The Effect on Serum Lipids and Oxidized Low-Density Lipoprotein of Supplementing Self-Selected Low-Fat Diets With Soluble-Fiber, Soy, and Vegetable Protein Foods

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An increased intake of soluble fiber and soy protein may improve the blood lipid profile. To assess any additional benefit on serum lipids of providing soy protein and soluble-fiber foods to hyperlipidemic subjects already consuming low-fat, low-cholesterol therapeutic diets, 20 hyperlipidemic men and postmenopausal women completed 8-week test and control dietary treatments in a randomized crossover design as part of an ad libitum National Cholesterol Education Program (NCEP) step 2 therapeutic diet (<7% saturated fat and <200 mg/d cholesterol). During the test phase, foods high in soy, other vegetable proteins, and soluble fiber were provided. During the control phase, low-fat dairy and low–soluble-fiber foods were provided. Fasting blood lipid and apolipoprotein levels were measured at 4 and 8 weeks of each phase. On the test diet, 12 ± 2 g/d soy protein was selected from the foods chosen. Direct comparison of test and control treatments indicated an elevated high-density lipoprotein (HDL) cholesterol concentration on the test diet (6.4% ± 2.4%, $P = .013$) and a significantly reduced total to HDL cholesterol ratio (−5.9% ± 2.3%, $P = .020$). The proportion of conjugated dienes in the low-density lipoprotein (LDL) cholesterol fraction was significantly reduced (8.5% ± 3.3%, $P = .020$) as a marker of oxidized LDL. A combination of acceptable amounts of soy, vegetable protein, and soluble-fiber foods as part of a conventional low-fat, low-cholesterol therapeutic diet is effective in further reducing serum lipid risk factors for cardiovascular disease.

CARDIOVASCULAR DEATH remains the major cause of mortality in North America and most Western nations.[1] Increasingly, active treatment is now advocated for all modifiable risk factors.[2-8] It is estimated that as many as one fourth of all middle-aged men may require treatment with cholesterol-lowering medications.[8-10] At the same time, there is concern that maximum use should be made of dietary modification to reduce the need for drug therapy and also reduce lipid risk factors in the intermediate- and lower-risk groups.[8,10] These groups initially are not considered for drug therapy[8] but, by virtue of their numbers, are the groups in which the majority of cardiovascular deaths occur.[10]

Current National Cholesterol Education Program (NCEP) dietary advice focuses on dietary fat and cholesterol[8] and may reduce low-density lipoprotein (LDL) cholesterol by as much as 18%.[11] Nevertheless, other modifications including increased intake of soluble fiber,[12] vegetable protein,[13] and plant sterols[14] may each reduce serum cholesterol by an additional 5% to 10% or more. Whether these effects are additive is not known. Furthermore, plant phenolics, flavonoids in fruit and vegetables, isoflavones in soy, and lignans in flaxseed have also attracted attention for their potential cardiovascular benefits as antioxidants,[15-17] possibly through reducing LDL cholesterol oxidation. We therefore considered it important to assess the effect on serum lipids and oxidized LDL cholesterol of providing hyperlipidemic subjects with a selection of foods high in soy, vegetable protein, and soluble fiber that are available in supermarkets and health food stores. These foods were then available for incorporation into the subjects' self-selected low-fat, low-cholesterol diet, and the nature of the blood lipid changes was determined.

SUBJECTS AND METHODS

Twenty hyperlipidemic subjects (15 men and five women) aged 56 ± 2 years (range, 39 to 66) with a body mass index of 24.2 ± 0.5 kg/m^2 (range, 20.5 to 29.0) participated in a two-phase dietary trial in which soy, other vegetable protein, and soluble-fiber foods (test) were compared with low-fat dairy protein, low–soluble-fiber foods (control). Both phases lasted 8 weeks and were completed by all subjects in a randomized crossover design. During the test and control phases, subjects maintained their habitual self-selected low-fat, low-cholesterol (NCEP step 2) diet, on which they were instructed at least 3 months previously. Subjects were familiar with the experimental foods offered for incorporation into their diet, since they were part of a larger group of 31 subjects in a previous metabolic study[18] in which the same foods were provided as in the present study. The last phase of the previous study directly preceded the first 8-week phase of the present study. In the present study, eight subjects underwent the test treatment in the first phase. The two 8-week diet treatments followed each other directly with no break, with the exception of seven subjects whose individual work or holiday schedule made adherence to this protocol impossible. For these subjects, the study periods were discontinuous with breaks between the test and control phases, representing a washout period of 2 to 13 weeks (mean, 7 weeks), during which time these subjects continued to maintain their NCEP step 2 diets but without supplements. Subjects were unblinded since it was not possible to disguise the nature of the soy and dairy foods.

All subjects had elevated serum LDL cholesterol (>4.1 mmol/L)[8]

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and a triglyceride level less than 4.0 mmol/L at the time of recruitment. None had clinical or biochemical evidence of diabetes or liver or renal disease. One subject was under treatment with a 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) reductase inhibitor, lovastatin 20 mg/d (Mevacor; Merck Frost Canada, Kirkland, Quebec); one woman was on hormone replacement therapy with progesterone 100 mg/d (Prometrium; Schering Canada, Pointe Claire, Quebec) and 17β-estradiol 50 mg/wk (Estroderm Patch; Ciba-Geigy Canada, Mississauga, Ontario); and one man was under treatment with a β-blocker, acebutolol 200 mg/d (Sectral; Rhône-Polenc-Rorer Canada, Ville St. Laurent, Quebec). Dosage levels for medications were held constant in both study periods. Subjects were instructed to keep their level of physical activity constant. Blood samples were obtained at weeks 4 and 8 of each phase. Subjects were provided with self-calibrating scales to weigh food and were instructed to record a 7-day diet history on weeks 2, 4, and 8 of each phase. Mean dietary intake data are shown in Table 1. Body weight was obtained at 2-week intervals throughout the study.

The study was approved by the Ethics Committee of the University of Toronto. Informed consent was obtained from all subjects.

Diets

The subjects were instructed to follow a NCEP step 2 diet (<30% total fat as % dietary energy, <7% energy as saturated fat, and <200 mg/d cholesterol)[8] and were on the diet for at least 1 month prior to the metabolic study and for the 2 months of the metabolic study itself which immediately preceded the present ad libitum study. The diet records on both the test and control ad libitum phases were consistent with adherence to this dietary advice (Table 1).

Supplements

Supplements were delivered by courier to the subjects' homes or were picked up by the subjects at 2-week intervals. Also at 2-week intervals, subjects were asked to place an order for foods on the supplement list based on personal preference and anticipated use. On the test list were vegetable protein foods derived from soy, other legumes, and cereal foods in the form of easy-to-prepare dishes or frozen dinners, meat substitutes, and vegetarian cold cuts (Too Good To Be True; Loblaw Brands, Toronto, Ontario; Yves Veggie Cuisine, Vancouver, British Columbia; and Cedar Lake-MGM Foods, Cedar Lake, MI). Soluble-fiber foods consisted of oat, barley, and legume dishes provided as breakfast cereals and dried soups (Too Good To Be True, Loblaw Brands; and Fantastic Foods, Petaluma, CA). On the control list, low-fat, low-cholesterol milk and egg products were provided, including skim-milk yogurt and low-fat cheese and cottage cheese (Westhill Dairy, Downsview, Ontario; and Nutrispring Farms, Dundas, Ontario), a low-cholesterol egg substitute (Eggbeaters; Lipton's, Toronto, Ontario), soups (Knorr; Best Foods Canada, Etobicoke, Ontario), and low-fat, low–soluble-fiber dishes (Lean Cuisine; Nestle Canada, North York, Ontario; and Weight Watchers; Heinz of Canada, North York, Ontario).

Analyses

Serum stored at −70°C was analyzed in a single batch for total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol after magnesium chloride precipitation[19] with an automated clinical chemistry analyzer (CH1000; Technicon, Tarrytown, NY) using techniques from the Lipid Research Clinics.[20] LDL cholesterol was calculated as previously described.[21] One subject had serum triglycerides higher than 4.0 mmol/L on two occasions, and his LDL cholesterol data therefore were not used in the final calculations. Serum levels of apolipoprotein A-1 (apo A-1) and apo B were measured with a Behring BN100 nephelometer (Behring Werke, Marburg, Germany).[22] For assessment of LDL oxidation, LDL particles were isolated by precipitation with buffered heparin at their isoelectric point (pH 5.05).[23] The LDL precipitate was centrifuged at 1,000× *g* and resuspended in saline. LDL cholesterol was estimated enzymatically[24] on an aliquot of the saline resuspension using a commercial cholesterol assay kit (Sigma Chemical, St Louis, MO). On another aliquot, LDL oxidation was estimated as conjugated dienes in LDL fatty acids. Lipids from isolated LDL were extracted with chloroform:methanol (2:1), dried under nitrogen, redissolved in cyclohexane, and analyzed spectrophotometrically at 234 nm using a molar extinction coefficient of 29,500. Oxidized LDL cholesterol was expressed as the ratio of conjugated dienes (micromoles) per micromole LDL cholesterol.[25] The coefficient of variation for this assay or six replicates was 2.5%.

The diets were analyzed on a system based on US Department of Agriculture food composition tables[26] but included additional foods used in the study that were analyzed for macronutrients[27] and fiber[28] by Association of Official Analytical Chemists' techniques and for fatty acids by gas chromatography.[29] The percentage figures for soluble and insoluble fiber were derived from published data[30] (Table 1).

Table 1. Calculated Dietary Intake for Test and Control Periods

Variable	Control	Test
Energy (kcal/d)	1,901 ± 82	1,795 ± 88
Protein		
Total		
g/d	89 ± 5	83 ± 5
% of energy	18.7 ± 0.3	18.5 ± 0.5
Vegetable		
g/d	29 ± 2	61 ± 5
% of energy	6.3 ± 0.3	13.7 ± 7
Soy		
g/d	1 ± 0	14 ± 2
% of energy	0.1 ± 0.1	3.1 ± 0.4
Available carbohydrate		
g/d	270 ± 11	254 ± 11
% of energy	57.2 ± 1.2	57.3 ± 1.5
Fiber		
Total		
g/d	30 ± 2	38 ± 2
g/1,000 kcal	15.7 ± 0.7	21.4 ± 0.7
Soluble		
g/d	7 ± 1	11 ± 1
g/1,000 kcal	3.9 ± 0.3	6.0 ± 0.3
Fat		
Total		
g/d	49 ± 4	49 ± 5
% of energy	23.1 ± 1.1	23.6 ± 1.4
SFA		
g/d	14 ± 1	11 ± 1
% of energy	6.9 ± 0.3	5.5 ± 0.4
MUFA		
g/d	17 ± 2	18 ± 2
% of energy	8.1 ± 0.5	8.7 ± 0.7
PUFA		
g/d	13 ± 1	16 ± 1
% of energy	6.0 ± 0.4	7.7 ± 0.5
Cholesterol		
mg/d	104 ± 12	74 ± 10
mg/1,000 kcal	54 ± 5	40 ± 5
Alcohol		
g/d	2 ± 1	2 ± 1
% of energy	0.7 ± 0.4	0.7 ± 0.3

NOTE. Values are the mean ± SEM.

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Statistical Analysis

The results are expressed as the mean ± SE. Weight change is expressed as kilograms per month. The percentage difference between baseline and treatment values (mean of weeks 4 and 8) for both diets was assessed by Student's *t* test (two-tailed) for paired data. The absolute difference between treatments was assessed using the General Linear Model in SAS (version 6.12), with diet, sex, sequence, sex and sequence interaction, and a random term representing the individual subject nested within the sex × sequence interaction included in the model.[31] Based on a standard deviation for the treatment effect of 11.1% for LDL cholesterol and 10.5% for HDL cholesterol, the number of subjects would allow approximately an 8% difference in LDL and HDL cholesterol to be detected as significant (assuming $\alpha = .05$ and $\beta = 0.8$).

RESULTS

No difference was found between diets for the percentage of energy derived from total fat, protein, or carbohydrate (Table 1). However, on the test phase, vegetable protein as a percentage of total protein was significantly higher versus the control (73% ± 3% *v* 33% ± 2%, $P < .001$), as were the mean intake levels of soy protein (11.5 ± 1.7 *v* 1.6 ± 0.5 g/d, $P < .001$) and soluble fiber (10.4 ± 0.7 *v* 7.2 ± 0.5 g/d, $P < .001$). There was no significant weight change during the 8 weeks on either phase (test, −0.3 ± 0.3 kg; control, −0.1 ± 0.2 kg).

Serum lipid data are presented in Table 2. The mean treatment difference between the test and the control (weeks 4 and 8) phases indicated a significantly higher value on the test phase for HDL cholesterol (6.4% ± 2.4%, $P = .013$) and a lower value for the total to HDL cholesterol ratio (−5.9% ± 2.3%, $P = .020$). In addition, conjugated dienes in the LDL fraction as a marker of oxidized LDL cholesterol were significantly reduced on the test compared with the control (−8.5% ± 3.4%, $P = .020$). The significance of these treatment differences was confirmed using the General Linear Model ($P = .016$, $P = .012$, and $P = .021$, respectively). There was a tendency for men to show a better treatment response than women, but only for serum total cholesterol was the sex difference significant ($P = .044$). Neither the man treated with a HMG CoA reductase inhibitor nor the woman on hormone replacement therapy responded differently compared with their peers. The order in which the diets were taken had no significant effect on the results.

Furthermore, despite the treatment differences in dietary cholesterol intake, after inclusion of dietary cholesterol as a covariant in the analysis of covariance, treatment differences in HDL cholesterol, the total to HDL cholesterol ratio, and conjugated dienes in the LDL fraction remained significant (Fig 1).

DISCUSSION

The inclusion of vegetable protein and soluble-fiber foods in the self-selected NCEP step 2 diets of hyperlipidemic subjects improved the cardiovascular risk profile by increasing HDL cholesterol and reducing the total to HDL cholesterol ratio and the proportion of oxidized products in the LDL fraction compared with the control phase. These changes in the lipoprotein ratio and oxidized LDL would be predicted to reduce the risk of cardiovascular disease,[15,32-39] and thus add support to the current interest in the cholesterol-lowering potential of soy protein and soluble-fiber foods. They also indicate that these additional benefits can be achieved with a modest amount of foods that are both acceptable to hyperlipidemic subjects and readily available in supermarkets and health food stores.

Vegetable proteins, principally soy,[13] and more recently, the yeast protein quorn,[40] have been shown to reduce serum cholesterol in man. In addition, wheat gluten has been shown to be less atherogenic than casein in rabbits, and a range of vegetable proteins have been demonstrated to produce a smaller

Table 2. Blood Lipid Data on the Control and Test Diet Periods

Variable	Control	Test	Treatment Difference	P*
Cholesterol (mmol/L)				
Total	6.33 ± 0.18	6.26 ± 0.20	−0.07 ± 0.13	.580
LDL	4.22 ± 0.16	4.20 ± 0.17	−0.02 ± 0.12	.861
HDL	1.25 ± 0.08	1.32 ± 0.08	0.07 ± 0.03	.012
Triglycerides (mmol/L)	1.89 ± 0.18	1.67 ± 0.19	−0.21 ± 0.11	.077
Apolipoproteins (g/L)				
Apo A-1	1.45 ± 0.06	1.48 ± 0.06	0.02 ± 0.02	.226
Apo B	1.54 ± 0.05	1.49 ± 0.05	−0.04 ± 0.03	.212
Ratios				
Total:HDL cholesterol	5.38 ± 0.32	5.00 ± 0.28	−0.38 ± 0.14	.016
LDL:HDL cholesterol	3.56 ± 0.25	3.36 ± 0.23	−0.20 ± 0.11	.096
Apo B:apo A-1	1.10 ± 0.06	1.04 ± 0.06	−0.06 ± 0.03	.070
Ox:LDL cholesterol	14.0 ± 0.9	12.9 ± 1.0	−1.1 ± 0.5	.021

NOTE. Values are the mean ± SEM. Control and test data are the mean of weeks 4 and 8. Treatment difference = test − control. To convert cholesterol and triglycerides to mg/dL, multiply by 38.67 and 88.57, respectively. To convert apo A-1 and apo B to mg/dL, multiply by 10.

Abbreviation: Ox:LDL-C, oxidized LDL cholesterol measured as the proportion of conjugated dienes (μmol) per mmol LDL cholesterol.

*Treatment difference *P* values assessed using the General Linear Model in SAS.

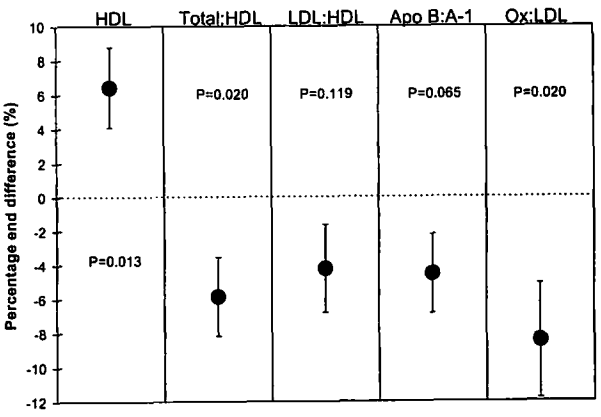

Fig 1. Percentage difference (mean ± SEM) between the control and test diets in serum HDL and the ratio of lipoproteins, apolipoproteins, and conjugated dienes in LDL (Ox:LDL). *P* values are for the significance of treatment differences.

increase serum cholesterol than animal proteins, again in rabbits.[41,42] The mechanism for the cholesterol-lowering effect of vegetable proteins is less clear, but may relate to the amino acid composition of plant versus animal proteins.[43-45] Despite the lack of a clear mechanism, a recent meta-analysis demonstrated a mean 12.9% reduction in LDL cholesterol for a mean 47-g/d soy protein intake.[13] The present study therefore suggests that even at these low dietary intake levels, when the two dietary components of soy protein and soluble fiber are combined, treatment differences in lipoproteins and their ratios may be found.

Isoflavonoids in soy foods have been strongly implicated by some,[13,46] but not all,[47,48] as influencing the serum lipid changes found after soy consumption. This aspect of soy action is of particular interest in the context of HDL cholesterol metabolism and the higher HDL cholesterol levels observed in the present study after soy consumption. The modest but nonsignificant decrease in triglyceride may partly explain this effect. However, soy isoflavonoids have sex hormone–like activity and belong to a class of compounds often referred to as phytoestrogens.[49,50] The presence of these compounds may create a hormonal environment similar to that which generally results in higher HDL cholesterol levels in women compared to men. In studies of soy, the isoflavonoids may have tended to preserve or even elevate HDL cholesterol levels despite significant reductions in LDL cholesterol.[13,51,52] Isoflavonoids may also act as antioxidants.[17] Oxidized LDL cholesterol is more readily taken up by the macrophages of the scavenger system in the arterial wall and so may contribute to plaque formation.[53-55] Consumption of antioxidant flavonoids, vitamin E, and lycopene has been shown to be associated with a reduced risk of coronary heart disease.[15,17,25] The isoflavonoid component of soy proteins may therefore be another factor conferring a cardiovascular benefit for soy products.

There is evidence that both purified viscous soluble fiber and soluble fiber in foods reduce serum cholesterol levels.[12,56-58] The primary mechanism of action of soluble fiber appears to relate to an increase in fecal bile acid loss[12,59-61] and an increase in the rate of chenodeoxycholate synthesis.[62-64] With increases of 5 to 15 g in soluble fiber, 4% to 10% reductions in serum cholesterol might be expected.[12,60,64] Depending on the soluble fiber, as little as 3 g/d may reduce serum cholesterol, but the results are much more variable.[65-69] No effects on HDL cholesterol or the total to HDL cholesterol ratio have been reported at the level of soluble fiber consumed in the present study. The treatment difference in soluble fiber intake is therefore unlikely to be responsible for the lipid changes observed here.

Within the limited range of treatment differences in vegetable protein and soluble fiber selected by the subjects, there was no significant relation to the corresponding treatment difference in blood lipids.

We believe that measures which increase the proportion of vegetable protein in the diet, especially from soy foods, can further improve the effectiveness of low–saturated-fat, low-cholesterol diets as currently recommended.[8] These additional dietary approaches added to the current advice may provide acceptable and effective options for blood lipid control for the larger section of the population at intermediate risk for cardiovascular disease, many of whom may not be suitable for drug therapy by current criteria.[8]

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REFERENCES

1. Higgins M, Thom T: Trends in CHD in the United States. Int J Epidemiol 18:58s-66s, 1989 (suppl)

2. Kannel WB: Blood pressure as a cardiovascular risk factor: Prevention and treatment. JAMA 275:1571-1576, 1996

3. Willett WC, Manson JE, Stampfer MJ, et al: Weight, weight change, and coronary heart disease in women. Risk within the 'normal' weight range. JAMA 273:461-465, 1995

4. Stefanick ML, Mackey S, Sheehan M, et al: Effects of diet and exercise in men and postmenopausal women with low levels of HDL cholesterol and high levels of LDL cholesterol. N Engl J Med 339:12-20, 1998

5. Lakka TA, Venalainen JM, Rauramaa R, et al: Relation of leisure-time physical activity and cardiorespiratory fitness to the risk of acute myocardial infarction. N Engl J Med 330:1549-1554, 1994

6. Rimm EB, Willett WC, Hu FB, et al: Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. JAMA 279:359-364, 1998

7. Ockene IS, Miller NH: Cigarette smoking, cardiovascular disease, and stroke: A statement for healthcare professionals from the American Heart Association. American Heart Association Task Force on Risk Reduction. Circulation 96:3243-3247, 1997

8. The Expert Panel: Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). JAMA 269:3015-3023, 1993

9. Smith GD, Song F, Sheldon TA: Cholesterol lowering and mortality: The importance of considering initial level of risk. BMJ 306:1367-1373, 1993

10. Lipid Research Clinics Program: The Lipid Research Clinics Coronary Primary Prevention Trial results II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 251:365-374, 1984

11. Schaefer EJ, Lamon-Fava S, Ausman LM, et al: Individual variability in lipoprotein cholesterol response to National Cholesterol Education Program step 2 diets. Am J Clin Nutr 65:823-830, 1997

12. Jenkins DJ, Wolever TM, Rao AV, et al: Effect on blood lipids of very high intakes of fiber in diets low in saturated fat and cholesterol. N Engl J Med 239:21-26, 1993

13. Anderson JW, Johnstone BM, Cook-Newell ME: Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 333:276-282, 1995

14. Miettinen TA, Puska P, Gylling H, et al: Reduction of serum

cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med 333:1308-1312, 1995

15. Hertog MG, Feskens EJ, Hollman PC, et al: Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. Lancet 342:1007-1011, 1993

16. Jenkins DJ, Kendall CW, Vidgen E, et al: Health aspects of partially defatted flaxseed, including effects on serum lipids, oxidative measures, and ex vivo androgen and progestin activity: A controlled crossover trial. Am J Clin Nutr 69:395-402, 1999

17. Tikkanen MJ, Wahala K, Ojala S, et al: Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc Natl Acad Sci USA 95:3106-3110, 1998

18. Jenkins DJ, Kendall CW, Mehling CC, et al: Combined effect of vegetable protein (soy) and soluble fiber added to a standard cholesterol-lowering diet. Metabolism 48:809-816, 1999

19. Warnick GR, Benderson J, Albers JJ: Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 28:1379-1388, 1982

20. Lipid Research Clinics Program: Manual of Laboratory Operations. Lipid and Lipoprotein Analysis (revised 1982). Washington, DC, US Department of Health and Human Services, US Government Printing Office, 1982 (NIH publication no. 75-678)

21. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499-502, 1972

22. Fink PC, Romer M, Haeckel R, et al: Measurement of proteins with the Behring Nephelometer. A multicentre evaluation. J Clin Chem Clin Biochem 27:261-276, 1989

23. Wieland H, Seidel D: A simple specific method for precipitation of low density lipoproteins. J Lipid Res 24:904-909, 1983

24. Allain CA, Poon LS, Chan CS, et al: Enzymatic determination of total serum cholesterol. Clin Chem 20:470-474, 1974

25. Agarwal S, Rao AV: Tomato lycopene and low density lipoprotein oxidation: A human dietary intervention study. Lipids 33:981-984, 1998

26. The Agriculture Research Service: Composition of Foods, Agriculture Handbook No 8. Washington, DC, US Department of Agriculture, 1992

27. Association of Official Analytical Chemists. AOAC Official Methods of Analysis. Washington, DC, Association of Official Analytical Chemists, 1980

28. Prosky L, Asp NG, Furda I, et al: Determination of total dietary fiber in foods and food products: Collaborative study. J Assoc Offic Anal Chem 68:677-679, 1985

29. Cunnane SC, Hamadeh MJ, Liede AC, et al: Nutritional attributes of traditional flaxseed in healthy young adults. Am J Clin Nutr 61:62-68, 1995

30. Anderson JW, Bridges SR: Dietary fiber content of selected foods. Am J Clin Nutr 47:440-447, 1988

31. SAS Institute: SAS/STAT User's Guide (ed 6.12). Cary, NC, SAS Institute, 1997

32. Kannel WB, Castelli WP, Gordon T: Cholesterol in the prediction of atherosclerotic disease: New perspectives based on the Framingham Study. Ann Intern Med 90:85-91, 1979

33. Grundy SM, Chait A, Brunzell JD: Familial Combined Hyperlipidemia Workshop. Arteriosclerosis 7:203-207, 1987

34. Stampfer MJ, Sacks FM, Salvini S, et al: A prospective study of cholesterol, apolipoproteins and the risk of myocardial infarction. N Engl J Med 325:373-381, 1991

35. Sacks FM, Pfeiffer MA, Moye LA, et al: The effect of prevastatin on coronary events after myocardial infarction in patients with average cholesterol levels. N Engl J Med 335:1001-1009, 1996

36. Arntzenius AC, Kromhout D, Barth JD, et al: Diet, lipoproteins, and the progression of coronary atherosclerosis. The Leiden Intervention Trial. N Engl J Med 312:805-811, 1985

37. Ornish D, Brown SE, Scherwitz LW, et al: Can lifestyle changes reverse coronary heart disease? The Lifestyle Heart Trial. Lancet 336:624-626, 1990

38. Stampfer MJ, Hennekens CH, Manson JE, et al: Vitamin E consumption and the risk of coronary disease in women. N Engl J Med 328:1444-1449, 1993

39. Rimm EB, Stampfer MJ, Ascherio A, et al: Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 328:1450-1456, 1993

40. Turnbull WH, Leeds AR, Edwards DG: Mycoprotein reduces blood lipids in free-living subjects. Am J Clin Nutr 55:415-419, 1992

41. Kritchevsky D, Tepper SA, Czarnecki SK, et al: Experimental atherosclerosis in rabbits fed cholesterol-free diets: Corn protein, wheat gluten and lactalbumin. Nutr Rep Int 26:931-936, 1982

42. Kritchevsky D, Tepper SA, Klurfeld DM: Dietary protein and atherosclerosis. J Am Oil Chem Soc 64:1167-1171, 1987

43. Carroll KK: Dietary proteins and amino acids—Their effects on cholesterol metabolism. Curr Top Nutr Dis 8:9-17, 1983

44. Kritchevsky D: Dietary protein, cholesterol and atherosclerosis: A review of the early history. J Nutr 125:589s-593s, 1995 (suppl)

45. Carroll KK: Review of clinical studies on cholesterol-lowering response to soy protein. J Am Diet Assoc 91:820-827, 1991

46. Baum JA, Teng H, Erdman JW, et al: Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. Am J Clin Nutr 68:545-551, 1998

47. Potter JM, Nestel PJ: Greater bile acid excretion with soybean than with cow milk. Am J Clin Nutr 29:546-551, 1976

48. Hodgson JM, Puddey IB, Beilin LJ, et al: Supplementation with isoflavonoid phytoestrogens does not alter serum lipid concentrations: A randomized controlled trial in humans. J Nutr 128:728-732, 1998

49. Setchell KD, Borriello SP, Hulme P, et al: Nonsteroidal estrogens of dietary origin: Possible roles in hormone-dependent disease. Am J Clin Nutr 40:569-578, 1984

50. Adlercreutz H, Hamalainen E, Gorbach S, et al: Dietary phytoestrogens and the menopause in Japan. Lancet 339:1233, 1992 (letter)

51. Kurowska EM, Jordan J, Spence JD, et al: Effects of substituting dietary soybean protein and oil for milk protein and fat in subjects with hypercholesterolemia. Clin Invest Med 20:162-167, 1997

52. Wilson TA, Behr SR, Nicolosi RJ: Addition of guar gum and soy protein increases the efficacy of the American Heart Association (AHA) step I cholesterol-lowering diet without reducing high density lipoprotein cholesterol levels in non-human primates. J Nutr 128:1429-1433, 1998

53. Steinberg D, Parthasarathy S, Carew TE, et al: Beyond cholesterol: Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 264:915-924, 1989

54. Jialal I, Grundy SM: Effect of dietary supplementation with alpha-tocopherol on the oxidative modification of low density lipoprotein. J Lipid Res 33:899-906, 1992

55. Witztum JL: The oxidation hypothesis of atherosclerosis. Lancet 344:793-795, 1994

56. Kritchevsky D: The role of dietary fiber in health and disease. J Environ Pathol Toxicol Oncol 6:273-284, 1986

57. Anderson JW, Garrity TF, Wood CL, et al: Prospective, randomized, controlled comparison of the effects of low-fat and low-fat plus high-fiber diets on serum lipid concentrations. Am J Clin Nutr 56:887-894, 1992

58. Yetley EA, Park YK: Diet and heart disease: Health claims. J Nutr 125:679s-685s, 1995 (suppl)

59. Kritchevsky D, Story JA: Binding of bile salts in vitro by non-nutritive fiber. J Nutr 104:458-464, 1974

60. Miettinen TA, Tarpila S: Effect of pectin on serum cholesterol, fecal bile acids and biliary lipids in normolipidemic and hyperlipidemic individuals. Clin Chim Acta 79:471-477, 1977

61. Lia A, Hallmans G, Sandberg A-S, et al: Oat β-glucan increases bile acid excretion and a fiber-rich barley fraction increases cholesterol excretion in ileostomy subjects. Am J Clin Nutr 62:1245-1251, 1995

62. Everson GT, Daggy BP, McKinley C, et al: Effects of psyllium hydrophilic mucilloid on LDL-cholesterol and bile acid synthesis in hypercholesterolemic men. J Lipid Res 33:1183-1192, 1992

63. Marlett JA, Hosig KB, Vollendorf NW, et al: Mechanism of serum cholesterol reduction by oat bran. Hepatology 20:1450-1457, 1994

64. Jenkins DJ, Wolever TM, Vidgen E, et al: The effect of psyllium in hypercholesterolemia at two levels of monounsaturated fat intake. Am J Clin Nutr 65:1524-1533, 1997

65. Palmer GH, Dixon DG: Effect of pectin dose on serum cholesterol levels. Am J Clin Nutr 18:437-442, 1966

66. Leadbetter J, Ball MJ, Mann JI: Effects of increasing quantities of oat bran in hypercholesterolemic people. Am J Clin Nutr 54:841-845, 1991

67. Davidson MH, Dugan LD, Burns JH, et al: The hypocholesterolemic effects of beta-glucan in oatmeal and oat bran. A dose-controlled study. JAMA 265:1833-1839, 1991

68. Ripsin CM, Keenan JM, Jacobs DR Jr, et al: Oat products and lipid lowering. A meta-analysis. JAMA 267:3317-3325, 1992

69. Brown L, Rosner B, Willett WW, et al: Cholesterol-lowering effects of dietary fiber: A meta-analysis. Am J Clin Nutr 69:30-42, 1999