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REVIEW

Nutrients: the environmental regulation of cardiovascular gene expression

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Abstract The complexity of nutrient–gene interactions has led to the development of a new branch in the nutrition sciences, the nutrigenomics. The individual susceptibility to nutrients based on environment → genotype → phenotype interplay makes this new research field extremely promising although complex. In this review, we highlight and examine recent findings and the most relevant hypotheses on the role of the diet in the onset and progression of cardiovascular diseases. The effect of unbalanced diets on the cardiovascular system is considered one of the most important risk factors both for ischemic and degenerative myocardial pathologies. The concept that nutrigenomics could help in improving public and personal health is becoming tangible indicating future directions for basic and applied research in the pathophysiology of cardiovascular disease.

Keywords Nutrigenomics · Diet · Cardiovascular disease · Polyunsaturated fatty acids

During the last two centuries, much progress have been achieved in understanding how food is metabolized. Carbohydrates, proteins and fats are oxidized by the body, and related energy values can be calculated. Since the early twentieth century, considerable research on energy exchange, nature of food components [36] and how nutrients influence the right balance between health and disease [27] has been carried out. Once the understanding of macronutrients was clarified, nutrition scientists turned their attention to the elucidation of the role of micronutrients in particular minerals and vitamins [23, 32, 33]. During the last half of the twentieth century, most work focused on the clarification of the functions of essential nutrients and the definition of the role of micronutrients as enzyme and hormone cofactors, and their subsequent roles in metabolic pathways [2]. Also, the relevance of carbohydrates and fats in different diseases, such as diabetes and atherosclerosis, was discovered, and their actual and potential mechanisms detailed [24, 51, 64]. However, the mechanism by which nutrients influence health and disease status remained unclear. For example, how can some individuals consume high fat diets and yet show no evidence of atherosclerotic disease? Genetic differences certainly were suspected, but the elucidation of cellular, molecular and ultimately genetic mechanisms in both healthy and unhealthy individuals proved to be a challenge.

Development of new tools enabling exploration of the cause-effect phenomena at the molecular level stimulated scientists to develop hypotheses and conduct experiments to lay the foundation for a deeper level of understanding of gene-diet interactions. Today, an emerging field of nutritional research focuses on identifying the molecular interactions between nutritional bioactive components and processes through which genome-encoded proteins are expressed. Discoveries in genomics offered unpredictable possibilities for more dynamic scientific investigations based on understanding the effects of nutrients in processes at molecular-level as well as the variable effects that nutrients and non-nutritive dietary components could have on each individual. The analysis of gene–nutrient interactions rapidly became a focal point of applicative research, since several types of environmental stimuli are able to

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modify genomes and diet is by far the most important of them. Furthermore, the gastrointestinal system is an interface between the external environment and the body and functions to extract nutrients from food as well as handle the non-nutrient components in foods. Investigation of how genes and gene products metabolize nutritional factors and, conversely, how dietary compounds regulate gene expression by determining phenotype modification has been defined as "nutrigenomics".

The tenets of nutrigenomics are essentially based on the concepts that: (1) diet can be an important risk factor for a wide number of diseases; (2) dietary compounds can directly or indirectly interact with the genome by altering gene expression; (3) the individual genetic pattern can influence the balance between physiological and pathological condition determined by diet; (4) several diet-regulated genes can play a fundamental role in the incidence and progression of many chronic diseases; (5) a personalized diet on the basis of nutritional status and genotype can be very helpful in preventing and curing chronic diseases. The eventual implementation of these concepts in every-day clinical practice promises to revolutionize the preventive and therapeutic approach to many degenerative diseases thereby reducing the need for conventional pharmaceutical therapy. At present, this highly innovative methodology is in its embryonic phase and needs extensive investigation and unquestionable confirmation by experimental and human studies before entering routine clinical use. Nevertheless, the body of knowledge about nutrient-gene interaction is rapidly increasing in different medical areas (cancer, metabolic diseases, cardiovascular diseases (CVD), etc.). Among others, great attention is paid to the potential effects of differently formulated diets on genes involved in the pathogenesis of CVD, the major cause of mortality and morbidity worldwide. An individual's likelihood of CVD is determined by his or her genetic profile, as well as on the individual's age, gender, and lifestyle. Therefore, the identification of genes potentially activated by specific dietary components is of paramount relevance in establishing efficient preventive strategies for patients at risk of CVD. Thus moderating environmental factors which we are exposed to over a lifetime, such as diet, potentially might have the greatest impact on CVD risk. Ordovas [42, 43] has identified so far several polymorphic loci in genes known to influence cardiovascular health. He estimated that hundreds of genes maybe ultimately introduced into a risk-analysis database. The author has also proposed four main components under genetic control that contribute to coronary artery disease risk: high blood lipids, impaired glucose tolerance and diabetes, high blood pressure and abdominal obesity [44] (Fig. 1).

In the last few decades, much attention has been focused on plasma lipoprotein composition as one of the most

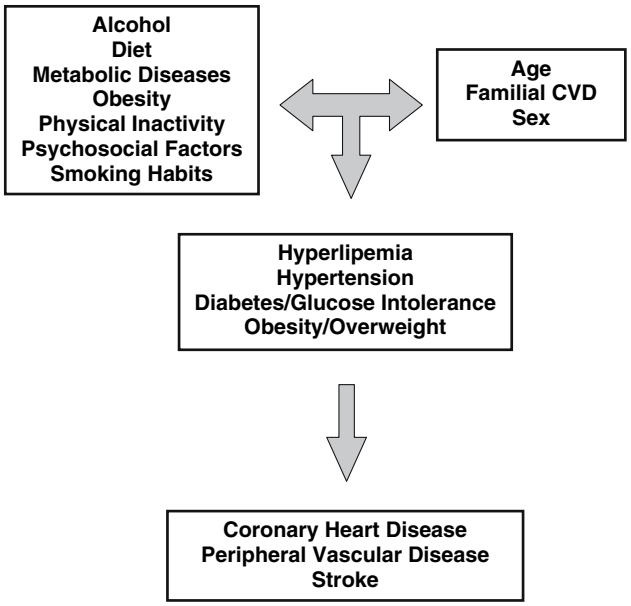

Fig. 1 Risk factors for cardiovascular diseases (after Ordovas [44])

important risk factors for CVD. Genetic variability in humans for all the known lipid-related genes and some variants associated with an abnormal lipid metabolism and plasma lipoprotein profiles have been extensively studied [7, 52]. Evidence suggests that variation in the genes for apolipoprotein (apo) A-I, apo A-IV, apo B, and apo E contributes to the heterogeneity in the lipid response to dietary intervention. However, the effects of genetic variation are not consistent and are sometimes conflicting, making recommendation of the use of genetic profiling to determine genetic responders to dietary interventions and thus tailoring therapeutic diets premature [37]. Dietary effects are not only confined to blood components. Antioxidant nutrients and related bioactive compounds common in fruits and vegetables as well as in high-fibre diets protect against environmental toxic insults to the vascular endothelium by down-regulating signalling pathways involved in inflammatory responses and atherosclerosis [21, 28]. Epidemiologic studies suggested that a high polyphenol intake from fruits and vegetables is associated with decreased risk for CVD by improving endothelial function and inhibiting platelet aggregation [61]. However, the biological mechanisms through which fibres and/or flavonoids influence the cardiovascular system are still to be fully elucidated.

Observational studies on Greenlandic population, in which the prevalence of cardiovascular pathologies were very low, supported subsequent studies which investigated whether marine ω-3 polyunsaturated fatty acids (PUFAs), such as EPA and DHA, could exert beneficial effects on the cardiovascular system [6, 29, 41]. This positive effect was hypothesized since Greenlandic typical diet is essentially

composed by fish containing high quantity of ω-3 PUFAs [5]. Subsequently, clinical trials and in vivo and in vitro experimental studies have demonstrated that ω-3 PUFAs protect against several cardiovascular disorders as well as myocardial infarction, arrhythmias, hypertension and atherosclerosis [3, 14, 54, 65]. Fundamental clinical trials, such as the GISSI prevention study (Italian Group for the Survival Study in the Infarction) [16] and the Diet And Reinfarction Trial (DART) [8], performed on patients with a history of ischemic stroke, have shown a positive direct relationship between ω-3 PUFAs consumption and a significant reduction, more than 30%, in the reinfarction risk. In order to identify basic mechanisms through which ω-3 PUFAs counteract CVD, several studies have been performed demonstrating that this class of lipids determine multiple effects on vascular structure and function. In vivo experimental studies showed a moderate blood pressure decrease in hypertensive rats [13] and in humans treated with low doses (4 g/day) of ω-3 PUFAs (particularly DHA), but not with high doses [1, 39]. Furthermore, EPA and DHA display endothelium-independent and endothelium-dependent vasorelaxing effects. In the latter case, it has been observed that ω-3 PUFAs suppress the synthesis of endothelium-derived contraction factors (EDCF) and increase the production of endothelium relaxing factors, such as nitric oxide (NO) and prostaglandin-1 [17, 30, 55]. Endothelium-independent mechanisms of ω-3 PUFAs are essentially based on the maintenance of low intracellular Ca^{2+} concentration in vascular smooth muscle cells (VSMCs) in order to reduce vasoconstriction [13]. Moreover, the ω-3 PUFAs-induced NO increase in endothelial cells can significantly reduce platelet aggregation, leukocyte adhesion and VSMC proliferation and migration [4, 12, 22]. This action is carried out by modulating the platelet-derived growth factor (PDGF) transduction pathway [56] or the cyclin-dependent kinase-2 activity [47]. In addition, the plasma triglyceride pro-atherosclerotic effect is strongly reduced by ω-3 PUFAs in a dose-dependent manner [20, 50]. To date, this effect is so well defined that EPA and DHA are currently used as therapeutic drugs in the hypertriglyceridemia treatment. The anti-inflammatory effect of ω-3 PUFAs has also beneficial repercussions in the development of atherosclerosis and thrombosis processes through the atherosclerotic plaque stabilization and the reduction of macrophages and lymphocyte infiltration [10, 57, 58]. In contrast, the knowledge concerning the anti-inflammatory effects of ω-3 PUFAs on the myocardium is limited to a potent anti-arrhythmic action that has been described both in vivo on myocardium [38, 40] and in vitro on cardiomyocytes [26, 31]. The presence of PUFAs in cardiomyocyte membrane phospholipids modulates Na^+, K^+ and Ca^{2+} channels' activity [62, 63] causing an electric stabilization of cells and thus prevention of arrhythmias. This stabilizing action influences also the heart rate throughout the autonomous nervous system [9]. No other substantial information is presently available concerning basic mechanisms supporting observations from epidemiologic studies. In particular, nutrient effects on genes expressed in the myocardium and their potential relevance in cardiovascular health and disease are totally unknown. Recently, as shown in Fig. 2, in an experimental model of hereditary cardiomyopathy, it has been demonstrated that ω3-PUFAs are able to counteract plasma membrane

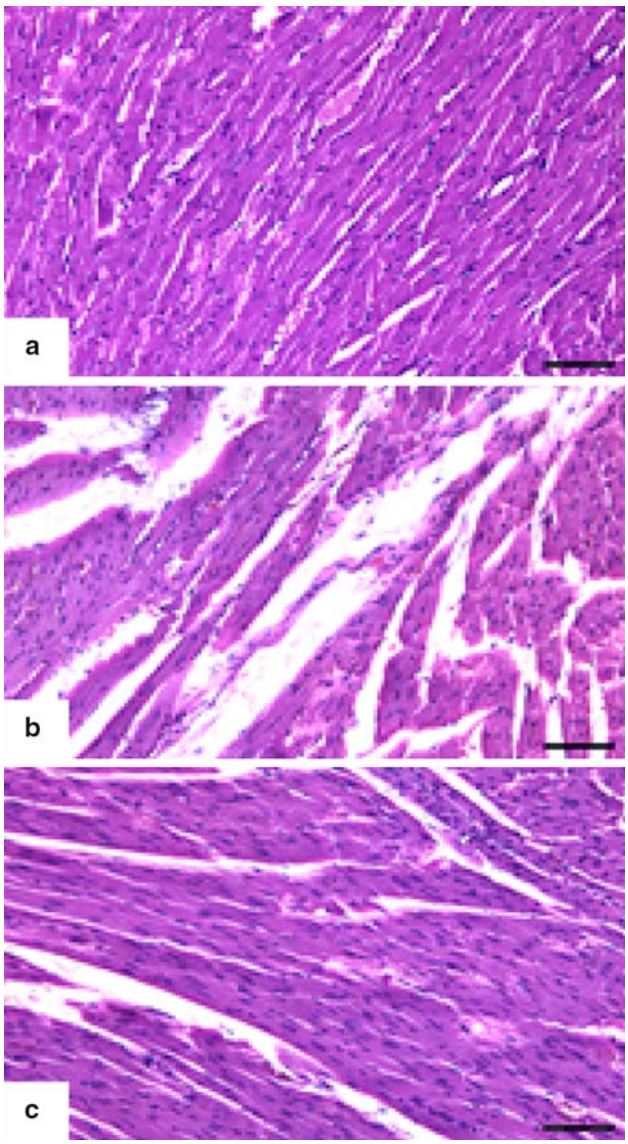

Fig. 2 Morphological analysis of hamster hearts. Light microscopy micrographs of paraffin-embedded ventricular sections (4 μm) stained with haematoxylin and eosin. Left ventricular sections displaying: **a** normal morphology in control healthy hamster; **b** large areas of myofibril loss in the myocardium of cardiomyopathic hamster fed with standard diet; **c** myofibril loss areas almost completely absent in the myocardium of cardiomyopathic hamster fed with a diet supplemented with ω3-PUFAs. *Scale bars* = 50 μm

degradation preserving physiological signals from the membrane surface to the nucleus and reactivating regular gene expression in otherwise damaged cardiomyocytes [15].

Another possible mechanism through which nutrients can directly modulate myocardial genes involves the activation of the transcription factor peroxisome proliferator-activated receptor-α (PPAR-α), but to date no conclusive data exist supporting this hypothesis. However, it can be speculated that nutrients could modulate cardiovascular function through dual signaling mechanisms: directly through binding or modulating transcription factors (e.g. PPAR alpha) or indirectly modifying cell membrane composition and triggering different intracellular signalling patterns [45].

In addition to PUFAs, several other nutrients can potentially be involved in CVD aetiology. It has been demonstrated for example that retinoic acid prevents medial thickening of intramyocardial and intrarenal arteries and perivascular and ventricular fibrosis in the heart [34]. Heart development is known to be sensitive to retinoid concentrations; a specific pattern of malformations is observed in both vitamin A-deficiency and retinoid-toxicity states. Dickman and Smith [11] suggested that retinoids may affect both morphogenesis and myofibril formation in the developing heart. Lycopene is one of the major carotenoids contained in a vegetable-rich diet. Recently the acyclic form of β carotene has been investigated in epidemiologic studies in which high circulating lycopene concentrations were associated with reductions in cardiovascular disease. In particular, lycopene plays a fundamental role in the early stages of atherosclerosis [49] and emerging evidences suggest its possible role in the primary prevention of CVD. Furthermore, the phytoantitoxin resveratrol (RV), a plant-derived polyphenol with phytoestrogenic properties, protects the cardiovascular system by numerous mechanisms including defence against ischemic-reperfusion injury, promotion of vasorelaxation, protection and maintenance of intact endothelium, anti-atherosclerotic properties, inhibition of low-density lipoprotein oxidation, suppression of platelet aggregation and estrogen-like actions [19]. A molecular study examining different pathways that may contribute to the beneficial effects of resveratrol demonstrated its possible inhibition of angiotensin II-induced VSMC hypertrophy, by interfering with the PI3K/Akt, $p70_{S6K}$ and the ERK 1/2 signaling pathways [18]. Moreover, the antiatherogenic (i.e. antiinflammatory) activity of RV on human endothelial cells interferes with nuclear factor-κB (NF-κB)-dependent transcription only when cells are stimulated at least overnight with RV alone or with TNFα, while a higher dose treatment does not influence such activity [48]. Finally, the dietary intake of methionine, the key amino acid in homocysteine metabolism, is suggested to be a risk factor for CVD. In a recent epidemiologic study, Virtanen et al. [60] concluded that long-term, moderately high dietary methionine intake may increase the risk of acute coronary events in middle-aged Finnish men.

Interestingly, in a very recent paper Ordovas and Mooser [46] focused their attention on the role of microbiota as a further determinant of the CVD risk, since it has been demonstrated that oral and intestinal microrganisms interact with the host genome and may play an important role in the development of diseases such as cancer, inflammatory, allergic and other age-related degenerative pathologies [25, 35, 59]. This concept places metagenomics—a new field of research that integrates molecular biology and genetics to identify and characterize the genetic material from environmental samples—as a relevant area of future research opening new perspectives to the knowledge of genome-environment interactions and related new approaches to human healthcare.

In recent years, significant advances have been made in the understanding of the complex interactions between lifestyle and genotype and their subsequent effects on health and disease. The increasing awareness of gene-nutrient interactions and the potential of an individual's genetic profile to alter nutrient requirements and responsiveness [53], as well as to modify their risk of developing diseases, will be the key to understanding the pathology and the progression of polygenic of metabolic and non-metabolic disorders [15]. The study of such interactions may provide therapeutic alternatives tailored to the individual and based on genetic background.

This new therapeutic frontier expands the current concept of personalized nutrition envisaged by several nutritionists in order to counteract "metabolic diseases"; thus in the future the most appropriate nutritional regimen should be tailored for each person at birth to maintain health and prevent disease, according to each individual genotype.

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