

Nutritional Approach for Designing Meat-based Functional Food Products with Nuts

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Meat and meat products are essential components of diets in developed countries and despite the convincing evidence that relate them to an increased risk for CVD, a growing consumption of meat products is foreseen. Epidemiological studies show that regular consumption of nuts, in general, and walnuts in particular, correlates inversely with myocardial infarction and ischaemic vascular disease. We assess the nutritional basis for and technological approach to the development of functional meat-based products potentially relevant in cardiovascular disease (CVD) risk reduction. Using the available strategies in the meat industry (reformulation processes) and a food-based approach, we address the design and development of restructured beef steak with added walnuts, potentially functional for CVD risk reduction. Its adequacy as a vehicle for active nutrients is confirmed by a pharmacokinetic pilot study in humans using γ -tocopherol as an exposure biomarker in chylomicrons during the post-prandial state. Effect and potential “functionality” is being assessed by a dietary intervention study in subjects at risk and markers and indicators related to CVD are being evaluated. Within the conceptual framework of evidence-based medicine, development of meat-based functional products may become a useful approach for specific applications, with a potential market and health benefits of great importance at a population level.

Keywords γ -tocopherol, nuts, functional foods, biomarkers, cardiovascular diseases

INTRODUCTION

Dietary Intake and Cardiovascular Disease

Diet and nutrition are important factors in the promotion and maintenance of health throughout life. According to the World Health Organization and the Food and Agricultural Organization,¹ several dietary patterns, along with lifestyle habits (exercise, smoking, and alcohol consumption), constitute major modifiable risk factors in relation to the development of non-communicable diseases: cardiovascular diseases (CVD), cancer, type 2 diabetes, obesity, osteoporosis, and dental diseases. Chronic diseases contribute to approximately 60% of all deaths, almost half of which are attributable to CVD.¹ Among the biological factors contributing to an increased risk for CVD are overweight, central obesity, hypertension, insufficient physical activity, tobacco consumption, and inadequate

nutrition. Unhealthy dietary practices include the high consumption of saturated fats, salt, refined carbohydrates, and low fruit and vegetables intake.^{1,2}

Meat and meat products are essential components of diets in developed countries, the consumption of which increases as the economic development of the populations improves. However, although these foods are important sources of high-quality proteins and several micronutrients (i.e. vitamin A, iron, zinc), there is convincing evidence that relate them to an increased risk for CVD. In this respect, although a lower intake of these foods, along with an increase in fruit and vegetable consumption, would be beneficial, a growing consumption of meat products is foreseen, despite the recommendations of public health experts pointing out that this circumstance may increase the relative contribution of nutrition to the development of chronic diseases (cancer, CVD, and obesity).

On the other hand, epidemiological studies show that regular consumption of nuts in general, and walnuts in particular, correlates inversely with myocardial infarction and ischaemic vascular disease regardless of other factors associated with risk such as age, sex, smoking, hypertension, weight, and exercise.^{3–6}

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Rationale for Intervention

Chronic diseases are largely preventable diseases and the public health approach involving primary prevention is considered to be the most cost-effective, affordable, and sustainable course of action to cope with the chronic disease epidemic worldwide.¹ The results from large intervention trials using single nutrients at pharmacological doses (i.e. α -tocopherol, β -carotene) have provoked a change towards a higher nutrient specificity (i.e. γ -tocopherol), the use of mixtures of phytochemicals and amounts achievable by diet, as approaches close to fruit and vegetable consumption.⁷ Moreover, while the failure of pill supplementation does not exclude the protective effects of dietary antioxidants, current evidence does not support supplementation of any of these antioxidant vitamins for prevention of coronary heart disease, and the intake of their primary food sources, especially fruits and vegetables, is encouraged.⁸

Observations relating to particular eating habits, mainly excessive intake of certain nutrients, with the etiology and development of chronic diseases, have led to the concept of "optimal nutrition." In this context, the development of "functional foods" is initiated with the aim that, being consumed within the habitual diet, they contribute to providing a beneficial effect on health and/or a risk reduction for certain diseases.⁹ The incorporation of these products into the diet is of great interest and may have a profound impact from a public health viewpoint, not just because it may affect wide groups of populations but because these diseases start at early ages, provoking a high cost to society and governments in financial terms, as well as in terms of disability-adjusted life years.

Within this context and considering the present state of and trends in meat consumption, we evaluate the nutritional basis for and the technological approach to the development of functional meat-based products potentially relevant in CVD risk reduction.

Scientific Basis for the Development of Functional Meat Products Containing Nuts for CVD Risk Reduction

The relationship between dietary fats and CVD, especially coronary heart disease, is strong and consistent. In industrialized countries, some 35–40% of the total energy in the food supply comes from fat, nearly half of which is from meat intake.^{10,11} Saturated fatty acids raise total and low-density lipoprotein (LDL) cholesterol, but individual fatty acids have different effects. Myristic and palmitic acids have the greatest effect and are abundant in diets rich in dairy products and meat, and it is suggested that the most effective replacements for saturated fats in terms of coronary heart disease outcome are polyunsaturated fatty acids (PUFA), especially linoleic acid.¹

Nuts and seeds are energy-dense foods and good sources of unsaturated fats, protein, fiber and microconstituents. On a population basis, there is a lack of reliable data regarding the consumption of nuts and seeds as a separate class of foods and regarding the effect of nut consumption on risk of CVD,^{6,12} although their differential nutritional composition supports a

potential beneficial effect on serum lipid profile, thrombosis and atherogenesis. Particularly, walnuts display a high fat content (62–68%), being rich in monounsaturated (oleic acid) and polyunsaturated fatty acids (with linoleic and α -linolenic acids constituting 58% and 12% of PUFA content, respectively), as well as fiber (5–10%), arginine-rich proteins, magnesium, potassium, phytosterols, and other micronutrients (i.e. selenium, ellagic acid).^{3,4,12–15}

Compared with other nuts (almonds, hazelnuts), walnuts contain very low amounts of α -tocopherol (ca. 2 mg vs 25 mg/100 g in hazelnuts and almonds) but a high content of γ -tocopherol (41 mg vs 2 mg/100 g),¹⁶ constituting an excellent dietary source of this vitamer. In different studies, low serum levels of γ -tocopherol (lipid-adjusted), but not α -tocopherol, have been observed in subjects with ischaemic heart disease.^{17–20} Based on the epidemiological evidence and the potential health benefits of several constituents of nuts, frequent or daily consumption of nuts, including walnuts, is recommended^{1,6,21} and, recently, "a sufficient basis for a qualified health claim about walnuts and reduced risk of heart disease" has been acknowledged.²²

Supporting the biological plausibility, γ -tocopherol exhibits a high antioxidant activity with potentially important physiological implications.^{23–26} Both γ -tocopherol and its major metabolite (γ -carboxyethyl hydroxychroman) have been shown to display other relevant activities including reactive nitrogen oxide species trapping, inhibition of ex vivo superoxide formation, natriuretic activity, inhibition of cyclooxygenase-2 activity (anti-inflammatory effect), up-regulation of endothelial nitric oxide synthase, and nitric oxide formation, reduction of platelet aggregation and delaying of arterial thrombus formation.^{24,27–30}

The Potential Role of the Meat Industry

Food fortification has proven to be an effective and low-cost way to increase the micronutrient supply and reduce the consequences of micronutrient deficiencies.^{31–33} This fact, along with other socioeconomic factors, has encouraged industries to improve the perceived value of their products through fortification.³³ As is the case in other food sectors, the possibility of developing healthier meat and meat products implies, on one hand, avoiding or limiting the presence of undesirable constituents and, on the other, increasing the content of those that are healthy or desirable. The strategies available for carrying out these changes include actions at the animal production level, those associated with the handling of meat raw material, and those related to processes of reformulation of meat products.^{34,35}

In this context, a practical approach to increase nut consumption is to incorporate them into frequently consumed foods, i.e. meat products. However, although the idea of introducing several ingredients into meat and reformulated meat products is not new,^{36–39} the development of derivatives of this type involves the careful consideration of numerous aspects since the newly developed meat product, among other things, must have the appropriate technological, sensory, and nutritional properties and be safe and convenient for consumption.^{35,40}

Table 1 Proximate analysis (%)^a, tocopherol content^b in raw restructured beef steak and average intake during the pilot intervention study

	Moisture	Protein	Fat	Ash	pH
Walnut-free ^a	74.0	22.2	2.2	1.0	5.7
Walnut-containing ^a (20%)	60.8	19.2	13.6	1.1	6.0
	γ -tocopherol (μ mol/100 g)		α -tocopherol (μ mol/100 g)		δ -tocopherol (μ mol/100 g)
Walnut-containing (n = 9)	8.64–10.10		0.07–0.21		1.89–2.73
Walnut-free (n = 3)	0.04–0.05		0.12–0.14		nd
	γ -tocopherol intake		Walnut consumption equivalence ¹		
	12.96–15.12 μ mol/steak serving ²		30 g (5–6 unshelled walnuts)		
	62.40–72.00 μ mol/week ³		136 g ³		

^a Taken from ref. 46.^b by means of a Quality-controlled HPLC method ⁵⁰

nd: not detected.

¹ Amount of walnut powder added.² Steak serving: 150 g.³ Based on the consumption of 4 steaks plus 1 sausage (80 g) per week.

Meeting Nutritional and Technological Challenges

Within a multidisciplinary project, we address the development of a functional meat product of this type using reformulation technology. Specifically, our aim was to design and develop meat products that were modified qualitatively and quantitatively, to provide products with a nutrient composition profile associated with a decreased risk for CVD. For this purpose, several aspects had to be addressed: how much meat could be supplied to be acceptable and sustainable within a balanced diet, while being compatible with nutritional recommendations (target groups); based on the available scientific evidence, which nut(s) and what amounts should and could be incorporated into the final meat product (technological challenge); and, finally, how to assess the efficacy of the product for the purpose for which it had been developed (human intervention trial and use of biomarkers).

Walnuts were selected as a good source of nutrients relevant to CVD (i.e. PUFA content, ω -6 and ω -3 fatty acids, γ -tocopherol, arginine-rich protein), compared to other nuts and plant foods and because of their generally good consumer acceptability. In addition, this approach provides several other constituents of walnuts (i.e. phytosterols, polyphenols, fiber) without substantially reducing other nutritionally important components of meat such as iron and zinc. Finally, the amount of walnuts to be incorporated into the final product was based, in part, on nut and meat consumption data in our country,^{41,42} data from epidemiological and clinical studies with nuts⁶ and recent recommendations about nut intake.^{21,43,44}

The technological approach, manufacturing and preparation procedures, sensory properties, and other characteristics of the new derivative have been described elsewhere⁴⁵ (Spanish Patent 2003–00367). Two complementary approaches were selected; firstly, the reduction of visible fat (and connective tissue) in the meat and, secondly, the incorporation of walnuts by means of reformulation technology. Briefly, beef top rounds were coarsely

ground and mixed with salt (1.5%) and sodium tripolyphosphate (0.5%), and walnut was added (up to 20% on a weight basis). Restructured beef steaks (150 g) were formed, frozen, and stored at -20°C until use. The amount of walnuts to be added was based on what was considered a practical level of intake for use in dietary intervention studies (Table 1) and consistent with the “scientific evidence that suggests, but does not prove, that eating 1.5 ounces of nuts, such as walnuts, a day as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease.”²²

The proportion of added walnut significantly affected the proximate analysis of the restructured beef steaks.^{45–47} Although protein and ash contents were similar, the addition of walnut gradually increased fat values and reduced moisture levels, consistently with meat product formulations where meat was replaced by added walnut. Although the fat content in the new products was increased (ca. 13%) at the highest percentages of walnut incorporated (i.e. 20%), >85% would be provided by walnuts and, therefore, from a health standpoint, the lipid profile in restructured beef is substantially improved.

Assessing the Effect of Meat-based Functional Products on Intermediate Biomarkers of CVD

Despite the great amount of development in the design and marketing of food products with functional properties, there is an important gap regarding the assessment of the “functionality” of the nutrients incorporated (i.e. bioavailability and their effects on intermediate risk markers in humans). As defined, “a food can be regarded as *functional* if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease.”⁹ Evidence supporting these “functional” properties may derive from epidemiological and experimental

in vitro studies, animal and human assays, and intervention trials using intermediate biomarkers with relevance in the prediction and/or prognosis of the disease.

In this context, we approached this goal from complementary perspectives: first, by evaluating the post-prandial (single-dose) bioavailability of different nut components contained in the new product (i.e. γ -tocopherol and fatty acid profile) as markers of exposure and, second, by assessing the long-term bioavailability of some nutrients (i.e. γ -tocopherol) in a group considered at risk for cardiovascular disease (>45 years, body mass index >25–<35, post-menopause, smoking habit, total serum cholesterol 220–290 mg/dl, 140–90 mg Hg).^{1,21} The impact of its regular consumption on several markers and indicators related to CVD (biomarkers of function and risk indicators) include those for lipid homeostasis, thrombosis, antioxidant and inflammatory markers, blood pressure, serum homocysteine, B₆, B₁₂, and folate.

The dietary intervention protocol consists of an unblinded, crossover, multiple-dose (regular consumption, 5 times/week for 5 weeks) bioavailability study, with a 1-month wash-out in between (reformulated products with versus without added walnut). This intervention study, however, considered two major factors in diet-health studies; the exposure dose and the potential occurrence of a time-lag effect similar to that observed in sodium restriction and blood pressure trials.⁴⁸ Subjects were advised not to modify their usual diets and lifestyle, except for avoiding regular consumption of nuts and the substitution of the new meat derivative for their habitual meat servings. Compared to previous human trials with walnuts, the protocol and “dose” used (5 steaks/week, ca. 150g/serving) is considered to be practical in the “real world” and easily incorporated into and sustainable in the diet. Based on 20% walnut-enriched reformulated products, the consumption of a 150 g portion/day would provide ca. 70% of the amount of nuts considered adequate,²² and this is consistent with recommendations regarding both the quantity and the frequency of nut consumption.^{21,43,44}

To date, *in vivo* evidence on the absorption of active substances (γ -tocopherol as a marker of walnut-containing meat products) has been obtained from a single-dose crossover bioavailability study where subjects consuming these products display an increase in γ -tocopherol in triglyceride-rich lipoproteins during the post-prandial period, a change not observed when these subjects consumed the same reformulated meat product without the addition of walnuts. The multiple-dose crossover intervention study is being carried out and it is worth mentioning that although many subjects are conscious of displaying three or more risk factors for CVD (target group), the response of the potential volunteers is far from ideal, with an intent to participate of less than 30% of those contacted.

FINAL CONSIDERATIONS

In our opinion, the nutritional relevance of the presented approach rests on testing the efficacy of the meat-based product

for the purpose for which it was developed (CVD risk reduction), incorporating short- and medium-term intervention trials, together with the use of intermediate cardiovascular markers in target groups. Within the conceptual framework of evidence-based medicine, we assess the impact on intermediate CVD biomarkers derived from its consumption.

Compared to previous human intervention trials with nuts, this study may provide relevant information on the potential role of the meat industry in using a food-based approach and reformulation technology to provide functional meat products. The dietary intervention used, involving the application of a food-based approach, is considered to be practical in the “real world” –easily incorporated into and sustainable in the diet. In this respect, results from the ongoing multiple dose study may not only be important in terms of the new product labelling; if, as estimated by Sabaté et al.,⁴ an increase in walnut consumption from 4 g/day to 28 g/day would lower cholesterol and LDL-C by 4% and 6%, respectively, then a similar, small, non-significant lowering effect, if observed in the present study, might also be sufficiently relevant at a population level.

At present, although many biomarkers for CVD risk have been proposed, when these are assessed in terms of effectivity criteria (i.e. biological plausibility, consistency with epidemiology, response in clinical trials, etc.), consensus among scientists exists only for LDL-cholesterol and blood pressure regarding a possible application as enhanced function claims.⁴⁹ For other parameters such as HDL-cholesterol, fasting triglycerides and homocysteine, however, firm proof that diet-induced changes in these biomarkers alter the risk of CVD is lacking and, in this respect, we should keep in mind the fact that risk factors are frequently related to each other and, thus, the best approach is to assess biomarkers related to different aspects of the disease process.

In summary, using a food-based rather than a nutrient-based approach, new meat-based products may incorporate several constituents that are potentially important in relation to CVD. The regular consumption of these products, as substitutes for other “natural” livestock products, is sustainable and compatible with a balanced diet, and may have a beneficial impact on intermediate markers of CVD. In this respect, although the effect on these biomarkers might not be significant on an individual basis, slight improvements in atherogenic lipid profiles, blood pressure and/or thrombosis, and inflammatory markers could be of great importance at a population level. Taking advantage of the nutritional scientific rationale and technological skills and, thus, specific applications, potential market and public health benefits may be enormous.

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CONFLICTS OF INTEREST

The authors declare that they have no competing financial interests.

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