Functional foods for coronary heart disease risk reduction: a meta-analysis using a multivariate approach[1–3]

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ABSTRACT

Background: It has been difficult to identify the appropriate bioactive substance for the development of new functional foods associated with coronary heart disease, because the results of many clinical studies are contradictory.

Objective: The objective of this study was to use the multivariate statistical approach known as principal component analysis (PCA) followed by a mixed model to process data obtained from a meta-analysis aimed at evaluating simultaneously the effect of ingestion of 1 of 3 types of bioactive substances (n−3 fatty acids, soluble fibers, and phytosterols) on 1 or more of 4 biomarkers (plasma total cholesterol, triacylglycerol, LDL cholesterol, and HDL cholesterol).

Design: Five independent variables (number of patients per study, dose, age, body mass index, and treatment length) and 4 dependent variables (percentage change in blood total cholesterol, LDL, HDL, and triacylglycerol) from 159 studies and substudies were organized into a matrix. The original values were converted to linear correlation units, which resulted in a new matrix.

Results: Two principal components were enough to explain 63.73% and 84.27% of the variance in the independent and dependent variables, respectively. Phytosterols and soluble fibers had a hypocholesterolemic effect, whereas n−3 fatty acids lowered triacylglycerol and increased total, LDL, and HDL cholesterol. The PCA and mixed model showed that this behavior was independent of dose, number of patients per study, age, and body mass index but was associated with treatment length.

Conclusions: PCA is useful for summarizing available scientific information in examinations of health claims for foods and supplements.   *Am J Clin Nutr* 2005;82:32–40.

KEY WORDS   n−3 Fatty acids, phytosterols, soluble fibers, cholesterol, multivariate analysis, functional foods

INTRODUCTION

The role of plasma lipids in the etiology of atherosclerosis and coronary heart disease has been well defined. A high plasma concentration of total cholesterol, triacylglycerol, and LDL cholesterol and a low plasma concentration of HDL cholesterol are considered important risk factors for the expression of coronary disease (1), and these plasma indexes or biomarkers must be jointly considered in the assessment of risk for populations (2).

Several studies have shown that the ingestion of bioactive substances—such as certain types of n−3 fatty acids (linolenic, eicosapentaenoic, and docosahexaenoic acids), soluble fibers (guar gum, psyllium, pectin, and oat products), and phytosterols (stanols and sterols)—may have a positive and significant lipidemic effect (3-5). However, reported contradictory results impair the choice of one or more substances for the development of new foods that could promote a reduction in the risk of coronary heart disease in humans (6-11). For the development of such foods, known as functional foods (12-14), in addition to satisfying all criteria necessary for the formulation of a regular food, one must also assess their functional efficiency on the basis of alterations in biomarkers.

Statistical techniques normally adopted in a meta-analysis, such as general regression models, summarize important information; however, they deal with one variable at a time. Multivariate approaches are statistical procedures capable of promoting data reduction or structural simplification, sorting and grouping, investigating the dependence among variables, predicting, and hypothesis testing. These approaches have been widely used in several areas of research such as medicine, sociology, business, education, psychology, and sports (15). In addition to being very efficient tools, especially in the study of correlations involving a large number of variables and sample units, these approaches have rarely been applied in nutritional research, despite this being an area in which multivariate correlation studies are essential.

The objective of this study was to present the multivariate statistical approach known as principal component analysis (PCA) followed by a mixed model to process data obtained from a meta-analysis aimed at evaluating simultaneously the effect of ingestion of 1 of 3 types of bioactive substances (n−3 fatty acids, soluble fibers, and phytosterols) on 1 or more of 4 biomarkers (plasma total cholesterol, triacylglycerol, LDL cholesterol, and HDL cholesterol).

SUBJECTS AND METHODS

Study characteristics

Studies of the effects of some n−3 fatty acids, phytosterols, and soluble fiber on blood total cholesterol, LDL-cholesterol,

TABLE 1
Inclusion criteria for the meta-analysis studies

Criterion	Specification
Sample	Humans
Treatment length	> 10 d
Functional ingredients	n−3 Polyunsaturated fatty acids (eicosapentaenoic acid, docosahexaenoic acid, or α-linolenic acid); phytosterols (plant sterol or stanol); soluble fiber (guar gum, pectin, oat products, or psyllium)
Health of subjects at baseline	Classified according to initial cholesterol and triacylglycerol concentrations measured under fasting conditions
Form of ingestion of the functional ingredient	Food, supplementation by capsules, or both
Experimental design	Placebo-controlled with a randomized, crossover, or parallel design
Blood biomarker concentrations	Net changes in blood cholesterol, LDL-cholesterol, HDL-cholesterol, and triacylglycerol concentrations were presented as relative values (%) between the final (posttreatment) and initial concentrations in the studies, with subtraction of the same effect observed in the respective placebo group, in both the studies with a parallel design and those with a crossover design

HDL-cholesterol, and triacylglycerol concentrations in adults were identified by a computerized literature search of the Internet [Web of Science, Elsevier, and MEDLINE (National Library of Medicine, Bethesda, MD)] of articles primarily published (96%) from January 1990 to April 2003 and by examining their respective cited reference sources. The full texts were obtained from the Library of the Institute of Chemistry of the University of São Paulo (USP), Brazil, and from the Louise M Darling Biomedical Library of the Center for Health Sciences, University of California. Only published trials reported in English were considered. Studies were selected for analysis according to the criteria shown in **Table 1**. For this meta-analysis, studies involving supplementation with more than one type of functional ingredient and studies that applied more than one dose or intervention time were partitioned to be treated as substudies. The dependence of these specific studies and substudies was considered in the mixed model.

Specification of the variables

In the present meta-analysis, 4 dependent variables (percentage change in cholesterol, LDL cholesterol, HDL cholesterol, and triacylglycerol) and 5 independent variables [number of patients per study, dose, age, body mass index (BMI), and treatment length] were obtained from 159 studies and substudies that were described in detail by the respective authors (**Table 2**) cited in the reference.

The change in biomarkers (Δ%) was calculated according to the following equation:

$$\Delta\%\text{Biomarker} = \{[(T_1 - T_0)/T_0] - [(P_1 - P_0)/P_0]\} \times 100 \tag{1}$$

TABLE 2
References reviewed in this meta-analysis categorized by the bioactive substance applied in the dietary interventions

Bioactive substance	Reference[1]	n[2]
Phytosterols	16–43	61
Soluble fibers	4, 7, 44–61	32
n−3 Fatty acids	10, 62–90	66

[1] The reference numbers correspond to the same studies presented in Figures 3 and 4.

[2] Corresponds to the total number of studies and substudies in each bioactive substance group.

where T_1, T_0, P_1, and P_0 were the final and initial blood concentrations of the specific biomarker for the experimental and the placebo groups, respectively. The number of patients per study was obtained from the total number of individuals included in the study or substudy, with ≈50% corresponding to the experimental and 50% to the placebo groups. Dose was considered as the functional ingredient in its pure form and was reported in grams. Age (y) and BMI (kg/m^2) were obtained from the mean for the experimental and placebo groups at baseline. The treatment length variable was computed with respect to the effective period of intervention, excluding both the adaptive and the washout periods in the crossover studies. Studies whose variables could not be included in this classification were not considered for this meta-analysis.

In general, the studies showed closely similar patterns concerning the form of bioactive substance ingestion, sex, experimental design, subject's baseline healthy conditions (cholesterol < 8 mmol/L and triacylglycerols < 3 mmol/L), diet (energy ≈2500 kcal, cholesterol ≈300 mg, and total fat ≈30% of energy), moderate practice of physical exercise, alcohol consumption, smoking habit, and use of mild drugs or other nutritional supplements, which were normally discontinued a few months before or maintained without changes during the intervention. Similarities in lifestyles allowed the conclusion that alterations observed in the biomarkers resulted basically from the nutritional interventions.

Statistical analysis

PCA was the multivariate technique applied to assess the association between the 4 dependent variables and the 5 independent variables and to categorize the studies and substudies according to the plane generated by the main components. PCA is only a descriptive statistical procedure, which does not involve any supposition about variance homogeneity. It is a simple and adequate descriptive technique to handle quantitative variables. The data matrices for independent variables (159 × 5) and for dependent variables (159 × 4), expressed in different units (g, kg/m^2, y, d, and %), were prepared by adopting the variables as columns and the studies and substudies as rows. First, statistical standardization was performed to obtain relativized data to which the multivariate technique was applied. The original values were converted into linear correlation units to form a new matrix, which was used as the base for PCA. The grouping

TABLE 3

Summary of variables observed in the studies included in the meta-analysis[1]

Variable	Median	$\bar{x} \pm$ SD	n
Independent			
Number of patients per study	39.00	84.28 ± 147.32	159
Dose (g)	2.76	3.77 ± 3.06	159
Age (y)	48.65	46.86 ± 11.63	151
BMI (kg/m^2)	25.94	25.64 ± 2.14	144
Treatment length (d)	42.00	61.49 ± 60.21	159
Dependent			
ΔCholesterol (%)	−3.76	−3.57 ± 5.34	130
ΔTriacylglycerol (%)	−11.29	−11.50 ± 13.97	125
ΔHDL cholesterol (%)	1.43	1.79 ± 5.72	111
ΔLDL cholesterol (%)	−4.50	−3.88 ± 6.87	115

[1] Δ, change.

variables were designated as n−3 fatty acids, soluble fiber, and phytosterols. Correlations between the variables of all selected studies and substudies were used, with the variables and "studies and substudies" being grouped as a function of similarities.

With respect to the variables, the number of factors obtained should be determined by the number of eigenvalues >1.0 (91). Eigenvalues correspond to vectors capable of holding part of the variation observed when the original values (4 and 5) are reduced into the principal components. The studies and substudies were plotted graphically on the two-dimensional plane generated by the variables. A mixed model was fitted using 2 principal components of the dependent variables as response. All calculations were performed by using STATISTICA software (version 6; Statsoft Inc, Tulsa, OK), except those in the mixed model, for which SAS software (SAS Institute Inc, Cary, NC) was used.

RESULTS

Study characteristics

The studies reported the effect of 3 different bioactive substances—n−3 fatty acids, phytosterols, and soluble fibers—on the percentage change in cholesterol, LDL cholesterol, HDL cholesterol, and triacylglycerol under different experimental conditions (**Table 3**). With this initial approach, it was possible to observe that the general dietary interventions promoted average net reductions in cholesterol, LDL cholesterol, and triacylglycerol of 3.57%, 3.88%, and 11.50%, respectively, and a net increase in HDL cholesterol of 1.79%.

TABLE 4

Partitioning of the factors into principal components for the independent variables (number of patients per study, dose, age, BMI, and treatment length)

Component	Eigenvalue	Total variance	Cumulative eigenvalue	Cumulative variance
		%		%
1	1.86	37.13	1.86	37.13
2	1.33	26.60	3.19	63.73
3	0.88	17.63	4.07	81.36
4	0.62	12.41	4.69	93.77
5	0.31	6.23	5.00	100.00

TABLE 5

Partitioning of the factors into principal components for the dependent variables: percentage change in cholesterol, LDL cholesterol, HDL cholesterol, and triacylglycerol

Component	Eigenvalue	Total variance	Cumulative eigenvalue	Cumulative variance
		%		%
1	2.33	58.21	2.33	58.21
2	1.04	26.06	3.37	84.27
3	0.55	13.68	3.92	97.95
4	0.08	2.05	4.00	100.00

Multivariate analysis

The eigenvalues obtained by PCA for the independent and dependent variables are presented in **Tables 4** and **5**, respectively. They were arranged in decreasing order, indicating the importance of the respective factor in explaining the variation of the data. On the basis of the decision criteria recommended by Piggot and Sharman (91), 2 factors (eigenvalues > 1.0) were selected and the factor coordinates of the variables, based on correlations for each one, are presented in **Table 6**. Two principal components were enough to explain 63.73% and 84.27% of the variance in the independent and dependent variables, respectively. The linear correlations between the dependent and independent variables, including the principal components PCD$_1$ and PCD$_2$ (first and second principal components for dependent variables) and PCI$_1$ and PCI$_2$ (first and second principal components for independent variables), are presented in **Table 7**. The correlations were low. The correlation between treatment length and the percentage change in cholesterol, LDL cholesterol, PCD$_1$, and PCD$_2$ and the correlation between PCI$_2$ and PCD$_2$ were significantly different from zero. The vectors relative to the centered and reduced variables, selected as active in this meta-analysis, were located on the circumference and are graphically represented in **Figures 1** and **2**.

The distribution of the 159 studies and substudies on the plane generated by the variables is shown in **Figures 3** and **4**. The contribution of BMI and age to the first principal component and of treatment length and number of patients per study to the second principal component are shown in Figure 1. The dose variable did not present a high correlation with the 2 principal components discussed in this study. Considering the dependent variables, the

TABLE 6

Factor-variable correlations (factor loadings) based on correlations[1]

Variable	Factor loading 1	Factor loading 2
Independent		
Number of patients per study	0.42	−0.72
Dose	0.40	0.43
Age	0.89	0.11
BMI	0.79	0.34
Treatment length	0.33	−0.71
Dependent		
ΔCholesterol	−0.83	−0.52
ΔTriacylglycerol	0.56	−0.70
ΔHDL cholesterol	−0.68	0.45
ΔLDL cholesterol	−0.92	−0.29

[1] Δ, change.

TABLE 7
Linear correlations between the independent and dependent variables, including the first and second principal component for dependent variables (PCD) and the first and second principal component for independent variables (PCI)[1]

Variable	Δ%Cholesterol	Δ%Triacylglycerol	Δ%HDL cholesterol	Δ%LDL cholesterol	PCD_1	PCD_2
Number of patients per study	0.069	0.003	−0.060	0.132	−0.078	−0.140
Dose	0.105	0.073	0.061	0.046	−0.070	−0.008
Age	−0.028	0.001	−0.163	−0.067	0.018	−0.115
BMI	0.035	−0.099	0.011	0.051	−0.119	0.084
Treatment length	0.189[2]	−0.040	0.041	0.261[3]	0.201[2]	0.325[3]
PCI_1	0.088	−0.049	−0.058	0.098	−0.132	−0.102
PCI_2	−0.074	−0.016	0.006	−0.156	0.056	0.238[2]

[1] Δ, change.
[2] $P < 0.05$.
[3] $P < 0.01$.

influence of the percentage change in LDL cholesterol, cholesterol, HDL cholesterol, and triacylglycerol on the first principal component and the influence of triacylglycerol on the second principal component are shown in Figure 2.

The mixed-model analysis was performed for the first 2 dependent principal components. The final equations are presented in **Table 8**. In the initial models, the bioactive substances (n−3 fatty acids, phytosterols and soluble fibers) were included as a fixed effect, studies as a random effect, and PCI_1 and PCI_2 as well as the interaction between type of study and independent principal components as exploratory variables. The mixed-model analysis showed that the bioactive substances and PCI_2 had a statistically significant effect. The interaction effects and the main effect of the first independent principal component were removed from the model because none of them were statistically significant at a 0.05 level of significance. A multiple comparisons Tukey's test was applied to compare the 3 intercepts

corresponding to the type of study. For the first dependent principal component, *P* values adjusted with the Tukey-Kramer test were as follows: phytosterols versus soluble fibers ($P = 0.2310$), n−3 fatty acids versus phytosterols ($P < 0.0001$), and n−3 fatty acids versus soluble fibers ($P < 0.0001$). This result indicates that the intercept for phytosterols and soluble fibers is equal and both variables are different from the intercept for n−3 fatty acids. For the second dependent principal component, *P* values adjusted with the Tukey-Kramer test were as follows: n−3 fatty acids versus phytosterols ($P = 0.7843$), n−3 fatty acids versus soluble fibers ($P = 0.1022$), and phytosterols versus soluble fibers ($P = 0.0154$). The conclusion is that the intercept for n−3 fatty acids and phytosterols is equal and both of them are different from the intercept for soluble fibers. A residual analysis was performed and it did not show any major departures from the assumptions, which indicated that the model was appropriate for the data.
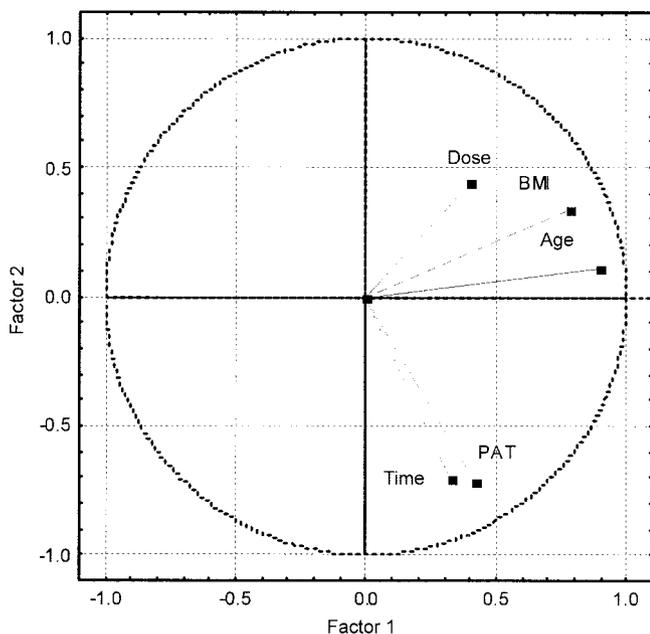
FIGURE 1. Projection of the independent variables on the factor plane [factor 1 (37.13%) compared with factor 2 (26.60%)]. PAT, number of patients per study; Time, treatment length.
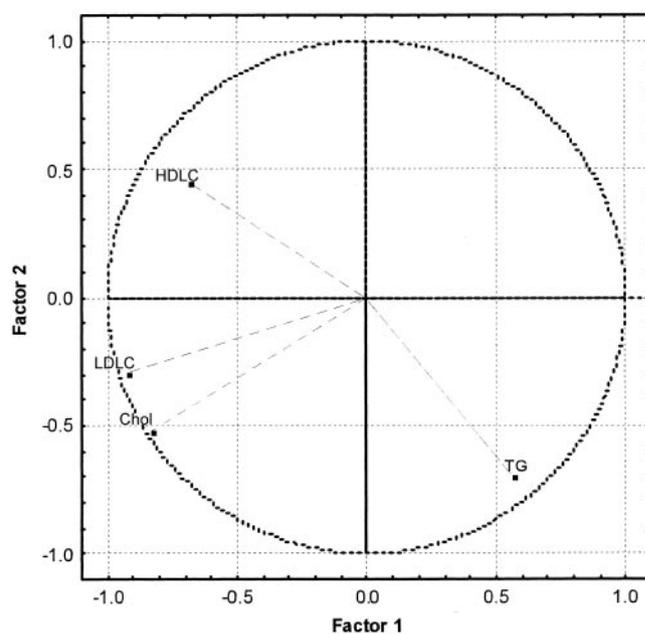

FIGURE 2. Projection of the dependent variables on the factor plane [factor 1 (58.21%) compared with factor 2 (26.06%)]. TG, triacylglycerol; Chol, cholesterol; HDLC, HDL cholesterol; LDLC, LDL cholesterol.
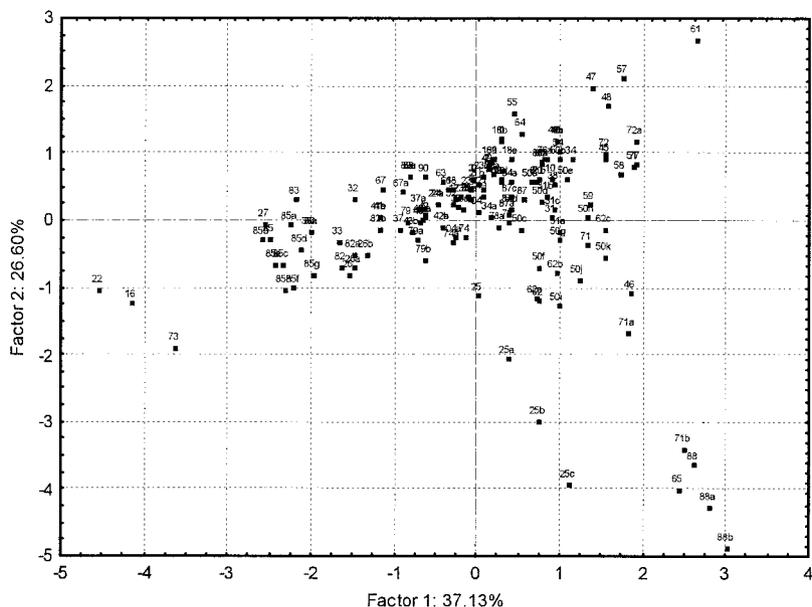
FIGURE 3. Projection of the studies and substudies on the factor plane produced by the independent variables (factor 1 compared with factor 2), where references 16–43 represent studies of phytosterols; 4, 7, and 44–61 represent studies of soluble fibers; and 10 and 62–90 represent studies of fatty acids. The letters following the reference numbers represent substudies.

DISCUSSION

Why submit the data obtained by this meta-analysis to multivariate statistical analysis?

It is impossible to use the simple ordering of information to reach the objective of this study. Multivariate analysis presents several alternative statistical procedures that permit a simplified structure of the data without relevant loss of information, which transforms an expressive number of original variables into a smaller number of new noncorrelated variables (15).

Regression models and univariate analysis have often been used in a statistical approach to a meta-analysis, which indicates important correlations between independent and dependent variables. However, many of these variables are correlated and could be substituted by principal components. Multivariate techniques are able to identify such correlations, taking into account a "p" number of variables of interest, with the same weight and at the same time, expressing these correlations graphically. It can be said that techniques such as PCA for quantitative variables present a complete picture of the study, the visual
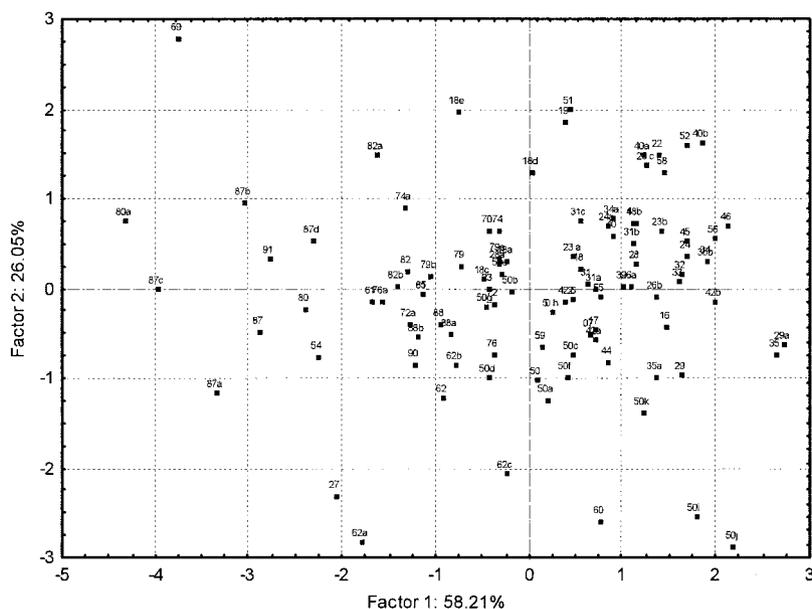

FIGURE 4. Projection of the studies and substudies on the factor plane produced by the dependent variables (factor 1 compared with factor 2), where references 16–43 represent studies of phytosterols; 4, 7, and 44–61 represent studies of soluble fibers; and 10 and 62–90 represent studies of fatty acids. The letters following the reference numbers represent substudies.

TABLE 8
Mixed model applied to the first and second principal component for dependent variables (PCD_1 and PCD_2)[1]

	Equation[2]	P	Equation[3]	P
Mixed model for first PCD_1				
n−3 Fatty acids	$PCD_1 = -1.6301^a - 0.2224\ PCl_2$	0.0001^4	$PCD_1 = -1.6329 - 0.2291\ PCl_2$	0.0001^4
	(0.2011) (0.1022)		(0.2031) (0.1031)	
Phytosterol	$PCD_1 = 0.9935^b - 0.2224\ PCl_2$	0.0001^4	$PCD_1 = 0.8233 - 0.2291\ PCl_2$	0.0001^4
	(0.1744) (0.1022)		(0.1428) (0.1031)	
Soluble fibers	$PCD_1 = 0.5156^b - 0.2224\ PCl_2$	0.0325^4	$PCD_1 = 0.8233 - 0.2291\ PCl_2$	0.0001^4
	(0.2326) (0.1022)	0.0345^5	(0.1428) (0.1031)	0.0311^5
Mixed model for second PCD_2				
n−3 Fatty acids	$PCD_2 = 0.0457^c + 0.2396\ PCl_2$	0.8081^4	$PCD_2 = 0.1409 + 0.2564\ PCl_2$	0.2468^4
	(0.1867) (0.0949)		(0.1198) (0.0912)	
Phytosterol	$PCD_2 = 0.2132^c + 0.2396\ PCl_2$	0.1958^4	$PCD_2 = 0.1409 + 0.2564\ PCl_2$	0.2468^4
	(0.1620) (0.0949)		(0.1198) (0.0912)	
Soluble fibers	$PCD_2 = -0.5659^d + 0.2396\ PCl_2$	0.0125^4	$PCD_2 = -0.5724 + 0.2564\ PCl_2$	0.0112^4
	(0.2160) (0.0949)	0.0150^5	(0.2151) (0.0912)	0.0071^5

[1] SE of the coefficient in parentheses. PCl_2, second principal component for independent variables. Intercepts with different superscript letters are significantly different, $P < 0.05$.

[2] Before the multiple comparisons Tukey's test applied to compare the 3 intercepts corresponding to the type of study.

[3] After the multiple comparisons Tukey's test applied to compare the 3 intercepts corresponding to the type of study.

[4] The intercept is significantly different from zero.

[5] The PCl_2 coefficient is significantly different from zero.

analysis of which contributes substantially to the interpretation of the results.

What information can be obtained from the reduction of factors and from grouping in multivariate analysis?

The data presented in Table 3 agree with those presented in other studies, which take into account the net effect of the experimental group. In a meta-analysis, Bucher et al (92) investigated the effects of dietary and supplemental intake of n−3 fatty acids on coronary heart disease. They observed that the antilipidemic effect was limited to an average 20% reduction in triacylglycerol concentrations; no effect on LDL- or HDL-cholesterol concentrations was observed. According to Quílez et al (93), over the past decade the possibility of using phytosterols as ingredients in functional foods has led to numerous studies of their ability to reduce blood cholesterol. The main conclusion was that the effective doses were between 1.5 and 3.0 g/d, which led to reductions in LDL of between 8% and 15%. The principal mechanism of action was hypothesized to be interference with the solubilization of the cholesterol in the intestinal micelles, reducing its absorption. In a meta-analysis conducted by Brown et al (94), the authors concluded that soluble fibers (2-10 g/d) were associated with small but significant decreases in total and LDL-cholesterol concentrations, with no effect on triacylglycerol and HDL-cholesterol concentrations.

The plane generated by the independent variables, where the vectors that present a good correlation to the 2 principal components, are located closer to the edge of the circumference (Figure 1). The independent variable dose did not correlate with the 2 first principal components. BMI and age were highly positively correlated with each other because BMI increases with age. However, there is no explanation for the high positive correlation between the number of patients per study and treatment length, which is a typical example that correlations may reflect a coincidental rather than a causal relation. The data in Figure 3 suggest that intervention studies providing the 3 bioactive substances do not follow a standard procedure according to age, BMI, sample

size, and dose. This fact leads to the conclusion that the biomarker alterations observed in Figure 4 were obtained from a randomized experimental condition. The mixed-model analysis (Table 8) showed that PCl_2 had a significant effect on both PCD_1 and PCD_2, and it is suggested that treatment length was the variable responsible for this effect (Table 7). That is, a shorter treatment length (PCl_2 negative) reduces PCD_1 and increases PCD_2. Although the second principal component represents the most desirable outcome for blood lipoproteins, ie, a reduction in triacylglycerol followed by a reduction in cholesterol and LDL cholesterol, the variation promoted by the dietary intervention assessed in this study was better represented in the first and second principal components. In the latter case, soluble fibers showed an effect different from that of n−3 fatty acids and phytosterols.

The dietary interventions promoted a positive correlation between the percentage change in cholesterol, LDL cholesterol, and HDL cholesterol and an inverse correlation with the percentage change in triacylglycerol (Table 6). n−3 Fatty acids have a different effect on PCD_1 compared with soluble fibers and phytosterols as a consequence of the different way in which these 3 bioactive substances act on human metabolism. Different mechanisms are responsible for the cholesterol-lowering effect of free and esterified phytosterols, such as competition for solubilization in dietary mixed micelles, cocrystallization with cholesterol to form insoluble mixed crystals, and interference with hydrolysis processes by lipases and cholesterol esterases (95). Evidence suggests that some soluble fibers bind bile acids or cholesterol during the intraluminal formation of micelles. The resulting reduction in the cholesterol content of liver cells leads to an upregulation of the LDL receptors and thus an increased clearance of LDL cholesterol. Soluble fibers could also promote the inhibition of hepatic fatty acid synthesis byproducts of fermentation as short-chain fatty acids (94). The hypolipidemia caused by n−3 fatty acid diets is well established and has been associated with various hepatic mechanisms such as increased fatty acid oxidation and inhibition of

de novo fatty acid synthesis secondary to decreased fatty acid synthase gene expression (96).

The percentage changes in cholesterol, LDL cholesterol, and HDL cholesterol were negatively correlated with the first principal component, whereas triacylglycerol made a positive contribution or that nutritional interventions that resulted in a reduction in the blood concentrations of triacylglycerol caused a considerable increase in LDL cholesterol, total cholesterol, and HDL cholesterol (Figures 2 and 4). This information should be printed on the labels of these functional foods because they can be purchased directly by consumers in supermarkets without medical supervision. In a study conducted in 6 hypertriglyceridemic patients supplemented with fish-oil concentrate, a 35% reduction in triacylglycerol was observed, which was accompanied by a 25% increase in LDL cholesterol, which suggested that n−3 fatty acids may enhance the propensity of VLDL cholesterol to be converted to LDL cholesterol (97). This could be a problem for those patients with modest elevations in triacylglycerol, in whom the elevation in LDL cholesterol impedes them from achieving their desired LDL-cholesterol concentration (8).

In summary, phytosterols and soluble fibers have a significant hypocholesterolemic effect, whereas n−3 fatty acids decreased triacylglycerol and increased total cholesterol, LDL cholesterol, and HDL cholesterol. The PCA and mixed models were able to show that this behavior is independent of dose, number of patients per study, age, and BMI but is associated with treatment length.

How should the results of this meta-analysis be used for the development of a functional food aimed at reducing the risk factors for coronary heart disease?

On the basis of the information generated by PCA and mixed models, the most adequate alternative for further studies should be the use of mixtures of the 3 bioactive substances to explore the maximum reduction of cholesterol, LDL cholesterol, and triacylglycerol with a maximum increase in HDL cholesterol. Other aspects of these 3 bioactive substances beside the hypolipidemic effect should be considered when developing function foods. For example, the major benefit of eating fiber-rich foods, including soluble fibers, may be a change in dietary pattern, resulting in a diet that is lower in saturated and *trans* unsaturated fats and cholesterol and higher in protective nutrients such as unsaturated fatty acids, minerals, folate, and antioxidant vitamins. Soluble fibers could promote slower absorption of macronutrients and increased satiety, which results in an overall lower energy intake (94). In addition, the contribution of n−3 fatty acids to the reduction in risk of coronary heart disease is mainly due to their effect at the vascular level. Eicosanoids synthesized from eicosapentaenoic acid are less potent in their ability to cause platelet aggregation or an inflammatory response than are corresponding eicosanoids derived from arachidonic acid (3, 83, 98, 99). Additional benefits of fish oils include improvements in endothelial function, better arterial elasticity, and modulation of inflammatory markers (100).

Conclusions

Although no health claims have been authorized on the basis of meta-analyses alone, they may be applied as supporting evidence for them. The multivariate statistical analysis applied in this study, PCA, showed correlations between 3 bioactive substances (n−3 fatty acids, soluble fibers, and phytosterols) and 4 blood biomarkers for coronary heart disease graphically. On the basis of these results, a more interesting proposal will be to develop further research that involves mixtures of these substances. The mixtures could be applied both for the purpose of developing new foods and for individual dietary planning, which will provide consumers with dietary alternatives capable of positively affecting plasma biomarkers and thus contribute to the control of coronary heart disease risk factors.

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