per week had a 52% lower risk of sudden cardiac death (RR = 0.48; 95%CI 0.24–0.96) compared with those who consumed fish less than once a month [105]. The results from the case–control and the prospective cohort studies suggest that eating fish once or twice a week – corresponding to an intake of about 200 mg/d EPA-DHA – compared to eating no fish, is associated with a lower risk of cardiac arrest and sudden death.

### *Omega-3 fatty acid supplementation trials and fatal CHD*

For the GISSI-Prevenzione trial more than 11,000 patients with a recent MI (<3 months ago) were randomized to an additional amount of 900 mg EPA-DHA per day and were followed for 42 months [88]. The control group did not receive a placebo. The additional amount of EPA-DHA reduced cardiovascular disease incidence by 20%, cardiac death by 35% and sudden death by 45%. The trial had an open label design and was liable to performance bias (i.e. differential behavior of participants between intervention and control groups or differential care provided by physicians). Therefore results from randomized, double-blind placebo-controlled trials were needed.

Using information from prospective cohort studies and controlled trials published up to 2002, we designed a randomized, double-blind, placebo-controlled trial, the Alpha Omega Trial, to test the effect of omega-3 fatty acids on major cardiovascular events [106]. The trial has a 2 × 2 factorial design and evaluated the effect of an additional amount of EPA-DHA and of the plant-oil based alpha-linolenic acid (ALA). The results from the prospective cohort studies had suggested that in cultures with a low level of fish consumption, an intake of 200 mg/d of EPA-DHA was associated with a significantly lower risk of fatal CHD and sudden death. The DART and the GISSI-Prevenzione trial suggested that, taking non-compliance into account, a dose of approximately 600 mg EPA-DHA per day was effective. We took the average of 400 mg/d, as the amount to be tested in the Alpha Omega Trial. For ALA, we used a dose of 2 g/d, equal to the recommended daily intake.

Between 2002 and 2006, we randomized from 32 centers in the Netherlands 4837 post–MI patients aged 60–80 who had had an MI up in the previous 10 years. The patients received either a margarine enriched with an additional amount of EPA-DHA or ALA or a combination of both, or placebo margarine. The fatty acid composition of the margarines were identical with the exception of the omega-3 fatty acids. In the experimental margarines, the neutral fatty acid oleic acid was replaced by the required amounts of either EPA-DHA, ALA or a combination of both. The margarines were identical in color, taste, odour and texture. The patients were followed for an average of 41 months, during which 671 developed a major cardiovascular event (fatal and nonfatal cardiovascular diseases, and revascularization). The additional amount of EPA-DHA and ALA did not reduce the rates of major cardiovascular events, fatal CHD or ventricular-arrhythmia-related events (placement of a cardioverter-defibrillator, cardiac arrest and sudden death).

Several meta-analyses of randomized controlled trials have been published. Two meta-analyses published before 2010 showed that EPA-DHA reduced fatal CHD significantly by 20% and sudden death non-significantly by approximately 15% [107,108]. The three trials with post–MI patients published in 2010 and the recently published trial in patients with multiple risk factors, did not show an effect of extra EPA-DHA on fatal CHD [90,109]. Two meta-analyses were published in 2012, one including 14 randomized, double-blind placebo-controlled trials [110] and the other 20 randomized clinical trials including those with a patient history of cardiovascular disease, presence of implantable cardioverter-defibrillator, with omega-3 fatty acid supplementation through fish oil supplements and dietary counseling [111]. In these meta-analyses the daily dose of EPA-DHA ranged from 0.4 to 4.7 g/day and the follow-up periods ranged from 1.0 to 4.7 years. Omega-3 fatty acid supplementation did not reduce major cardiovascular events but did reduce fatal CHD significantly by approximately 10% in both meta-analysis, if no adjustment was made for multiple comparisons. Supplementation with EPA-DHA reduced sudden death by 7% in the meta-analysis by Kwak et al. and by 13% in the meta-analysis by Rizos et al., and therefore in both cases not to a significant extent [110,111].

An intriguing question to be answered is why did an additional amount of EPA-DHA in the early clinical trials

significantly reduce the rate of fatal CHD and sudden death and why did the recent ones show no effect? One of the explanations could be the earlier mentioned confounders due to the open label design of the early trials. Another explanation could be that the patients in the recent trials were very well treated not only by antithrombotic drugs as in the early trials, but also by state-of-the-art treatment with antihypertensive drugs and statins [112]. Compared to the recent trials, the treatment level of statins was low (29%) in the GISSI-Prevenzione trial [88]. Therefore, we carried out a subgroup analysis in non-statin users in the Alpha Omega Trial [113]. The average LDL cholesterol level was 0.8 mmol/l higher in the non-statin users compared to statin users, and the LDL cholesterol level of the non-statin users in the Alpha Omega Trial was similar to that of the participants in the GISSI-Prevenzione trial (3.38 vs. 3.55 mmol/l). An additional amount of 400 mg/d EPA-DHA was not enough to reduce major cardiovascular events in non-statin users, but an additional amount of 400 mg/d EPA-DHA in combination with 2 g/d ALA reduced the major cardiovascular events by 54% (hazard ratio = 0.46; 95% CI 0.21–1.01, $P = 0.051$). These results suggest that the low level of statin treatment could be the major reason for the protective effect of EPA-DHA on major cardiovascular events observed in the GISSI-Prevenzione trial.

Compared to the Alpha Omega Trial, the absolute risk in the GISSI-Prevenzione trial was almost twice as high for fatal CHD and three times higher for sudden death [90]. This could be another explanation for the positive results in the GISSI-Prevenzione trial and the negative results in the Alpha Omega Trial. Therefore we selected in the Alpha Omega Trial a subgroup of patients who had had not only an MI, but who also had diabetes [114]. These patients had a similar rate of fatal CHD as the patients with a recent MI in the GISSI-Prevenzione trial. In the patients with the double burden of MI and diabetes, an additional amount of 400 mg/d EPA-DHA reduced the composite endpoint of ventricular-arrhythmia-related events by 42% and therefore not to a significant extent (hazard ratio = 0.58; 95%CI 0.24–1.39); conversely an additional amount of 2 g/d ALA significantly reduced this endpoint by 53% (hazard ratio = 0.47; 95%CI 0.18–1.24). The combination of 400 mg/d EPA-DHA plus 2 g/d ALA was able to reduce ventricular-arrhythmia-related events by 84% (hazard ratio = 0.16; 95%CI 0.04–0.69). The combination of extra EPA-DHA plus ALA reduced also the composite endpoint of ventricular-arrhythmia-related events plus fatal MI by 72% (hazard ratio = 0.28; 95%CI 0.11–0.71). These results suggest that omega-3 fatty acids protect against severe arrhythmias and fatal MI only in high-risk patients.

### *Fish or fish oil supplements?*

Prospective cohort studies showed that eating fish once a week vs. less than once a month was associated with a significantly lower risk of fatal CHD. The protective effect of fish was confirmed in a randomized controlled trial in cardiac patients. In contrast to the earlier trials, recent trials did not find a protective effect on fatal CHD of extra EPA-DHA in trials with fish oil supplements. Based on these results I recommend to both individuals without and with a history of cardiovascular disease to eat fatty fish at least once or lean fish twice a week. The advantage of this recommendation above the use of fish oil supplements is that fish, in particularly fatty fish, is a rich source not only of the omega-3 fatty acids EPA and DHA, but also of, for example minerals such as potassium and calcium, and vitamins such as vitamin D and vitamin B12. However, fish is not a panacea for CHD prevention. The largest risk reduction will be obtained if the recommended fish consumption will be a component of a nutritionally adequate diet.

## Is it useful to recommend phytosterol-enriched foods to patients already taking lipid-lowering drugs?[5]

### *Introduction*

### Plant sterols and stanols

Phytosterols (PSs) are non-nutritive compounds that are structurally analogous to cholesterol with the same function in plants that cholesterol has in animals. There are more than 250 different phytosterols, including pure sterols (PSRs) and stanols (PSNs), which are the saturated form of sterols. Common dietary sources of PSRs are plant-based foods that are rich in stigmasterol, b-sitosterol and campesterol, all members of the triterpene family [115]. Phytostanols are found naturally at lower concentrations than sterols and can be produced by the hydrogenation of PSRs. Due to large differences in the consumption and composition of vegetables throughout the world, the dietary intake of plant sterols ranges from 160 mg/dl in Britain to 375 mg/day in Japan; populations following vegetarian diets have been reported to intake more than 600 mg/dl. Typically, the dietary intake of PSN is approximately 50 mg/day. Vegetables with a high percentage in PSs include vegetable oils, nuts, seeds, legumes and whole-grains. Molluscs (oyster 264 mg/100 g), crustaceans (lobster 137 mg/100 g) and egg yolks (95 mg/100 g) are the most important non-vegetable sources of PS.

The precise PSR/PSN mechanism of action is partially understood. PS shave been shown to be effective in reducing the absorption of both dietary and biliary cholesterol from the intestinal tract by displacing cholesterol from micelles, hence limiting the intestinal solubility of cholesterol and decreasing the hydrolysis of cholesterol esters in the small intestine. Cholesterol absorption is regulated by controlling both in flux and efflux. The uptake of cholesterol from micelles into intestinal mucosal cells is mediated by Niemann-Pick C1-Like 1 protein (NPC1L1), which is partially inhibited by PSs [116]. The efflux of cholesterol is determined by adenosine triphosphate-binding cassette transporter proteins (ABCG5 and ABCG8), and the up-regulation of these proteins decreases net absorption by increasing cholesterol efflux. Moreover,

[5] The authors of this section are Merino J and Masana L.

in vitro studies have also shown that ACAT-mediated esterification inside the enterocyte is less efficient for PSs than for cholesterol [117]. The partial inhibition of cholesterol absorption by the above mechanisms produces a state of relative cholesterol deficiency, which is followed by an up-regulation of cholesterol biosynthesis and LDL-receptor activity.

**Phytosterol-enriched functional foods**

The production of PSR- and PSN-fortified food has become popular because of the ability of these compounds to decrease LDL cholesterol concentrations. The first description of the use of phytosterol-enriched foods is from 1977. Lees et al. tested a commercial preparation derived from soybean oil (3 g/d) and observed a significant decrease in LDLc after consumption of this preparation [118]. Nine years later, in 1986, Heinemann et al. reported the first description of the use of plant stanols to lower plasma cholesterol [119]. These investigators showed that dispersed sitostanol in sunflower oil at a dose of 1.5 g/day lowered LDLc by 15% in hypercholesterolaemic adults. Both sterols and stanols are frequently used in esterified forms because this form increases their solubility and allows for their incorporation into lipid-based foods, such as margarines, yoghurts, mayonnaise, milk, salad dressings and snacks bars. Recent studies have investigated the consumption of regularly consumed foods enriched with phytosterols esters, concluding that these products have been shown to be an effective means of reducing total cholesterol and LDLc in adults and children with hyperlipidaemia [120]. Many randomized controlled trials (RCT) have clearly established that LDL cholesterol plasma concentrations are reduced by approximately 10% with PSR/PSN fortified-foods in a dose-dependent manner, reaching a maximum plateau at approximately 1.5–2 g per day. In this review, we focus on the effect of PSRs/PSNs in conjunction with lipid lowering drugs.

***Effect of phytosterols add-on lipid lowering treatment***

**Statins**

Statins (3-hydroxy-3-methyl-glutaryl coenzyme-A reductase inhibitors) are the most effective lipid-lowering medications for the prevention of coronary artery disease. These compound function by blocking the limiting enzyme HMG CoA reductase, leading to an increase in the number of LDL receptors and augmenting LDL plasma clearance. To balance this inhibition in cholesterol synthesis, there is a concomitant increase in intestinal cholesterol absorption. Therefore, PSRs/PSNs have been speculated to play a synergistic role with statins. The first study on the effect of combining PSN supplementation with statins was conducted in 1994 by Vanhanen et al. In a specific cohort of statin treated hypercholesterolaemic subjects (on increasing pravastatin doses from 40 to 80 mg/d), the addition of 1.5 g/day of a sitostanol ester-enriched mayonnaise was associated with an additional 10.8% decrease in LDLc after the follow-up period [121]. More marked reductions were observed in patients with coronary heart disease or familial hypercholesterolaemia, the general population or postmenopausal women who were receiving stable statin lipid-lowering treatment. In these studies, plant stanol esters at a dose of 2–3 g/day lowered LDLc by an additional 16%–20% compared to statins alone, although these studies were open labeled.

The best evidence for the beneficial effect of PSs in patients undergoing statin treatment has come from different double-blind, randomized, placebo-controlled trials, as shown in Table 5. Plant sterol esters were used in 10 studies [122–131] and plant stanol esters in 14 [121–123,125–128,132–138], each administered in doses of 1.5–3 g/day for 4–26 weeks; one study had a long term follow-up of 85 weeks [123]. Reductions in LDLc ranged from 4% to 15%. In these studies, PSR supplementation was associated with an additional reduction in total cholesterol ranging from 5% to 11% and in LDLc ranging from 8% to 17%. Similarly, dietary PSN supplementation in statin treated patients was related to a significant decrease of between 4% and 11% in total cholesterol and 6% and 16% in LDLc. Data from a previous meta-analysis of 8 randomized controlled trials that included more than 300 statin-treated hypercholesterolaemic patients concluded that the addition of PSRs/PSNs at doses of 1.7–6g/day significant lowered both total cholesterol (14 mg/dl) and LDLc (13 mg/dl) compared to statin use alone [120]. No significant impact on either HDLc or triglycerides has been shown. Regarding the question about the differential effect on LDLc by PSNs or PSNs, previous systematic reviews have grouped plant sterols and plant stanols together without regard to potential differences in comparative efficacy [120,139]. Plant sterols have a higher bioavailability than plant stanols [129,134], which may suggest differences in the degree of cholesterol displacement in the intestinal micelles. A meta-analysis of 14 randomized controlled trials evaluating the effect of plant sterols vs. plant stanols at doses of 0.6–2.5 g/day in healthy patients and patients with hypercholesterolaemia showed no significantly different effects between the two on total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels [25]. It appears that the overall results from many randomized controlled studies support that PSRs/PSNs maintain their LDL lowering capacity when administered with statins. No safety concerns were reported in these medium-length studies. Moreover, no major differences were observed between PSRs and PSNs.

**Ezetimibe**

Ezetimibe is the first member of a new pharmacological family for the treatment of hypercholesterolaemia developed since the discovery of statins. The lipid-lowering mechanism of ezetimibe is mediated by cholesterol absorption inhibition. Although the molecular drug target was initially under controversy, today it is generally acknowledged that ezetimibe inhibits cholesterol absorption by binding an extracellular loop of NPC1L1. Ezetimibe

**Table 5** Characteristics of studies in already statin treated patients with the additional effect of phytosterols on serum lipid parameters.

| Author(s), (reference), year, ($n$) | Study design | Population | Follow-up | Statin dosing | PSR/PSN dosing | Changes in LDLc after follow-up | | Concurrent diet |
|---|---|---|---|---|---|---|---|---|
| Hallikainen et al. [138] 2011 ($n = 24$) | Double-blinded parallel | Type 1 Diabetes and controls | 4 weeks | Stable doses of atorvastatin ($n = 4$), rosuvastatin ($n = 2$) and simvastatin ($n = 18$) | Vegetable oil-based test spreads (3 g/d PSN) ($n = 12$) | −3.8%[a] | | Usual diet |
| Kelly et al. [126] 2011 ($n = 30$) | Double blinded parallel | Hypercholesterolaemia | 85 weeks | Stable statin treatment for several years | PSR enriched margarine (2.5 g/d) ($n = 11$) PSN enriched margarine (2.5 g/d) ($n = 8$) | PSR: 9.6%[a] | PSN: −9.7%[a] | Usual diet |
| De Jong et al. [122] 2008 ($n = 41$) | Double-blinded parallel | Hypercholesterolaemia | 16 weeks | Stable doses of atorvastatin, simvastatin or pravastatin | PSR ester-enriched margarine (2.5 g/d) ($n = 15$) PSN ester-enriched margarine (2.5 g/d) ($n = 15$) | PSR: −7.6% | PSN: −12.2%[a] | Usual diet |
| Fuentes et al. [124] 2008 ($n = 30$) | Double-blinded crossover | Familial Hypercholesterolaemia | 4 weeks | Atorvastatin or simvastatin 40 mg/d | PSR ester-enriched margarine (2.5 g/d) ($n = 30$) | −7.1% | | 4 dietary low-fat interventions |
| De Jong et al. [123] 2008 ($n = 54$) | Double-blinded parallel | Hypercholesterolaemia | 85 weeks | Stable doses of atorvastatin, simvastatin, pravastatin or rosuvastatin | PSR ester-enriched margarine (2.5 g/d) ($n = 18$) PSN ester-enriched margarine e (2.5 g/d) ($n = 19$) | PSR: −8.7% | PSN: −13.1%[a] | Usual diet |
| Takeshita et al. [131] 2008 ($n = 44$) | Double-blinded parallel | Hypercholesterolaemia | 12 weeks | Pravastatin 10 mg/d | PSR dissolved in DAG oil (0.4 g/d) ($n = 14$) | −5.1%[a] | | Usual diet |
| Castro-Cabezas et al. [134] 2006 ($n = 20$) | Single-blinded parallel | Hypercholesterolaemia | 6 weeks | Maximal dose of statins (A80 or S80) for at least 6 months before inclusion | PSN enriched margarine (3 g/d) ($n = 11$) | −15.6%[a] | | Intensive dietary intervention |
| Goldberg et al. [136] 2006 ($n = 26$) | Double-blinded parallel | Hypercholesterolaemia | 6 weeks | Stable statin doses | Soy PSN tablets (1.8 g/d) ($n = 13$) | −6.3% | | American Heart Association Healthy diet |
| Hallikainen et al. [125] 2006 ($n = 76$) | Double-blinded crossover | Hypercholesterolaemia | 20 weeks | Stable statin doses | PSN and PSR oil based spreads (2 g/d) ($n = 39$) | PSR: −9.6%[a] | PSN: −6.6%[a] | Usual diet |
| Cater et al. [135] 2005 ($n = 10$) | Double-blinded crossover | Hypercholesterolaemia with CHD | 8 weeks | Stable doses of atorvastatin or simvastatin | PSN ester-enriched margarine (3 g/d) | −14.9%[a] | | Low saturated fat and cholesterol diet |
| Ketomaki et al. [127] 2004 ($n = 3$) | Double-blinded parallel | Family with FH, including homozygous child | 157 days | LDL apheresis once per fortnight | PSN and PSR ester-enriched spread (2 g/d both) ($n = 1$ HoFH) | PSR: −17.4%[a] | PSN: −6.5% | Low-fat diet |
| Amundsen et al. [132] 2004 ($n = 57$) | Open labeled | Familial Hypercholesterolaemia | 26 weeks | Same doses of statins during the whole study period | PSN ester-enriched spread (1.76 g/d) ($n = 20$) | −11.2%[a] | | Usual diet |
| Ketomaki et al. [128] 2004 ($n = 5$) | Double-blinded crossover | Familial hypercholesterolaemia | 4 weeks | Stable statin doses, A80 ($n = 3$) A20 ($n = 1$) L60 ($n = 1$) | PSN and PSR rapeseed oil spread (2 g/d) | PSR: −14.5%[a] | PSN: −16.4%[a] | Usual diet |

**Table 5** (*continued*)

| Author(s), (reference), year, (*n*) | Study design | Population | Follow-up | Statin dosing | PSR/PSN dosing | Changes in LDLc after follow-up | Concurrent diet |
|---|---|---|---|---|---|---|---|
| Gylling et al. [137] 2002 ($n = 11$) | Open labeled crossover | hypercholesterolemic coronary patients | 16 weeks | Stable statin doses (S20). Cholestyramine 8 g/d was added for the last 8 weeks | PSN ester-enriched margarine (2.25 g/d) ($n = 11$) | −13%[a] | Low-fat low-cholesterol diet |
| Simons et al. [130] 2002 ($n = 154$) | Double-blinded parallel | Hypercholesterolaemia | 4 weeks | Cerivastatin 400 μg | PSR ester-enriched margarine (2 g/d) ($n = 37$) | −6.1% | American Heart Association diet step 1 |
| Neil et al. [129] 2001 ($n = 62$) | Double-blinded crossover | Hypercholesterolaemia | 8 weeks | Stable statin doses | PSR fortified fat spread (2.5 g/d) ($n = 31$) | −10%[a] | Usual diet |
| Blair et al. [133] 2000 ($n = 167$) | Double-blinded parallel | Hypercholesterolaemia | 8 weeks | Stable doses of atorvastatin, simvastatin, pravastatin or lovastatin | PSN enriched spread (3 g/d) ($n = 83$) | −10%[a] | Usual diet |
| Vanhanen et al. [121] 1994 ($n = 14$) | Double-blinded parallel | Hypercholesterolaemia | 6 weeks | Increaseing doses of pravastatin from 40 to 80 mg/d | Sitostanol ester-enriched mayonnaise (1.5 g/d) ($n = 7$) | −10.8%[a] | Usual diet |

PSR: phytosterols, PSR phytostanol.
[a] *p*-Value <0.005 compared with the baseline lipid levels.

binding precludes the internalization of NPC1L1, thereby preventing it from chaperoning the transport of cholesterol from the plasma membrane to the endoplasmic reticulum. Therefore, PSRs/PSNs could be considered mechanistic competitors. To address this issue, two important studies were conducted to evaluate if the combination of ezetimibe and phytosterols was more effective than ezetimibe alone in altering cholesterol metabolism [140,141]. Jakulj et al. first assessed this question in a double-blind, placebo-controlled, crossover study in which 40 subjects with mild hypercholesterolaemia received 10 mg/d ezetimibe and either 25 g/d of a spread containing phytosterols (2 g/d) or 25 g/d of a control spread. There were also two more passive study arms in which subjects ate 25 g/d of a spread containing phytosterols (2 g/d) without receiving ezetimibe or the same amount of a control spread without ezetimibe. The authors concluded that the LDLc reducing effect of the combination (25%) did not significantly differ from that the ezetimibe monotherapy (22%) [140]. In contrast, Lin et al. enrolled 21 mildly hypercholesterolaemic subjects in a double-blind, placebo-controlled, triple-crossover study in which individuals received a phytosterol-controlled diet plus (A) a ezetimibe placebo and a phytosterol placebo, (B) 10 mg/d ezetimibe and a phytosterol placebo or (C) 10 mg/d ezetimibe and 2.5 g phytosterols for 3 weeks each. The authors observed that the addition of PSs to ezetimibe significantly enhanced the effects of ezetimibe, including lower intestinal cholesterol absorption, greater faecal excretion and significant decreases in plasma LDLc. The authors discussed that the mechanism of PS actions differs from ezetimibe, because it has been observed that phytosterols may act by substituting cholesterol from intestinal micelles and enterocytes rather than by blocking the molecule mediating cholesterol absorption NPC1L1 [141]. On the other hand, ezetimibe blocks phytosterol absorption, which has been considered a controversial safety issue.

### Fibrates

Patients with mixed dyslipidaemias (increased LDLc and triglycerides and low HDLc) could benefit from a combination of lipid-modifying drugs, such as fibrates. The pharmacological mechanisms of fibrates are mediated by PPARα activation. Several animal and human studies have shown no interactions between PSRs/PSNs and fibrates on LDL cholesterol metabolism. Clinical studies have shown an increase in the hypocholesterolaemic effect of PSRs/PSNs added to fibrates. On the other hand, their effects on lipid profiles are complementary; PSRs/PSNs act on LDL cholesterol, whereas fibrates modulate HDL and triglycerides concentrations, leading to global lipid control [142].

### *Bile acid sequestrant resins*

The primary mechanism by which humans remove excess cholesterol is catabolism into bile acids. Approximately

95% of the bile acids secreted into the intestinal lumen are reabsorbed in the distal digestive tract. Bile acid sequestrant resins (BASRs) induce bile acid precipitation, and these acids are not able to be reabsorbed in the distal ileum and are excreted into the faeces. Not enough data are available to determine the impact of BASRs on PSRs/PSNs. BASRs interact with lipophilic substances that interfere with intestinal phytosterols. On the other hand, dietary PSRs but not PSNs have been suggested to suppress bile acid synthesis, most likely by altering the cholesterol-lowering efficacy of BASRs [142].

### N-3 fatty acids

Fish oils rich in long-chain PUFAs (N-3) can reduce circulating triglycerides and raise HDLc. The combination of both phytosterols and N-3 fatty acids results in a complementary beneficial effect on the lipid profile. Accordingly, in a single-blinded crossover study in which 21 moderately hyperlipaemic subjects were randomized to 4 experimental isoenergetic diets for 4 weeks each, LDLc significantly decreased by 13% after supplementation with fish oil fatty acid esters of plant sterol. A recent study provided hypercholesterolaemic children with a daily dose of an emulsified preparation containing plant sterols esters (1300 mg), fish oil (providing 1000 mg of eicosapentaenoic acid [EPA] plus docosahexaenoic acid [DHA]) and vitamins B12 (50 μg), B6 (2.5 mg), folic acid (800 μg) and coenzyme Q10 (3 mg) for 16 weeks. In these children, a significant decrease in both total cholesterol and LDLc of 15% and 10%, respectively, was observed [143]. Independent of the direct effect on cholesterol metabolism, several mechanisms have been proposed to explain how N-3 PUFAs might beneficially affect risk factors implicated in the pathogenesis of atherosclerosis and thrombotic disease. These mechanisms include improving vascular reactivity, decreasing platelet aggregation, lowering plasma triglycerides, decreasing blood pressure, preventing arrhythmias and reducing inflammation.

### Niacin

A complementary effect of niacin and PSRs/PSNs on lipid profiles has been shown that is similar to the effects described for fibrates. In ApoE knockout mice, niacin has been shown to increase the lipid-lowering effect of PSRs/PSNs and to slow atherosclerosis progression.

### *Clinical indications*

Reducing elevated LDLc is a key public health challenge, and dietary modification is the first step toward improving serum lipid levels. In response to the growing evidence supporting the significant cholesterol lowering effect of phytosterol-enriched foods, the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII) guidelines recommended the inclusion of 2g/day of phytosterols in the diet along with other dietary modifications, such as decreasing the intake of saturated fat (<7% of daily calories), trans-fat (less than 2%) and dietary cholesterol (<200 mg/day) [144]. These recommendations are also endorsed by other scientific organisations, such as the European Atherosclerosis Society (EAS) [144].

PSR/PSN supplementation is indicated in individuals with low cardiovascular risk and high cholesterol for whom drug therapy is not indicated or is optional. However, in high-risk patients for whom drug therapy is mandatory, the indication is not clear. According to our review, the evidence suggests that adding phytosterol enriched foods to the diet of patients on lipid-lowering therapy will result in an incremental LDL decrease of approximately 10%, which is more than the effect of doubling the statin dose [129,130,133,134]. Other groups of patients who might benefit from PSRs/PSNs are those who refuse to take statins and those who stop statin therapy because of adverse effects, which occur in up to 10% of patients.

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## Treatment of hyperlipemic patients at low cardiovascular risk[6]

The 2012 European Guidelines (GL) on cardiovascular (CV) disease prevention in clinical practice stress the role of LDL cholesterol (LDL-c) and the target level to reach in subjects with a different degree of CV risk, that is low, moderate, high and very high risk. The GL also specify that there are some pathological conditions (e.g. familial hyperlipidemia; presence of kidney dysfunction; occlusive peripheral artery disease), which should also be considered as an expression of very high CV risk and therefore need a pharmacological treatment. On the contrary, in subjects with low CV risk there is no strict indication to drug therapy despite an LDL-c level up to 190 mg/dl. The last suggestion is based on the observation that these subjects show a low probability of developing a CV event. Nevertheless, according to the so called Rose paradox, it's well known that the large proportion of CV events occurs in this population, since the individual risk is low but the number of subjects carrying such a risk is very high, thus, multiplying a small risk for a large number of subjects there is a lot of events in the whole population.

However, there are some observations which can account for the suggestions of the 2012 European GL. In particular, the consideration that the decision to treat implies the use of statins, fibrates etc. to the highest recommended dose or, at least, the highest tolerable dose, or to combine two molecules with the objective to reach the target LDL-c plasma concentration. The choice to start a pharmacological treatment represents the conclusion of an accurate evaluation of the expected benefit and the possibility of adverse events. The results of large intervention

[6] The authors of this section are Rozza F and Trimarco B.

studies have clearly demonstrated that statins are able to markedly improve cardiovascular prognosis in patients with high and very high CV risk [145], but it is also well documented that the magnitude of the beneficial effect depends on the basal conditions of the patients. In fact, the slope of the correlation between LDL-c plasma concentrations and cardiovascular events calculated in primary prevention studies is significantly smaller as compared to that recorded in secondary prevention trials, which include patients at higher CV risk [145]. So, the question is: treatment of patients with low LDL-c levels does allow a significant reduction of the incidence of CV events? In the Anglo-Scandinavian Cardiac Outcomes Trial – lipid lowering arm (ASCOT-LLA), patients with an average – or lower than average – plasma cholesterol concentration received 10 mg of atorvastatin/die. Despite the low cholesterol level, treatment was able to reduce fatal and non-fatal MI by 36% [146].

On the other hand, although statin-induced rhabdomyolysis has an incidence of less than 1 per 10,000 patient-years for monotherapy and increases sharply only with the combination of two different statins or a statin combined Wight a fibrate, the discontinuation rate, mainly due to the adverse events (AE) related to the lipid lowering agents reaches the 30% level.

Thus, moving to patients with a low LDL c plasma level and a low probability to develop CV events, the weighed benefit of LDL c reduction is reduced, while the probability of AE remains unchanged. It's not only a theoretical speech, since it has been recently demonstrated that statins increase the risk of developing diabetes [147]. Although it has been recently demonstrated that this effect parallels the cholesterol lowering action of statins, since this risk increases by raising the dose of each statins [148] or moving from a less to a more effective statin [149], this observation has not changed the clinical practice in patients with moderate or high CV risk, since the expected benefit is greater than the possible negative consequences. On the contrary, in patients with a low CV risk a greater probability to develop diabetes should be taken into account and so, to balance this element, it is important to look for something with a comparable beneficial effect and a very low probability to induce AE.

The solution may be represented by nutraceuticals, which are concentrated forms of presumed bioactive substances originally derived from foods but now present in non-food matrix and used to enhance health in dosages exceeding those obtainable in normal food.

The available nutraceuticals are a lot, but the choice should rely on the evidence-based medicine, since the knowledge that a natural substance exerts a beneficial effects when introduced as food does not necessarily implies that the administration at higher dose as a pharmaceutical supplementation will induce the same effect. In fact, the HOPE study has demonstrated that supplementation of Vitamin E, that in foods is able to exert an antioxidant effect, do not modify cardiovascular prognosis [150].

Recently, Affuso and coworkers [151] investigated in a double blind, placebo controlled study performed in hypercholesterolemic patients the effect of a nutraceutical combination (red yeast rice, berberine and polycosanols) on plasma lipids, endothelial function and insulin sensitivity, and showed a significant reduction (vs. controls) in total cholesterol, LDL-c and triglycerides, with no change in HDL-c.

This nutraceutical combination led to an improvement in endothelial function, known to be impaired in patients with dyslipidemia. Moreover, in patients with insulin resistance (baseline HOMA index $> 2.6$) treatment with such nutraceutical combination significantly reduced HOMA, and significantly increased the QUICKI and McAuley indices, thus indicating enhanced insulin sensitivity.

In another study, patients with metabolic syndrome or dyslipidemia not tolerating statins or not requiring the use of statins were treated with the nutraceutical combination tested by Affuso [151]. After a two-week running period with a standard diet, patients were randomized to placebo ($n = 662$) or to the combination of nutraceuticals ($n = 682$) for 8 weeks of treatment. The results showed 20% reduction in total cholesterol plasma concentration, 25% reduction of LDL-c, 7% increase of HDL-c, 20% reduction of plasma triglycerides and a fall in diastolic blood pressure, which in keeping with the observation by Affuso may be ascribed to an improvement of endothelial function and insulin sensitivity. This interpretation is also corroborated by the observation of a significant reduction in waist circumference. At the end of the treatment period, the percentage of patients with metabolic syndrome was significantly reduced (from 70% to 35%) in the study group, but not in the placebo group, thus suggesting a reduction in the risk of developing CV events.

In the era of the evidence-based medicine it is required that the improvement in cardiovascular prognosis induced by a pharmacological treatment is supported by the evidence obtained in large intervention placebo controlled trial that such a therapy induces a reduction of cardiovascular mortality and non-fatal myocardial infarction and stroke. However, in low risk patients it is very difficult to obtain data on hard end-points, since the low probability to develop such events would require the enrollment of a huge number of subjects and a very long follow-up to reach a satisfactory statistical power. On the other hand, in clinical practice the decision to start a pharmacological treatment is based on the calculated cardiovascular risk of the single patient. Thus, in order to assess the effect of nutraceutical combination on cardiovascular prognosis Izzo and coworkers [152] evaluated the change in Framingham risk score induced by nutraceutical treatment. For this purpose, they excluded patients with diabetes mellitus since the presence of this pathological condition would have precluded the possibility to reduce the global cardiovascular risk score. Looking at the baseline Framingham Risk Score *versus* the final Framingham Risk Score in single patients, it was clear that those treated with nutraceuticals moved from higher baseline values to lower values at the end of the follow-up, while in the placebo group the Framingham risk score remained unchanged

**Table 6** Change in lipid profile and CV risk in patients treated with atorvastatin or nutraceuticals.

| | Atorvastatin (ASCOTT-LLA) | NUT (effects of NUT on calculated FRS) |
|---|---|---|
| TC (mg/dl) | −50 | −48 |
| Ldl-c (mg/dl) | −45 | −43 |
| HDL-c (mg/dl) | −1 | +5 |
| TG (mg/dl) | −30 | −43 |
| CV risk (%) | −36 | −35 |

TC: total cholesterol; LDL-c: LDL cholesterol; HDL-c: HDL cholesterol; TG: triglycerides; NUT: nutraceuticals; CV risk: Reduction of cumulative incidence of primary end point (fatal or non-fatal MI) in patients treated with Atorvastatin vs placebo and Reduction of calculated Framingham Risk Score in patients treated with NUTs vs placebo.

during treatment. In particular, in the study group there was a reduction of the Framingham risk score of 35% which reached the statistically significance level.

Putting together the changes in lipid parameters obtained in the ASCOT-LLA and in the Izzo trial, it's possible to observe that the changes in total and LDL-c were quite similar, while an increase in HDL-c was detected only during treatment with the combination of nutraceuticals and was associated with a reduction in plasma triglyceride concentration greater than that observed in the atorvastatin-treated group (Table 6).

It is interesting to note that the reduction in the Hazard Ratio (i.e. the probability of developing CV events) obtained in the ASCOT-LLA study (primary end point: fatal or non-fatal MI) was 36%, which is very similar to the reduction in Framingham risk score observed in the Izzo study (35%) (Table 6).

### *Conclusion*

International Guide Lines for cardiovascular prevention have been traditionally based on the results of randomized, placebo controlled large intervention trial, however more recently there is a general consensus that the results of registers and large studies performed in the real word should be taken into consideration for the appraisal of guide lines. This concept seems to fit with the use of nutraceuticals in the management of dyslipidemia in subjects at low CV risk which may allow the reduction of cardiovascular events in the general population. Furthermore, the studies performed following the suggestions of International GL have documented a 30% risk reduction induced by statin treatment, thus suggesting that the risk of CV events still remains high and defines the so called "forgotten majority". A possible explanation for this observation is that following the GL strategy, the treatment of dyslipidemia starts to late when the vascular damage is already established. Therefore, the availability of a nutraceutical treatment with a documented efficacy and tolerability which allows an early reduction of plasma LDL c levels may possibly improve not only the strategies of population prevention but also induce a further improvement of the individual cardiovascular prognosis.

## Functional foods and cardio-metabolic diseases: the role of dietary fiber[7]

### *Introduction*

Cardio-metabolic diseases are one of the major causes of mortality worldwide. Hence, huge efforts have been made to find easy strategies for the prevention of these diseases and the management of disease-related risk factors.

Diet and its components, having pleiotropic effects, represent one of the best approach to reduce cardio-metabolic risk. In this light, dietary fiber (DF) has been extensively studied and, so far, available evidence supports the health benefits of its consumption.

As a matter of fact, several prospective studies have highlighted the inverse association between DF intake and cardiovascular risk. A remarkable pooled analyses of ten cohort studies ($n$ = 2,506,581 from USA and Europe) [153] showed that consumption of DF was inversely associated with risk of coronary heart disease. In particular, a 14% reduction of the risk was observed for every 10 g/day increase in DF intake, especially those from cereal and fruits. Similar trends have been found more recently. First of all, in the NIH-AARP cohort [154], the highest quintile DF intake (28 g/day) was associated with a lower risk of death from cardiovascular disease compared to the lowest quintile (12 g/day). Moreover, the National Health and Nutrition Examination Survey [155] showed the association between high intake of DF and low prevalence of cardio-metabolic risk factors (metabolic syndrome, inflammation and obesity).

Hence, evidence-based guidelines recommend a daily fiber intake from 25 to 30 g, preferring fiber-rich foods such as fruits, vegetables, whole grains, legumes and nuts [156–158]. However, worldwide the mean fiber intake is still lower than the recommended daily dose, even in the Mediterranean countries [155,159–161]; in this light, functional foods, as additional sources of fibers, may contribute to health promotion in the general population.

Functional foods are by definition similar in appearance to traditional products but have been modified to bring beneficial effects beyond nutrients supplying [162]. In addition, in 2002 the Institute of Medicine (IOM) introduced the concept of functional fiber as "isolated, non-digestible carbohydrates that have beneficial physiological effects in humans" [156]; it can be added to processed foods to improve health benefits of goods. Of course, functional foods or fiber need a scientifically based effect to get a claim.

So far, evidence available from clinical trials focusing on the effects of functional foods or fibers is still insufficient. This conclusion may be due, in part, to two major factors: a) short duration of the trials does not allow relevant

[7] The authors of this section are Riccardi G, Rivellese AA and Vetrani C.

changes in clinical outcomes; b) different types and sources of fiber have not the same physiologic roles.

Therefore, until now, only few types of fiber have achieved a "health claim" by the Food and Drugs Administration (FDA) and the European Food Safety Agency (EFSA).

In addition, very few studies have focused on the evaluation of mechanisms of fibers as primary goal.

Thus, this viewpoint aims to 1) briefly summarize the effects of fiber, from fiber-rich foods or dietary supplements, on major cardio-metabolic risk factors (overweight/obesity, glucose tolerance, blood lipids, inflammation); 2) highlight the evidence on mechanisms of action of fiber; 3) identify possible strategies to increase fiber consumption.

### *Effects of dietary fiber on cardio-metabolic risk factors*

DF consumption has been associated with a better body weight control, even in the long term [163,164]. A landmark review by Wanders and colleagues [165] have summarized the evidence on the effect of different type of DF on body weight, appetite and energy intake. The main conclusions of the review were that more viscous fibers, especially β-glucans, influence appetite and acute energy intake, whereas no clear association between type of fiber and body weight control in the long-term was observed.

Fiber-rich foods are often characterized by a lower glycemic index (GI) compared with other foods. This characteristic avoids acute increase of blood glucose levels in the postprandial period, inducing beneficial effects on glucose tolerance in both diabetic and non-diabetic people [166].

Among fibers, β-glucan (4 g) from whole or processed oat and barley seems to have the greatest effect on plasma glucose [167]; however, there is evidence that other sources of fiber may affect glycemic homeostasis. In particular, a high intake of fruits, vegetables, legumes and whole grain has shown to reduce significantly postprandial glucose and insulin and glycemic variability [168].

Moreover, a significant reduction of postprandial plasma insulin levels was observed after 12-week consumption of whole grain-based products [169].

In addition, the so called "second meal effect" of fiber, especially from whole grains and legumes, has been demonstrated. As a matter of fact, there is evidence that having fiber-rich foods at breakfast brings down plasma glucose levels during the rest of the day, whereas their intake at dinner leads to lower plasma glucose levels before breakfast the following morning [170].

Furthermore, the lowering effect on LDL-cholesterol of DF is well documented; a meta-analyses of randomized controlled trials clearly showed that legumes consumption can decrease total and LDL-cholesterol (−11.8 mg/dl and −8.0 mg/dl, respectively) [171].

Interestingly, this effect has been obtained also with other fiber sources (whole grains, fruits and vegetables) in healthy subjects [172] as well in diabetic patients [168,173].

Price et al. [174] showed a significant reduction of LDL-cholesterol levels after the consumption of aleurone-enriched products (bread and breakfast cereal; aleurone 27 g/day) suggesting a potential role of functional foods in the modulation of LDL-cholesterol.

The effect of fiber on triglycerides levels is still not convincing even if an improvement of postprandial levels was observed after a test meal rich in oat or wheat fiber. Moreover, a significant reduction of triglycerides in chylomicron was detected after wheat-meal [175].

Finally, inflammation is a well-established risk factor for cardiovascular disease (CVD). Overall the evidence has shown that DF intake significantly influences C-reactive protein (CRP), a marker of inflammation [176].

Moreover, the source of fiber may have an important role in influencing inflammation. As a matter of fact, the consumption of whole wheat-fiber products had no effect on inflammation markers [169], whereas de Mello et al. [177] have shown that whole rye bread intake leads to a significant reduction in CRP levels.

Recently, an improvement of inflammation markers was observed after the 4 weeks-supplementation of aleurone-enriched goods (bread and breakfast cereals; aleurone 27 g/day) [178].

The main mechanisms behind these benefits are shown in Fig. 5. DF affects gastric emptying, influencing absorption rate of dietary carbohydrates and lipids, gut hormones secretion and satiety feelings. Besides it reach the colon where they are fermented by gut microbiota with the consequent production short chain fatty acids (acetate, propionate and butyrate) that improve glucose homeostasis and lipid metabolism and influence microbiota profile [179].

### *Functional foods enriched with dietary fiber*

Although the health benefits of DF are well established, in western countries DF consumption does not fit with recommendations, as reported before [155,159–161].

This lack of compliance may be due to several reasons. As a matter of fact fiber-rich food are less appealing than the refined ones, especially for taste and flavor [180]; in

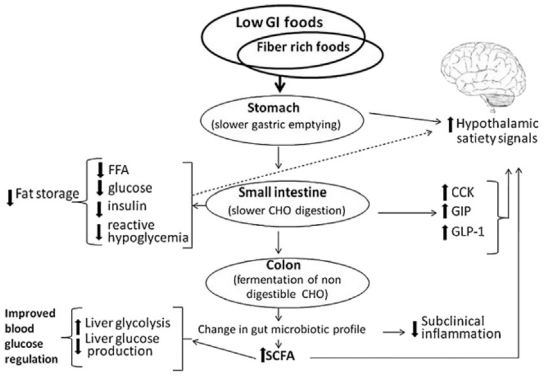


**Figure 5** Possible mechanism of action of dietary fiber. (Source: Rivellese et al. [179]). CCK: cholecystokinin; CHO: carbohydrates; FFA: free fatty acids; GI: glycemic index; GIP: gastric inhibitory polypeptide; GLP-1: glucagon-like peptide 1; SCFA: short chain fatty acids.

addition, many foods which are good sources of fiber require long cooking time, or may induce intestinal discomfort. Moreover, socio-cultural habits have changed over the years: many people are used to have their meals in restaurants and cafeterias where the choice of fiber rich foods is often rather problematic [181]. Ready-to-eat functional foods enriched with fiber can circumvent these problems, thus improving the overall dietary habits of western populations. Against this background, functional foods resembling usual bakery products for their taste and flavor, have been additioned with fibers extracted from legumes, cereals or other vegetable products. These products have been tested for their health benefits in vivo trough clinical trials and have been shown to be effective on glucose and lipid metabolism.

Within this context, we have shown that bakery products enriched with β-glucan and other bioactive compounds (ω3 fatty acids, folates and tocopherols) reduce fasting plasma triglycerides, postprandial chylomicrons, and increase satiety feeling, thus possibly preventing overfeeding and contributing to reduce the risk of overweight. Besides, in our study they decreased elevated plasma homocysteine levels which are indicated as a marker of an increased cardiovascular risk [182].

Thence, the introduction of functional foods enriched in DF – alone or in combination with other bioactive compounds – in the habitual diet may represent an useful strategy to improve cardio-metabolic state in high-risk subjects but also to prevent cardio-metabolic diseases; promoting the use of both natural foods and functional foods might facilitate compliance to a healthy diet with a significantly higher fiber intake than it is usually consumed in western populations.

## The Mediterranean diet: an historical perspective and where we are now[8]

### *Nutrigenomics, nutrigenetics and functional foods*

The genetic revolution that we have experienced in the past several decades has created new and exciting areas of investigation related to the interaction between foods, nutrients and our genetic make up to determine our susceptibility to disease and our ability to prevent or treat common conditions (i.e. cardiovascular disease, cancer, diabetes, obesity, cognitive decline and dementia, inflammatory bowel disease, etc. etc.) [183–185]. Environment and foods in particular have been shown, through experimental data, that they may have the ability to regulate gene expression and genetic structure [186]. The ultimate goals of these disciplines is to develop the ability to provide nutritional counseling to individuals based on a detailed study of their genetic profile and the specific effects that certain foods, food constituents and nutrients may have on the structure and function of genes and their ability to prevent or cause specific conditions [187].

While promising and exciting, at the moment however these new disciples are in their infancy and we have not have yet definite scientific evidence that the effects observed in experimental and small clinical studies have real preventive and clinical implications.

While we wait for this evidence we have to rely on the existing knowledge and provide counsel to individual based on the dietary and food patterns that have been identified and proven to have healthful and beneficial effects on the life of both individuals and populations. One of these patterns that has received substantial amount of attention is the so called “Mediterranean diet”

### *Mediterranean diet*

The term Mediterranean used together with diet, food or cuisine has become a synonymous of a healthful and tasteful pattern of eating. The press is full of articles, book, monographs that exalt the Mediterranean diet as a way to “enjoy food” while ensuring a long and healthy life.

This interest and fascination with the “Mediterranean Diet” is the result of more than fifty years of medical and nutritional studies conducted by researchers around the world. It is commonly accepted that the interest in the food of the southern European countries that are bordering the Mediterranean sea, started in the early fifties with the work by one of the famous nutritionists of the twentieth century, Dr. Ancel Keys. He launched and organized the Seven Countries Study, an epidemiological investigation on the role of diet and other cardiovascular risk factors in the etiology of cardiovascular disease and death [188–190]. The study was initiated based on his observations that in countries like Greece, Italy and Japan the cases of myocardial infraction (ate least those in the hospitals) were much lower than those he had observed in Minnesota and those in Finland. Another important figure in the design and launching of the seven countries study was Dr. Paul Dudley White, by many considered the father of modern cardiology.

The study, composed of sixteen cohorts in seven countries (Finland, Greece, Italy Japan, the Netherlands, USA and former Yugoslavia), was one of the first examples of a successful international collaboration in medical research and, while with important study design limitations (especially by today’s standards), represented a formidable task for the early fifties and has represented, over the years, an important source of information on the effect of diet on health and in cardiovascular and chronic disease epidemiology. Moreover has represented the training ground for generations of health scientists in multiple disciplines.

The Seven countries study was able to show a significant and relevant association between diet and Coronary Heart Disease (CHD) (incidence and mortality). In particular a significant and direct relationship between % of calories from saturated fat and CHD and a significant and inverse relationship between the consumption of mono-unsaturated fats and CHD. The data from the 15 year mortality follow-up were particularly significant in

[8] The authors of this section are Trevisan M, Misciagna G, Krogh V, Panico S, Della Valle E, and Farinaro E.

showing an inverse association between Coronary Deaths and the ratio of the dietary Monounsaturated/Saturated fats [188–190].

While olive oil has been considered the main component of the MD, many constituents of this dietary pattern have been identified to have potential beneficial effects on health, these include: wine, garlic, fish, vegetables, legumes, almonds and other nuts [191,192].

The data of 15-year follow-up of the Seven Countries Study have been followed by numerous scientific articles showing relevant inverse relationships between the Mediterranean Diet, and/or its constituents, and either CHD or its risk factors [193–196].

More recent investigations have been able to find protective effects of the MD or its constituents in other chronic disease like stroke, colorectal cancer, diabetes, dementia, wheeze and atopy in children, and all-cause mortality. The bulk of the observations to date come from observational studies, therefore leaving open the doubt regarding a cause-effect relationship; however a recent randomized population based clinical trial were able to show a significant effect of the MD (either olive oil or mixed nuts) in the prevention of Cardiovascular Disease in individual at high risk for this disease [197].

Based on these findings, nutritionist at the Harvard School of Public health developed the Mediterranean Diet Pyramid [198], an attempt to define the component of a healthy diet based on the food choices of a traditional MD. This diet is characterized by large amounts of fruit, vegetables, and vegetarian proteins, moderate amounts of whole grains, small amounts of red meat, and regular use of fish, olive oil, and nuts [199].

This pyramid was developed in contrast to the USDA Food Guide Pyramid that emphasized low fat foods.

The scientific and public interest in the “Mediterranean Diet” has been recently heightened by the UNESCO through the inclusion of the Mediterranean Diet among its list of “Intangible Cultural Heritages”.

The clinical and epidemiological investigations on the association between MD and health have been accompanied by a vast number of basic science and clinical investigation exploring the detailed mechanisms that could link the consumption of a Mediterranean type of diet with beneficial health outcomes. This detailed studies, while useful in helping us understand the true mechanisms underlying the beneficial effects of MD, have focused the attention often on a minimalistic and reductionist approach to the role of MD on health, focused on the foods per se and the nutrients and lost track of the fact that the MD, like all other dietary patterns, is not just the compilation of the foods and nutrients ingested but is strictly linked to social factors and norms and the forces that shaped the dietary pattern over the centuries i.e. the territory, the life style habits, economic conditions, climate, etc.

In order to address this issue it may be of value to analyze the status of the MD and the current health status in some of the areas that have historically been characterized by an MD type diet.

The Mediterranean basin is the region of the world composed of the lands around the Mediterranean Sea. The basin covers portions of three continents, Europe, Asia and Africa, and the lands surrounding the water share a similar weather pattern “Mediterranean climate” with mild, rainy winters and hot, dry summer. The vegetation is characterized by forests composed generally of broadleaf trees and coniferous and scrublands. The olive tree is very prevalent in many areas of the Mediterranean basin and has come to represent this region of the world.

Many of the countries surrounding the Mediterranean Sea experience big variations in climate and geography within their boundaries, with the lands surrounding the sea characterized by the typical Mediterranean climate and other portions, far from the sea, experiencing very different weather pattern. Italy and France are good example of these within country variations with dietary pattern being strongly influenced by the climate i.e. southern Italy is characterized by a typical MD pattern while the northern part of the country is characterized more by a continental dietary pattern, a pattern where traditionally olive oil is substituted by butter, fish is substituted by meat, and the consumption of vegetables is much reduced.

Italy therefore represents an ideal place where the variation in dietary pattern can be exploited for scientific purposes.

The southern part of Italy was one of the regions that Dr. Keys visited as part of his scientific travels and where he noticed the paucity of people suffering from heart attacks compared to his Minnesota. The rural diet of the small towns of southern Italy (i.e. Nicotera) inspired him and became one of the launching steps of the seven countries study.

Unfortunately as we embark in analysis of the current situation of southern Italy we find disappointing and disheartening news. The region that once was characterized by low CHD mortality and was one of the centerpieces of the seven countries study is experiencing now the highest mortality for cardiovascular Disease and Diabetes in the country [200]. These disturbing facts are made even more frightening by the data showing that southern Italy is the part of Italy with the highest prevalence of overweight and obesity [201], pointing out to a future potential worsening of the current unhealthy trends. The progressive improvement in socio-economic conditions that has taken place in industrialized countries in the second half of the 20th century has produced substantial life style changes. This change has brought about the mechanization of most working activities and transportation means, a remarkable decrease in physical activity – and therefore, in energy expenditure, together with an increase in food availability; as a result eating habits are now characterized by excess and inadequacy. The main change is represented by a massive exposure to energy dense diets, rich in animal fat, cholesterol, refined sugars, salt and alcohol, and a low nutrient/calorie ratio. These are the features of modern lifestyles that have led to overweight and obesity epidemics and, consequently, to an increase in the incidence

of diabetes, hyperlipidemia and arterial hypertension. In addition, the number of women and youngsters who smoke has gone up, and this has further increased cardiovascular disease risk.

Clearly part of the observed negative trends is the result of a national trend in lifestyle change in particular in food consumption that is moving away from the traditional MD, with the ratio of calories intake/calories expended becoming more and more unhealthy do the increased sedentary life habits, the advent of fast foods, etc. This phenomenon has affected both urban and rural areas of southern Italy; however important role in this worsening of the health profile of southern Italian, especially those living in the urban areas, is played by the living conditions (traffic, air pollution, violence etc.) that is plaguing the urban areas and that cannot be overcome by ingestion of few healthy foods.

Another important aspect of the deterioration of the diet is the nature of the foods consumed that is the result of globalization and the industrial trends in the food industry.

One of the main reasons why the people leaving in the lands surrounding the Mediterranean basin consumed a healthy diet was the climatic conditions of the lands they inhabited and the effect of these conditions on the agricultural productions. Traditionally vegetable were plentiful and available from consumption within hours from the picking; today the vegetables and fruits may travel thousands of miles, fruits are artificially ripened and months can go by before consumption, canned and frozen foods have become more popular.

Grains in the traditional MD were whole grain and non-reconstituted, today they have been replaced by refined and reconstituted grains.

Cheeses and meats were used in moderation and they were coming from animals raised through grazing and therefore the meat was characterized by a lower content of fats [202].

Similar negative trends of food and lifestyle habits, social circumstances and health indicators have been shown in other areas of the Mediterranean basin [203]. We are therefore witnesses to a paradox where, while the world is praising and attempting to adopt the Mediterranean lifestyle, the region that was characterized by the MD are losing it with the expected significant negative health consequences.

What's happening should help us understand that our effort to improve the health of individuals and populations should not and cannot focus on individual food items choices but needs to consider the food we eat in the social context.

The relevance of the social context is well described by the UNESCO document describing the rationale for the inclusion of the MD in its list of the world "Intangible Cultural Heritages".

> … "The Mediterranean diet constitutes a set of skills, knowledge, practices and traditions ranging from the landscape to the table, including the crops, harvesting, fishing, conservation, processing, preparation and, particularly, consumption of food. The Mediterranean diet is characterized by a nutritional model that has remained constant over time and space, consisting mainly of olive oil, cereals, fresh or dried fruit and vegetables, a moderate amount of fish, dairy and meat, and many condiments and spices, all accompanied by wine or infusions, always respecting beliefs of each community. However, the Mediterranean diet (from the Greek diaita or way of life) encompasses more than just food. It promotes social interaction, since communal meals are the cornerstone of social customs and festive events. It has given rise to a considerable body of knowledge, songs, maxims, tales and legends. The system is rooted in respect for the territory and biodiversity, and ensures the conservation and development of traditional activities and crafts linked to fishing and farming in the Mediterranean communities".....

## Conclusive remarks[9]

Mounting evidence supports the hypothesis that functional foods containing physiologically-active components may enhance health. This field, however, is at its very beginning, additional research is necessary to substantiate the potential health benefit of those foods for which the diet-health relationships are not scientifically validated to a sufficient extent.

Diet exerts a strong effect on diseases known as "single-gene autosomal recessive disorders", examples of which are phenylketonuria, galactosemia, and fructose intolerance. These conditions can be managed by personalized nutrition strategies.

More than 6000 human monogenic disorders have been identified, including over 100 protein-based metabolic disorders. Some are rare and complex dietary diseases, namely, fatty-acid oxidation disorders, organic acid metabolism disorders, urea cycle defects and glycogen storage disease. Patients may reduce their intake of the dietary substrates or metabolites that accumulate in these conditions and nutrigenomics will improve prevention and treatment by identifying specific mutations or haplotype combinations that modulate the dietary response in affected patients. In multifactorial pathologies like cardiovascular disease, obesity, type 2 diabetes mellitus, cancer etc., nutrigenomic studies have shown that most of them are amenable to dietary intervention that may modulate their onset and progression.

In the last ten years, there has been remarkable progress in the study of gene–environment interaction, which has led to a new area of knowledge: nutrigenetics and nutrigenomics. This field is now available to patients to help them to improve their health.

*Nutrigenetics* aims at identifying the genetic susceptibility to the effect of nutrients based on the architecture of the genome of each individual.

*Nutrigenomics* studies the effects of food and food constituents on gene expression.

[9] The authors of this section are: Farinaro E, Assmann G.

Lastly, nutritional genomics holds great promise for disease identification and prediction as well as for disease prevention and treatment.

Use of dietary supplements has increased markedly in recent years, across several countries, because of the perception that antioxidant vitamins and minerals may reduce the risk of cardiovascular disease (CVD), cancer and other chronic diseases. However, there is inconclusive trial evidence for a role of dietary supplements in chronic disease prevention, at least among healthy individuals in the general population. Nevertheless, from a public health perspective, it is extremely important to understand the health effects of a nutrient, such as selenium-frequently supplemented; however, at present the widespread use of dietary supplements such as selenium for cardio-metabolic disease prevention in the general population is not justified and should not be encouraged.

There is evidence from longitudinal cohort studies that certain classes of food and dietary patterns are beneficial in primary prevention. The results of these studies have led to the identification of putative functional foods. The mechanisms by which these foods exert effects are complex, but are probably related to the macro- and micronutrient content of the food, and to the presence of other constituents of the diet. The benefits observed may depend on the baseline risk factors, and state of existing diseases, may be dose dependent, and may be affected by the preparation of the food. The benefits of functional food have seldom been reproduced by providing isolated components of foods as supplements. It appears essential that before health claims are made for particular foods, in vivo randomized, double-blind, placebo-controlled trials of clinical end-points are necessary to establish clinical efficacy; these studies can demonstrate efficient absorption and beneficial effects on the cardiovascular risk profile.

Prospective cohort studies have shown that eating fish once a week compared to eating fish less often is associated with a significantly lower risk of fatal CHD. Based on these results it should be useful recommend to both individuals without and with a history of cardiovascular disease to eat fatty fish at least once or lean fish twice a week. The advantage of this recommendation on the use of fish oil supplements is that fish, in particularly fatty fish, is a rich source of the omega-3 fatty acids, as well as minerals such as potassium and calcium, and vitamins, such as vitamin D and vitamin B12. However, fish is not a *panacea* for CHD prevention. The largest risk reduction will be obtained if the recommended fish consumption is a component of a nutritionally adequate diet.

Diet and its components, having pleiotropic effects, represent one of the best approach to reduce cardio-metabolic risk. In this light, dietary fiber (DF) has been extensively studied and, so far, available evidence supports the health benefits of its consumption.

In fact, several prospective studies have highlighted the inverse association between DF intake and cardiovascular risk. A remarkable pooled analysis of ten cohort studies has shown that consumption of DF was inversely associated with risk of coronary heart disease. Thence, the introduction of functional foods enriched in DF – alone or in combination with other bioactive compounds – in the habitual diet may represent an useful strategy to improve the cardio-metabolic state in high-risk people and thus prevent cardio-metabolic diseases; promoting the use of both natural and functional foods might facilitate adherence to a healthy diet with a significantly higher fiber intake compared with the common nutritional habits of western populations.

Since there is need for research work aimed at devising personalized diet, it seems more than reasonable the latter be modeled on Mediterranean diet, given the large body of evidence of its healthful effects.

A wealth of recent international research studies has provided clear evidence of the crucial role played by dietary habits for cardiovascular prevention. There is strong evidence in favor of the protective role of a balanced diet, based on fruit and vegetables and low in animal fat, in reducing important risk factors for cardiovascular disease, such as high cholesterol levels.

The Mediterranean diet – which captured the attention of the scientific community involved in nutrition and preventive cardiology – is a nutritional model whose origins go back to the traditional diet adopted in European countries bordering the Mediterranean sea, namely central and southern Italy, Greece and Spain; this diet has become quite widespread, especially in the last decades in countries with highly developed economies, such as the US. These models all share a large intake of bread, fruit, cereals, olive oil, and fish; the populations living in the Mediterranean regions have a lower incidence of cardiovascular diseases than the North American ones, whose diet is characterized by high intake of animal fat. A possible explanation is that most of the fat used in Mediterranean cuisines derives from olive oil, which has been proven to reduce serum cholesterol levels.

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