The genetic factors involved in functional food efficacy on cardiovascular disease etiology

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Abstract

While the impacts of modifiable and non-modifiable risk factors on chronic diseases such as cardiovascular disease (CVD) are widely established, the interactions between such coexisting risk factors and their subsequent effects on the promotion or suppression of CVD are less known. As part of the diet, functional foods are considered a modifiable factor that influence health beyond their basic nutritional value. The relationship between these functional foods and the underlying genome, along with their joint implication in health and disease, forms the focus of the emerging field of nutrigenomics. Reviewed in this paper are some prominent gene-diet interactions demonstrated in CVD etiology. Specifically, the interaction between foods such as phytosterols and isoflavones with genetic factors of the consuming population are examined in relation to CVD. By determining how nutritional intake affects genetics and vice versa, we create the potential to offer improved dietary guidelines to certain individuals, subgroups, or populations in order to maximize health benefits of specific diets.

Keywords: cardiovascular disease, heart disease, phytosterols, isoflavones, nutrigenomics, nutrigenetics

Introduction

The multi-faceted nature of chronic diseases prompts the examination of the biological underpinnings that influence disease progression, along with better preventative strategies and treatment options. Cardiovascular disease (CVD) is known to be the leading cause of death worldwide and is subject to well-characterized environmental influences (Basson, 2008). For instance, differences in CVD prevalence across countries may be accounted for by non-modifiable socio-economic factors such as disparities in healthcare systems. However, a universal factor that is modifiable to some extent is the diet, but unlike drug treatment, there exist few strict dietary guidelines for prevention and treatment of CVD. As discussed in this review, food offers considerable potential for alleviating some of the risk associated with chronic diseases. While this does not necessitate an extreme approach of food-for-health only, we can certainly reap the advantages of particular foods for our everyday lives.

In the past several decades, the field of nutrigenomics has been of particular focus, forming the basis of how nutrition interacts with our genetics and vice versa (Ferguson, 2013). Nutrigenomics lends itself well to understanding how some individuals and some populations respond differently to similar treatments. Notably, nutrigenomics aims to explain

these sources of variation with respect to nutrient-genome interactions.

While it is clear that individual responses to nutrients will differ, there has been little in the way of offering clinical guidelines for precise nutritional approaches in confronting chronic diseases. CVD is no exception, and thus forms the basis of this discussion (**Figure 1**). Understanding that a pyramid-like interaction exists between food (in this case, phytosterols and isoflavones), CVD, and underlying genome is essential to elucidating the mechanisms and treatments for chronic illnesses such as CVD.

In the following sections, each piece of this pyramid is broken down starting with CVD, then functional foods that have been shown to modulate CVD, and finally the nutrient-gene interactions that are the basis of specific CVD responses.

Cardiovascular Disease Etiology

The study of CVD involves many aspects and implications, more widely encompassing a series of diseases that feature dysfunction in the vascular system and target organs such as the heart and brain. Diseases of the cardiovascular system include coronary heart disease, stroke, heart arrhythmias, rheumatic heart disease, cardiomyopathy,

and others (Celermajer et al., 2012). The etiologies underlying many of these diseases involve conditions such as atherosclerosis, vascular inflammation, and high blood pressure (Celermajer et al., 2012; Stefansson & Bakovic, 2014). Together, these facets form the primary focus of this paper with the interaction of modifiable (diet) and non-modifiable (genetic) risk factors further considered in their joint propagation of CVD (**Figure 2**).

The development of atherosclerosis is usually an intrinsic component in the progression of CVD. In this process, blood constituents such as specific serum lipids (LDL cholesterol and triglycerides), calcium, and white blood cells accumulate on the inner walls of arteries, forming plaque-like deposits which can induce the pathophysiology observed in CVD (McBride, 2007). The rate of plaque development is highly dependent on risk predisposition influenced by specific factors including genetic background, hypertension and lifestyle habits such as tobacco smoking and diet (Insull, 2009; Modkad et al., 2003). Atherosclerosis onset is gradual and often silent, involving decades of plaque accumulation that is influenced chronically by these aforementioned factors (Modkad et al., 2003).

During the atherogenic process, a characteristic chronic inflammatory response occurs in both lipoprotein metabolism and the arterial wall composition (Kleeman et al., 2008). Oxidation of LDL lipids results in endothelial cell activation and recruitment of various inflammatory cells, namely T-lymphocytes and monocyte-derived macrophages. These are involved in producing a wide array of inflammatory cytokines and mediators of atherosclerosis (Kleeman et al., 2008; Frostegård, 2013). The type of cytokine species produced can induce anti-inflammatory or pro-inflammatory effects, with the latter being more predominant in CVD. Kleeman et al. (2008) have reviewed the consistency in effect of these cytokines, showing that regardless of the experimental condition, IL-1, IL-12, IL-18, TNF- α , MIF, IFN- γ , and M-CSF display potent pro-atherogenic characteristics while IL-10 demonstrates anti-atherogenic properties. The response of IL-4, IL-6, and GM-CSF have been inconsistent and are dependent on disease progression (Kleeman et al., 2008). Many of the pro-atherogenic cytokines have also been implicated in adverse alterations of plasma cholesterol, showing that inflammation is not only characteristic of atherosclerosis, but exacerbates the cholesterol imbalance that promotes formation of plaque (Kleeman et al., 2008).

Hypertension, which can arise from a multitude of factors including genetics and diet, also contributes to CVD through the promotion of endothelial injury, vascular inflammation, and accelerated plaque development (Díez, 2014). Elevated blood pressure accounts for one of five characteristic components that constitute the metabolic syndrome, a known precursor and common comorbidity of CVD. In this disorder, there is occurrence of central obesity, insulin resistance, hypertension, dyslipidemia (low HDL and high LDL levels), and excessive abdominal fat. These factors usually complement one another's occurrence and increase the risk of various types of CVD, including rheumatic heart

disease, coronary heart disease, stroke, ischemic heart disease and others, as defined by the International Classification of Diseases (9th revision, codes 390-459) (Malik et al., 2004).

The pathophysiology accompanying the above etiologies can lead to the adverse effects associated with CVD. Atherosclerosis, in particular, may lead to arterial stenosis which results in ischemia of tissues and organs (Frostegård, 2013). Further, atherothrombosis may present if plaques are ruptured, leading to prothrombotic material that may coagulate and form blood clots (Frostegård, 2013). This occurs either in a blood vessel or organ site, which may impart severe consequences such as stroke or heart attack. An individual's age plays into enhancing this pathophysiology due to the changes in molecular and cellular functions associated with increased vascular age (Lakatta, 2003). While hypertension can promote increased vascular resistance which, in itself, can lead to ischemic heart disease, it also acts via a separate mechanism associated with myocardial remodelling and left ventricular hypertrophy (Nadruz 2015). Under conditions of chronic hypertension, the wall of the left ventricle compensates by thickening and subsequently dilating in a way that reduces left ventricular ejection fraction, thereby leading to cardiac failure (Drazner, 2011). While these are some of the most prominent ways in which CVD may develop, many other mechanisms exist in the breadth of the disease pathogenesis.

To assess CVD more accurately, certain guidelines are in place such as those for cholesterol levels. For example, an LDL/HDL cholesterol ratio > 3.3 qualifies an individual for cholesterol-controlling pharmacotherapy such as statins (Insull, 2009; Fernandez & Webb, 2008). However, fewer guidelines exist in terms of preventing CVD risk with the use of diet, and compared to pharmacotherapy, relatively little work has been done to control CVD in specific populations using functional foods.

Functional Foods Modulating Cardiovascular Disease

The notion of using functional foods (FFs) for health benefits has long been around as a dietary therapeutic measure. Research on the potency of FFs that may be used to prevent or treat CVD, however, is not on par with the many pharmacotherapies that exist. According to the American Heart Association, a FF is any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains (Hasler, 2002). Such foods often contain dietary bioactives that enhance but are not essential for health. As such, they are often left out of the diet entirely despite many proven advantages. Health Canada maintains a database of health claims that have been accepted for market on product labeling (Health Canada, 2017), with notable claims towards mitigating CVD as follows: phytosterols for blood cholesterol lowering (2010), soy proteins for blood cholesterol lowering (2015), oat products for blood cholesterol lowering (2007), vegetables and fruit for heart disease reduction (2016), and

more. Similarly, the U.S. Food & Drug Administration (FDA) has a distinctive list of authorized health claims that meet significant scientific agreement, and each of the aforementioned foods is also approved by the FDA for similar heart-claims (Center for Food Safety and Applied Nutrition, 2017).

For this discussion, the FFs identified to modulate CVD in a nutrigenomic-dependent manner involve phytosterols and isoflavones. The foundation for the main cardio-protective mechanisms of each of these compounds is outlined in this section, while the following section will tie together the less established role of the genome in affecting both the efficacy of the FFs and the etiology of CVD. In terms of CVD pathogenesis, the FFs that have been authorized may lower serum cholesterol (as indicated by most of the above claims), hypertension, or inflammation. Beyond these FFs, there certainly exist many more compounds with numerous distinct mechanisms, many of which are yet to be studied.

Phytosterols

A dietary bioactive shown to modulate the aforementioned phenotype of CVD includes phytosterols. Structurally similar to cholesterol, phytosterols are found as constituents of plant cell walls and may yield beneficial effects when consumed in the diet (Ostlund, 2002). Phytosterols may also be referred to as plant sterols or plant stanols (unsaturated and saturated derivatives of phytosterols, respectively) (Marangoni & Poli, 2010). Since phytosterols are derived from plant sources, natural foods with the highest levels include oils such as corn or canola, seeds, nuts, whole grains, flours, and soybeans, with supplements commonly available in the form of specific phytosterol species such as β -sitosterol and campesterol (Racette et al., 2009).

Through the corroboration of multiple animal and human studies, phytosterols have been well established as functional foods that reduce serum LDL cholesterol (LDL-c) and high blood pressure levels. Andersson et al. (2004) performed an extensive cross-sectional study with over 22 000 men/women that demonstrated an inverse relationship between dietary plant sterol intake and serum cholesterol concentration. While this study was performed in Norfolk, England, similar LDL-c lowering has been observed in other populations across the world. Notably, a meta-analysis of 84 randomized controlled trials established an 8.8% decrease in LDL-c for a daily dose of 2.15 g/day (Demonty et al., 2008).

Plat and Mensink (2002) have elucidated the mechanism with which phytosterols exert their cholesterol-lowering effect. In their study, the altered expression of certain intestinal proteins was probed using an intestinal Caco-2 cell line. A membrane transporter protein ABCA1 involved in the shuttling of cholesterol from the enterocyte to the lumen (thereby decreasing cholesterol absorption into micelles) was of particular importance. The study indicated that in response to phytosterols, there was increased luminal excretion of cholesterol (i.e. decreased absorption) (Plat & Mensink, 2002). This process involved two distinct effects of

phytosterols in lowering cholesterol absorption. First, there was a competition that phytosterols provided against cholesterol incorporation into mixed micelles—a mechanism widely accepted. The second mechanism involved a nutrigenomic interaction which increased luminal transporter ABCA1 expression in response to sitostanol (a phytosterol) supplementation. Specifically, this was due to sitostanol persisting for a longer amount of time in the enterocyte, causing upregulated expression of ABCA1 protein, and ultimately leading to greater luminal excretion of cholesterol. As a net result, there was a reduction in circulating LDL-c and an increase in fecal cholesterol excretion (Plat & Mensink, 2002; Repa et al., 2000).

These results were further supplemented by a randomized controlled trial (RCT) conducted in a Chinese population (Cheung et al., 2017). The RCT offered greater evidence in a double-blinded, placebo-controlled study design that controlled for confounding variables. The study compared phytosterols in a low-fat milk supplemented matrix (at 1.5 g phytosterols/day), against conventional low-fat milk consumption (control). This was significant because the Chinese study population traditionally have a higher prevalence of lactose intolerance. Delivery of phytosterols was intentionally provided via milk in order to test if dysfunction in lactose absorption could potentially affect phytosterol absorption as well. The critical findings suggested otherwise, with results showing that those in the phytosterol supplemented group indeed had significant reduction in LDL-c (by 9.5% on average), total cholesterol, and diastolic blood pressure. Occurrences of lactose intolerance were similar in both groups suggesting that the condition, which is associated with intestinal enzyme dysfunction, does not implicate phytosterol absorption (Cheung et al., 2017; Scrimshaw & Murray, 1988).

With the role of phytosterol supplementation well established in LDL-c reduction, other studies have further shown beneficial effects in modulating additional CVD etiology. For instance, certain studies have elicited a blood pressure lowering effect of phytosterols in animal models (Vaskonen et al., 2002). In rats with hypertension induced via an atherogenic diet, phytosterol supplementation resulted in a lowering of blood pressure through enhancing the endothelium response to acetylcholine, thereby allowing for vascular relaxation and increasing lifespan considerably (Vaskonen et al., 2002). However, the acute induction of hypertensive conditions in rats may be less applicable to a human population where chronic CVD propagation can occur over many decades.

To a lesser extent, certain components of inflammation have also been proposed to interact with phytosterol consumption. Data from cell models suggests that phytosterols are involved in reducing the inflammatory activity of immune mediators such as macrophages and neutrophils (Rocha et al., 2016). Such immune cells produce prostaglandins, which have created debate in regards to their effect on atherogenesis. Awad et al. (2004) have concluded that PGE₂ facilitates enhanced platelet aggregation and

vasoconstriction, while PGI₂ has offered opposite protective effects through inhibiting platelet aggregation. These researchers have further concluded that phytosterol supplementation resulted in a reduced secretion of PGE₂ from macrophages and enhanced effects of PGI₂, which may offer atheroprotective benefits in terms of inhibiting plaque formation. However, this study was performed in macrophages grown in cell culture, which may not fully represent physiological conditions in aortic components that are undergoing atherosclerotic lesions. As such, other studies show a non-significant effect of phytosterol supplementation on inflammatory plasma markers such as C-reactive protein (CRP) (Rocha et al., 2016). Further consideration would be beneficial to clarify how phytosterols influence prostaglandin levels and vascular inflammation in-vivo.

Isoflavones

Isoflavone(s) (ISF) are another functional food component, comprising a subclass of flavonoids which are capable of exerting phytoestrogenic effects in the body (Rimbach et al., 2008; Xiao, 2008). Despite many bioactive forms, the basic structure of ISF molecules includes a heterocyclic pyrone ring that links two benzene rings with minor substitutions of side chains, meanwhile resembling estrogenic structure.

ISF are a well-studied compound, closely associated with soy proteins from soybean legumes. The glycosidic forms of ISF, daidzin and genistin, are converted by gut microbiota into the bioactive aglycone forms, daidzein and genistein (Xiao, 2008). Of importance is the regional disparity in ISF consumption; while intakes of 25-50 mg ISF/day are common in eastern Asia where soy is a staple, less than 1 mg ISF/day are consumed on average in Western countries (Rimbach et al., 2008). Since ISF resemble estradiol structurally, this enables their binding to both α and β isoforms of the estrogen receptor which inspires the notion that ISF exhibit physiological actions similar to natural estrogens (Xiao, 2008). Our interest, however, focuses on the protective quality of ISF on LDL-c levels as well as their anti-inflammatory effects. As discussed in the next section, the specific nutrigenomic interactions of ISF with gut microbiota can also be considered in relation to CVD etiology.

The effects of ISF on gene expression have been associated with an increase in antioxidant properties (Mahn et al., 2005). Specifically, enzymes for endothelial nitric oxide synthase (eNOS) as well as certain antioxidant enzymes have shown upregulation after soy treatment in the rat's aortic tissue. This indicates a heightened ability to scavenge free radicals and chelate metals through enhancement of cytochrome c oxidase, superoxide dismutase, glutathione peroxidase, and other antioxidant enzymes (Rimbach et al., 2008; Mahn et al., 2005). While these antioxidant effects are not strictly tied to CVD in humans, there lies potential in further research to look into vascular benefits through ISF consumption, since the vasodilating effects of eNOS and the

antioxidant properties required for atheroprotection are well understood.

Albeit less potent than phytosterols, soy protein consumption has been shown to induce LDL-c lowering, with the U.S. Food & Drug Administration approving heart health claims for soy proteins (Hu, 2011). Nevertheless, the contribution of ISF specifically to this cholesterol lowering effect has remained controversial, with ISF showing discord in potency of delivery as either supplements or as part of soy proteins. The American Heart Association has compiled a meta-analyses of 22 randomized trials, concluding that ISF may only slightly decrease LDL-c with no effect on HDL-c, triglycerides, or blood pressure (Xiao, 2008). Jenkins et al. (2010) have attributed the LDL-c lowering effects to both intrinsic and food-displacement mechanisms, with isoflavones providing an intrinsic lowering effect while the considerable intake of soy proteins themselves may displace a diet that is heavier in saturated fats and refined sugars. While neither of these are as potent as phytosterol mechanisms, ISF use does offer some health benefits, especially in certain populations.

Nutrigenomic Factors

To format a discussion focused around nutrigenomics, we must clarify the way in which the term is used. Strictly, 'nutrigenomics' refers to nutritional genomics, studying the impact of nutrients on gene expression, while 'nutrigenetics' investigates how genetic variability affects the body's response to a nutrient (Rimbach et al., 2008). These terms thereby approach the nutrition-gene interaction via opposing starting points. However, this may seem to be an artificial division in terminology, and for the purpose of this discussion, all 'nutrient-gene' interactions are considered under the 'nutrigenomic' umbrella in order to avoid the exclusion of significant yet meaningful research findings.

According to the American Heart Association, nutrigenomics represents a suitable approach to confronting CVD by offering both prevention and treatment benefits through individually-optimized diets (Corella & Ordovas, 2009). Yet, relative to research in fields such as pharmacotherapy, nutrigenomics is still in its infancy, with little more than a foundation placed in a pursuit of offering precise, scientifically-backed nutrition guidelines.

Fundamentally, we understand well that genomics affect disease, but we are only starting to decipher how nutrition affects both genomics *and* disease, with these interactions occurring bidirectionally. As visualized in the pyramid of Figure 1, functional foods are rooted deeply in their nutrigenomic interactions to jointly influence risk of CVD. The distinct genetic background of an individual largely involves the presence of single nucleotide polymorphisms (SNPs) which promote or protect against disease risk, while the alteration of genetic expression itself may result from the induction of certain transcription factors.

Single Nucleotide Polymorphisms Involved with Cardiovascular Disease

An example of SNPs affecting CVD risk involves polymorphisms in the apolipoprotein E (ApoE) gene. ApoE comprises the 'protein' component of plasma lipoproteins, which are responsible for shuttling cholesterol and fats in the bloodstream (Eichner et al., 2002). Interestingly, in different populations ApoE can interact differently with lipoprotein receptors, which can influence circulating cholesterol levels as a result of unique ApoE isoforms created by SNPs. Multiple studies have established that generally, carriers of E2 alleles of ApoE are slower in transferring VLDLs and chylomicrons to the liver due to a lowered binding affinity, while E4 carriers offer better efficiency in transfer (Eichner et al., 2002). Therefore, carriers of E2 show a delayed clearance of lipoproteins and an increase in LDL receptors at the liver, resulting in lower plasma LDL levels (Weintraub et al., 1987). As established, this can result in differential disease etiology for CVD.

With respect to FFs, there is some evidence to suggest that the foods previously discussed are affected by genotype. ISF supplementation (43.5 mg/day for 12 months) in women aged 49-65 has been shown to lower total cholesterol and LDL-c but only in the E2/E3 genotype (Atkinson et al., 2004). However, this specific data did not consider any estrogen receptor activity and was further limited by the use of smaller sample sizes (i.e. the E2/E3 group had only 25 women), while the few other studies looking into ISF-ApoE interactions also use smaller sample sizes. Rimbach et al. (2008) have however studied polymorphisms in the estrogen receptor, showing that individuals with certain SNPs are good candidates for ISF therapy. Specifically, evidence suggests that ISF consumption differs in impact on CVD biomarkers in a SNP-dependent fashion through estrogen receptor genes, ER α and ER β (Hall et al., 2005). In particular, beneficial ISF-genotype interactions were observed only for the ER β *AluI* genotype on the biomarker for vascular cell adhesion molecule 1 (VCAM1), which has shown implications in human atherosclerosis (Hall et al., 2005). In addition, it has been demonstrated that SNPs in ER β *AluI* are significant for CVD in both sexes due to the AA genotype existing in a relatively higher frequency for those with coronary artery disease, with an associated increase in CVD biomarkers (Mansur et al., 2005). Therefore, it appears that in addition to SNPs existing in genes for apolipoproteins, the estrogen receptor may also play a role in the nutrient-gene influences of ISF supplementation.

Additionally, epidemiological data suggests that Northern Europeans tend to have higher frequencies of the less beneficial ApoE4 allele than Southern Europeans, and that Nigerian, Japanese, Mexican American, and Finnish populations have relatively low levels of the beneficial E2 allele (Eichner et al., 2002). While such data may help in advancing nutrigenomic recommendations, there is insufficient epidemiological evidence to introduce dietary guidelines based on ApoE genotypes. Yet, it seems plausible

to hypothesize that individuals with E4 alleles, for instance, who have greater predisposition towards CVD may benefit most from a diet that provides cholesterol-lowering effects through supplementation with phytosterols or ISF.

Nutrigenomic Interactions due to Intestinal Composition

It is important to also consider the intestinal effects of FF consumption. Both phytosterols and ISF have been shown to interact with aspects of intestinal composition, including specific transporter enzymes and the ecosystem of the gut microbiota. As established, phytosterols exert part of their action mechanistically by increasing the expression of luminal transporter ATP-binding cassette 1 (ABCA1) (Repa et al., 2000). This is possible because ABCA1 (also referred to as ABC1 in the literature) is subject to transcriptional regulation by nuclear receptors LXR/RXR, both of which act as sensors for sterol substrates (Repa et al., 2000). Because phytosterols are able to persist for a longer amount of time in intestinal cells, they are sensed by LXR/RXR, which act as transcription factors to upregulate transporter expression for greater cholesterol excretion in the feces (Oram et al., 2000). This is made evident by Plat and Mensink (2002) who demonstrated that increasing sitostanol supplementation also increases ABCA1 expression. While studies have not shown phytosterol action on polymorphic forms of ABCA1, it is understood through Tangier's Disease that deficiency in ABCA1 may cause prevalent atherosclerosis (Oram et al., 2000). Thus, nutrigenomic induction is observed with the use of phytosterols.

ISF, on the other hand, interact with gut microbiota in the large intestine to reach their bioactive forms. Unique gut microbiota signatures have been established in their relevance to inflammatory processes and chronic conditions such as obesity, inflammatory bowel disease, CVD, and Type 2 diabetes (Cho and Blaser, 2012). Studies comparing the influence of environmental vs. genetic factors on microbiota composition have yielded inconsistent results. However, there is agreement that both factors have considerable impact on gut signatures. A recently published review considered human twin studies, inbred mouse lines, and gene-deletion studies to demonstrate that there may be substantial effects on the composition of the gut microbiota due to as little as a single host gene (Spor et al., 2011). The study concluded that the gut microbiome should be considered a phenotypic trait of the host, signifying that both environmental and genetic factors are in effect (Spor et al., 2011).

Furthermore, it should be considered that while nutrients do not necessarily alter genetic expression through the gut microbiome, they may in fact have indirect modulatory effects on the genome via downstream products created by the gut. This relates with the FF aspects discussed above as some FFs have potency dependent on the microbiome of the consuming population. For example, data has shown that only 25-30% of Western adults are able to convert isoflavones into their more potent bioactive form, equol, while 50-60% of

Japanese/Chinese adults can undergo the same conversion (Atkinson et al., 2005). This variance in conversion of daidzein to equol has been attributed largely to the gut microbiota (Atkinson et al., 2005). Incidentally, CVD risk is lower in Japan and China relative to Western countries (Rimbach et al., 2008). While it has not been discerned if differences in CVD risk depend on equol's differential conversion across these populations, the wide variance in ISF consumption may play a role. A plausible framework for a nutritional guideline may then involve a more reserved recommendation of soy proteins for Western populations, since these populations cannot convert the non-bioactive forms of the ISF as effectively. Instead, it may be suggested to take equol in supplemental form rather than through soy products. However, this certainly requires greater understanding to address the effectiveness of supplement vs. whole-food delivery, with such recommendations offering greater benefit to the populations they serve.

Induction of Specific Genes

In addition to being dependent on SNPs and gastrointestinal composition, FFs may also modulate the specific expression of genes. A good example elucidated by Mahn et al. (2005) involves the phytoestrogenic action of ISF supplementation. In this study, soy-deficient rats were re-fed with soy protein for 6 months, showing a two-to-threefold increase in mRNA levels for eNOS, cytochrome c oxidase, and other antioxidant enzymes (Mahn et al., 2005). Mechanistically, ISF such as genistein were shown to be acting directly on estrogen receptors, enabling transcription factors to upregulate these enzymes and lead to improved vascular relaxation and decreased blood pressure, both of which are determinants of CVD risk.

Other studies have shown the gene-altering effects of genistein with respect to vascular tone. Rimbach et al. (2008) treated human umbilical vein endothelial cells (HUVEC) with genistein, which resulted in considerable down-regulation for genes that encoded endothelin-2 as well as endothelin converting enzyme 1—both of which are factors associated with powerful vasoconstrictive effects in the body. Endothelins are further responsible for enhancing platelet aggregation, chemotaxis for inflammatory cells, and production of cytokines (Pedram et al., 1997). The nutrigenomic down-regulation of endothelins can thus offer protection against various CVD etiologies, from vascular inflammation to hypertension and vasoconstriction (Cassidy et al., 2003). This has recently been substantiated by novel pharmacological treatments such as endothelin receptor antagonists which target the more particular condition of pulmonary arterial hypertension (Sidharta et al., 2015). Progress thus continues to be desired to incorporate genomics into treatment for CVD.

Discussion & Conclusions

To conclude a discussion on the complex nutrigenomic interactions that form the basis for influencing CVD, we must

carefully consider research established through the literature. As summarised in Figure 3 and apparent throughout this discussion, the association between functional foods and certain risk factor reduction has been established over many years. Further, these risk factors have been well recognized in relation to CVD reduction. However, gaps continue to exist in examining the nutrigenomic interactions that govern CVD in different individuals and populations. For common FFs discussed, we have been able to show certain nutrient-gene interactions, including the effects of SNPs in apolipoprotein genes and estrogen receptors, the effect of intestinal composition (transporters and gut microbiota) on FF efficacy, as well as the induction of target genes to alter their expression.

While studying the effects of single SNPs is beneficial, the future of the field may necessitate recognizing the complex interactions of metabolic pathways involving numerous genetic influences, including overall genetic variation, epigenetics, the microbiome, etc. Per the example demonstrated in this review, diverse microbiome environments are conducive to varying levels of ISF conversion and energy homeostasis, which may impact CVD outcomes differently, especially considering downstream interaction with different genotypes for apolipoproteins and estrogen receptors. While single studies have not been able to establish such multi-step interactions in depth, the pooling and connecting of data points with multiple intermediate markers (such as specific SNPs from pathways that are related) will continue to assist future research. This may then offer enhanced identification of clinical endpoints for determining the nutrigenomic interactions underlying chronic diseases such as CVD.

Importantly, it should be considered that continuing research and investing into the therapeutic use of foods may be meaningless if practitioners do not adapt new treatment regimes. Even with organizations such as the American Heart Association collating relevant research guidelines, there may continue to exist implicit attitudes in promoting drugs over food for reasons based on the diversity of research and practices that exist. Ideally, we should appreciate the benefits of both drug and dietary interventions to treat individuals over the spectrum of health or disease. Indeed, this is especially significant for disease prevention in healthier individuals, who may only consume food rather than drugs. While research may not be at a point to definitively tailor one's entire diet to their genes (i.e. precision nutrition), it may seem more feasible in the near future to group individuals based on genetic factors and provide a stratified nutrition approach.

Thus, nutrigenomics represents a growing but underdeveloped field of study, especially in relation to pharmaceutical research and in light of the FFs discussed here being few of many potential modulators for human health. After all, dietary intake is universal. Thus, the field would benefit from greater interest, with greater benefits being passed on to individuals, subpopulations, and the global community at large.

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References

- Andersson SW, Skinner J, Ellegård L, Welch AA, Bingham S, Mulligan A, Andersson H, Khaw KT. 2004. Intake of dietary plant sterols is inversely related to serum cholesterol concentration in men and women in the EPIC Norfolk population: a cross-sectional study. *European Journal of Clinical Nutrition*. 58(10): 1378-1385.
- Atkinson C, Frankenfeld CL, Lampe JW. 2005. Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. *Experimental Biology and Medicine*. 230(3): 155-170.
- Atkinson C, Oosthuizen W, Scollen S, Loktionov A, Day NE, Bingham SA. 2004. Modest protective effects of isoflavones from a red clover-derived dietary supplement on cardiovascular disease risk factors in perimenopausal women, and evidence of an interaction with ApoE genotype in 49–65 year-old women. *The Journal of Nutrition*. 134(7): 1759-1764.
- Awad AB, Toczek J, Fink CS. 2004. Phytosterols decrease prostaglandin release in cultured P388D1/MAB macrophages. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 70(6): 511-520.
- Babu PA, Liu D. 2008. Green tea catechins and cardiovascular health: an update. *Current Medicinal Chemistry*. 15(18): 1840-1850.
- Basson M. 2008. Cardiovascular disease. *Nature*. 451(7181): 903.
- Cassidy A, de Pascual Teresa S, Rimbach G. 2003. Molecular mechanisms by which dietary isoflavones potentially prevent atherosclerosis. *Expert Reviews in Molecular Medicine*. 5(24): 1-15.
- Celermajer D, Chow CK, Marijon E, Anstey N, Woo K. 2012. Cardiovascular disease in the developing world. *Journal of the American College of Cardiology*. 60(14): 1207-1216.
- Center for Food Safety and Applied Nutrition [Internet]. 2017. Food & Drug Administration; [cited 2017 Nov 19]. Available from <https://www.fda.gov/Food/IngredientsPackagingLabeling/ucm2006876.htm>
- Cheung C, Ho DK, Sing C, Tsoi M, Cheng VK, Lee GK, Ho Y, Cheung BM. 2017. Randomized controlled trial of the effect of phytosterols-enriched low-fat milk on lipid profile in Chinese. *Scientific Reports*. 7: 41084.
- Cho I, Blaser MJ. 2012. The human microbiome: at the interface of health and disease. *Nature Reviews Genetics*. 13(4): 260-270.
- Corella D, Ordovas JM. 2009. Nutrigenomics in cardiovascular medicine. *Circulation: Cardiovascular Genetics*. 2(6): 637-651.
- Demonty I, Ras RT, van der Knaap HCM, Duchateau GSMJE, Meijer L, Zock PL, Geleijnse JM, Trautwein EA. 2008. Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. *Journal of Nutrition*. 139(2): 271-284.
- Díez J. 2014. Arterial hypertension in patients with heart failure. *Heart Failure Clinics*. 10(2): 233-242.
- Drazner MH. 2011. The progression of hypertensive heart disease. *Circulation*. 123(3): 327-334.
- Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. 2002. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. *American Journal of Epidemiology*. 155(6): 487-495.
- Ferguson LR. 2013. Nutrigenomics and nutrigenetics in functional foods and personalized nutrition. 1ST ED. Boca Raton (FL): CRC Press.
- Fernandez ML, Webb D. 2008. The LDL to HDL cholesterol ratio as a valuable tool to evaluate coronary heart disease risk. *Journal of the American College of Nutrition*. 27(1): 1-5.
- Frostegård J. 2013. Immunity, atherosclerosis and cardiovascular disease. *BMC Medicine*. 11: 117.
- Hall WL, Vafeiadou K, Hallund J, Bügel S, Koebnick C, Reimann M, Ferrari M, Branca F, Talbot D, Dadd T, et al. 2005. Soy-isoflavone-enriched foods and inflammatory biomarkers of cardiovascular disease risk in postmenopausal women: interactions with genotype and equol production. *The American Journal of Clinical Nutrition*. 82(6): 1260-1268.
- Hanis CL, Hewett-Emmett D, Douglas TC, Bertin TK, Schull WJ. 1991. Effects of the apolipoprotein E polymorphism on levels of lipids, lipoproteins, and apolipoproteins among Mexican-Americans in Starr County, Texas. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 11(2): 362-370.
- Hasler C. 2002. Functional foods: benefits, concerns and challenges - a position paper from the American Council on Science and Health. *The Journal of Nutrition*. 132(12): 3772-3781.

- Health Canada [Internet]. 2017. Government of Canada; [cited 2017 Nov 13]. Available from <https://www.canada.ca/en/health-canada/services/food-nutrition/food-labelling/health-claims/assessments.html>
- Hu FB. 2011. Do functional foods have a role in the prevention of cardiovascular disease? *Circulation*. 124(5): 538-540.
- Insull W. 2009. The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. *The American Journal of Medicine*. 122(1): 3-14.
- Jenkins DJA, Mirrahimi A, Srichaikul K, Berryman CE, Wang L, Carleton A, Abdounour S, Sievenpiper JL, Kendall CWC, Kris-Etherton PM. 2010. Soy protein reduces serum cholesterol by both intrinsic and food displacement mechanisms. *The Journal of Nutrition*. 140(12): 2302S-2311S.
- Kleemann R, Zedelaar S, Kooistra T. 2008. Cytokines and atherosclerosis: a comprehensive review of studies in mice. *Cardiovascular Research*. 79(3): 360-376.
- Lakatta EG. 2003. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part III: cellular and molecular clues to heart and arterial aging. *Circulation*. 107(3): 490-497.
- Mahn K, Borrás C, Knock GA, Taylor P, Khan IY, Sugden D, Poston L, Ward JP, Sharpe RM, Viña J, et al. 2005. Dietary soy isoflavone-induced increases in antioxidant and eNOS gene expression lead to improved endothelial function and reduced blood pressure in vivo. *The FASEB Journal*. 19(12): 1755-1757.
- Malik S, Wong N, Franklin S, Kamath T, L'Italien G, Pio J, Williams R. 2004. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation*. 110(10): 1245-1250.
- Mansur AD, Nogueira CCM, Strunz CMC, Aldrighi JM, Ramires JAF. 2005. Genetic polymorphisms of estrogen receptors in patients with premature coronary artery disease. *Archives of Medical Research*. 36(5): 511-517.
- Marangoni F, Poli A. 2010. Phytosterols and cardiovascular health. *Pharmacological Research*. 61(3): 193-199.
- McBride PE. 2007. Triglycerides and risk for coronary heart disease. *JAMA*. 298(3): 336-338.
- Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, Marks JS. 2003. Prevalence of obesity, diabetes, and obesity-related health risk factors. *JAMA*. 289(1): 76-79.
- Nadruz W. 2015. Myocardial remodeling in hypertension. *Journal of Human Hypertension*. 29(1): 1-6.
- Oram JF, Lawn RM, Garvin MR, Wade DP. 2000. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. *Journal of Biological Chemistry*. 275(44): 34508-34511.
- Ostlund Jr RE. 2002. Phytosterols in human nutrition. *Annual Review of Nutrition*. 22(1): 533-549.
- Pedram A, Razandi M, Hu R, Levin ER. 1997. Vasoactive peptides modulate vascular endothelial cell growth factor production and endothelial cell proliferation and invasion. *Journal of Biological Chemistry*. 272(27): 17097-17103.
- Plat J, Mensink R. 2002. Increased intestinal ABCA1 expression contributes to the decrease in cholesterol absorption after plant stanol consumption. *The FASEB Journal*. 16(10): 1248-1253.
- Racette SB, Speare CA, Phillips KM, Lin X, Ma L, Ostlund Jr RE. 2009. Phytosterol-deficient and high-phytosterol diets developed for controlled feeding studies. *Journal of the American Dietetic Association*. 109(12): 2043-2051.
- Repa JJ, Turley SD, Labaccaro JMA, Medina J, Li L, Lustig K, Shan B, Heyman RA, Dietchy JM, Mangelsdorf DJ. 2000. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science*. 289(5484): 1524-1529.
- Rimbach G, Boesch-Saadatmandi C, Frank J, Fuchs D, Wenzel U, Daniel H, Hall WL, Weinburg PD. 2008. Dietary isoflavones in the prevention of cardiovascular disease – a molecular perspective. *Food and Chemical Toxicology*. 46(4): 1308-1319.
- Rocha VZ, Ras RT, Gagliardi AC, Mangili LC, Trautwein EA, Santos RD. 2016. Effects of phytosterols on markers of inflammation: a systematic review and meta-analysis. *Atherosclerosis*. 248: 76-83.
- Scrimshaw NS, Murray EB. 1988. The acceptability of milk and milk products in populations with a high prevalence of lactose intolerance. *The American Journal of Clinical Nutrition*. 48(4): 1142-1159.
- Setchell KD, Clerici C. 2010. Equol: history, chemistry, and formation. *The Journal of Nutrition*. 140(7): 1355S-1362S.
- Sidharta PN, Krähenbühl S, Dingemanse J. 2015. Pharmacokinetic and pharmacodynamic evaluation of macitentan, a novel endothelin receptor antagonist for the treatment of pulmonary arterial hypertension. *Expert Opinion on Drug Metabolism & Toxicology*. 11(3): 437-449.
- Spor A, Koren O, Ley R. 2011. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews Microbiology*. 9(4): 279-290.

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- Stefansson A, Bakovic M. 2014. Dietary regulation of Keap1/Nrf2/ARE pathway: focus on plant-derived compounds and trace minerals. *Nutrients*. 6(9): 3777-3801.
- Vaskonen T, Mervaala E, Krogerus L, Karppanen H. 2002. Supplementation of plant sterols and minerals benefits obese zucker rats fed an atherogenic diet. *The Journal of Nutrition*. 132(2): 231-237.
- Weintraub MS, Eisenberg S, Breslow JL. 1987. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. *Journal of Clinical Investigation*. 80(6): 1571.
- Westerby R. 2011. An overview of cardiovascular disease risk assessment. *Nursing Standard*. 26(13): 48-55.
- Xiao CW. 2008. Health effects of soy protein and isoflavones in humans. *The Journal of Nutrition*. 138(6): 1244S-1249S.

Tables and Figures

Figure 1. The interactions depicted in this pyramid form the basis for this discussion. Functional foods (placed in the centre), like any treatment regime, are rooted deeply in their nutrigenomic interactions to jointly influence the upward risk of CVD. Note that in this case, discussion of functional foods will focus on phytosterols and isoflavones.

Figure 2. The interaction of modifiable and non-modifiable risk factors are implicit in their joint propagation of CVD. The highlighted portions are focused on for the purpose of this discussion.

Figure 3. The general consensus obtained in the literature regarding the interaction between functional foods, nutrigenomics, and CVD. Note that the thin black lines represent associations that have been well established, while the thicker grey lines represent areas still under study and essential for future direction. The bidirectional relationship between nutrigenomics and foods as well as disease risk is of importance in illustrating the complex interactions that take place with these key components.