MEMORANDUM OF CALL

Between Dan Dwyer (Keinfeld, Kaplan and Becker for Lipton)
and Dr. Sharon Ross (DSNP, ONPLDS, CFSAN, FDA)

0506 '00 MAY 12 P1:53

May 1, 2000 (2 pm)

Re: Petition for Health Claim: Vegetable Oil Sterol Esters

A call was placed to Dan Dwyer by Sharon Ross to ask that Lipton submit two recently published studies as a supplement to their health claim petition. The two studies are: (1) Spreads enriched with plant sterols, either esterified 4,4-dimethylsterols or free 4-desmethylsterols, and plasma total- and LDL-cholesterol concentrations. Sierksma A, Westrate JA and Meijer GW. Br J Nutr (1999) 82:273-282, and (2) Plant sterol and stanol margarines and health. Law M. BMJ (2000) 320:861-864. Dan Dwyer stated that he will follow-up on this request to Lipton as soon as possible.

00P-1275

MT2

LAW OFFICES
KLEINFELD, KAPLAN AND BECKER
1140 NINETEENTH STREET, N.W.
WASHINGTON, D. C. 20036-6601
TELEPHONE (202) 223-5120
FACSIMILE (202) 223-5619
E-MAIL: kkb@kkblaw.com

ALAN H. KAPLAN
THOMAS O. HENTELEFF
RICHARD S. MOREY
PETER O. SAFIR
KINSEY S. REAGAN
PETER R. MATHERS
BONNIE A. BEAVERS
DANIEL R. DWYER
GLENN E. DAVIS
PRESCOTT M. LASSMAN
STACY L. EHRLICH
JENNIFER A. DAVIDSON
STACEY L. VALERIO

WEST COAST OFFICE:
ONE MARKET STREET
STEUART TOWER, SUITE 1450
SAN FRANCISCO, CA 94105-1313
TELEPHONE (415) 538-0014
FACSIMILE (415) 538-0016

VINCENT A. KLEINFELD
1907-1993

6507 '00 MAY 12 P1:12

May 3, 2000

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MAY 3 2000

Via Hand Delivery

Sharon A. Ross, Ph.D.
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20004

Re: Petition submitted by Lipton for a Health Claim regarding Vegetable Oil Sterol Esters and Coronary Heart Disease

Dear Dr. Ross:

This follows up on our telephone conversation of May 1, 2000 in which you requested copies of two journal articles, pursuant to your review of the above-referenced health claim petition. These articles (Sierksma et al, 1999 and Law, 2000) are enclosed.

In addition, we are enclosing for your information a copy of an abstract of a study by Judd et al. of the effect of vegetable oil sterol esters in salad dressings on blood cholesterol that was presented at a recent conference.

Please contact me or Nancy Schnell if you have any further questions.

Sincerely,

Daniel R. Dwyer

Counsel to Lipton

enclosures

cc: Nancy L. Schnell, Esq.
Lipton

British Journal of Nutrition (1999), 82, 273–282

NOTICE
THIS MATERIAL MAY BE PROTECTED BY COPYRIGHT LAW (TITLE 17 U.S. CODE)

Spreads enriched with plant sterols, either esterified 4,4-dimethylsterols or free 4-desmethylsterols, and plasma total- and LDL-cholesterol concentrations

Aafje Sierksma, Jan A. Weststrate* and Gert W. Meijer

Unilever Nutrition Centre, Unilever Research Vlaardingen, Olivier van Noortlaan 120, 3133 AT Vlaardingen, The Netherlands

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In a 9-week study seventy-six healthy adult volunteers with an average age of 44 (SD 11) years, with baseline plasma total cholesterol levels below 8 mmol/l, received in a balanced, double-blind, crossover design, a total of three different table spreads for personal use. Two spreads were fortified either with free (non-esterified) vegetable-oil sterols, mainly from soyabean oil (31 g sterol equivalents/kg; 0·8 g/d) or sheanut-oil sterols (133 g sterol equivalents/kg; 3·3 g/d). One spread was not fortified (control). Average intake of spread was 25 g/d for 3 weeks. None of the spreads induced changes in blood clinical chemistry or haematology. Plasma total- and LDL-cholesterol concentrations were statistically significantly reduced by 3·8 % and 6 % (both 0·19 mmol/l) respectively, for the spread enriched with free soyabean-oil sterols compared with the control spread. The spread enriched with sheanut-oil sterols did not lower plasma total- and LDL-cholesterol levels. None of the plant-sterol-enriched spreads affected plasma HDL-cholesterol concentrations. Plasma-lipid-standardized concentrations of α- plus β-carotene were not statistically significantly affected by the soyabean-oil sterol spread in contrast to lipid-standardized plasma lycopene levels which showed a statistically significant decrease (9·5 %). These findings indicate that a daily intake of free soyabean-oil sterols as low as 0·8 g added to a spread is effective in lowering blood total- and LDL-cholesterol levels with limited effects on blood carotenoid levels. The lowering in total- and LDL-cholesterol blood levels due to consumption of the vegetable-oil-sterol-enriched spread may be helpful in reducing the risk of CHD for the population.

Plant sterols: Lipids: Carotenoids

The major manifestation of cardiovascular disease is CHD, which remains the leading cause of death in the developed world. Atherosclerosis, which damages the coronary arteries, is the primary disease mechanism of CHD (Reitsma, 1995). Hypercholesterolaemia contributes to the atherosclerosis and its clinical manifestations such as coronary artery occlusion and ischaemic myocardial infarction (Steinberg & Witztum, 1990).

Lowering blood total- and LDL-cholesterol levels has been advocated as a method to decrease the incidence of CHD (Probstfield & Rifkind, 1991; Cucherat & Boissel, 1993). The benefits of serum cholesterol concentration reduction are related to age; a 10 % reduction in total serum cholesterol concentration produces a reduction in IHD of 50 % at age 40, 40 % at age 50, 30 % at age 60, and 20 % at age 70. The benefit can be realised quickly, the greater part after 2 years and the full benefit after 5 years (Law *et al.* 1994). Reduction in the blood cholesterol concentration not only slows the process of atherosclerosis, but can also result in regression of atherosclerosis (Levine *et al.* 1995).

Dietary modification is the first step in lowering blood cholesterol levels, for example, replacing saturated fatty acids by unsaturated fatty acids (Mensink & Katan, 1992) and increasing the intake of soluble fibres (Ripsin *et al.* 1992). In addition to unsaturated fatty acids and soluble fibres, plant sterols, such as sitosterol, may also reduce blood cholesterol levels (Pollak & Kritchevsky, 1981). Sitosterol at intakes of 3–20 g/d reduces blood cholesterol levels by 5–15 % in human studies without major side-effects (Farquhar & Sokolow, 1958; Oster *et al.* 1976; Lees *et al.* 1977; Schwartzkopff & Jantke, 1978). Plant sterols are naturally occurring plant compounds. The most important dietary sources are vegetable oils and vegetable-oil-based products such as margarines (Ling & Jones, 1995). The most predominant plant sterols are sitosterol, campesterol and

* Corresponding author: Dr Jan Weststrate, fax +31 10 460 5993, email Jan.Weststrate@Unilever.com

stigmasterol, and intake of these is estimated to be about 250 mg/d in non-vegetarians and about 500 mg/d for vegetarians (Ling & Jones, 1995). Plant sterols reduce the absorption of cholesterol in the gut possibly by displacing cholesterol from the micelles, by limiting the intestinal solubility of cholesterol or by decreasing the hydrolysis of cholesterol esters in the small intestine (Ling & Jones, 1995). Daily consumption of about 3 g of a mixture of vegetable-oil sterol esters, in particular esters of sitosterol, campesterol and stigmasterol, in a commonly used food, margarine, was recently reported to be effective in lowering plasma total- and LDL-cholesterol levels in normocholesterolaemic and mildly hypercholesterolaemic subjects (Weststrate & Meijer, 1998). There have been very few studies published on the effect of doses of plant sterols less than 1 g/d on plasma cholesterol levels in normocholesterolaemic subjects using commonly consumed foods (Pelletier *et al.* 1995).

The objective of the present study was to compare the effects of the consumption of two types of spreads (margarines) enriched with plant sterols, either sheanut-oil sterol (133 g sterol equivalents/kg), mainly 4,4-dimethylsterols, or non-esterified soyabean-oil sterols (31 g sterol equivalents/kg), mainly 4-desmethylsterols, in healthy volunteers on plasma total- and LDL-cholesterol levels. Flora, a spread not enriched with plant sterols but containing similar amounts and types of fatty acids, was used as the control. Our previous study (Weststrate & Meijer, 1998) had shown a substantial plasma total- and LDL-cholesterol-lowering action of a spread enriched with esterified vegetable-oil sterols, mainly derived from soyabean oil (110 g sterol equivalents/kg). Non-esterified soyabean-oil sterols might be somewhat more effective in lowering plasma total- and LDL-cholesterol levels compared with esterified soyabean-oil sterols, as sterols need to be in an unesterified or free state to inhibit cholesterol absorption (Peterson *et al.* 1953; Swell *et al.* 1956; Best & Duncan, 1958). However, solubility of free plant sterols in spread is limited to approximately 30 g/kg. In our previous study a sheanut-oil-sterol-enriched spread (101·5 g sterol equivalents/kg) was not effective in lowering blood cholesterol levels (Weststrate & Meijer, 1998). A different fatty acid composition of this spread compared with the control product (less linoleic fatty acid and more oleic fatty acid) could have contributed to this result. In the present study, in contrast to our previous study, the fatty acid composition of the sheanut-oil-sterol-enriched spread was similar to the control spread. Blood chemistry and haematology were performed to assess possible adverse effects of spread consumption. Plasma α- plus β-carotene and lycopene levels were assessed only for the spreads which gave a statistically significant reduction in plasma total- and LDL-cholesterol concentrations, as we found in our previous study (Weststrate & Meijer, 1998) a reduction of between 15 and 19 % in plasma lipid-standardized levels of these lipophilic dietary compounds after intake of spreads enriched with plant sterols.

Subjects and methods

Subjects

Volunteers were recruited by advertisement from inhabitants of Vlaardingen (The Netherlands) and its surroundings, and from employees of Unilever Research Vlaardingen, with the exception of employees of the Unilever Nutrition Centre. Subjects' characteristics are shown in Table 1. They ranged in age from 18 to 62 years and their BMI at the start of the study were between 19·2 and 29·6 kg/m^2. Alcohol drinking was below 21 units/week for females and below 28 units/week for males. Volunteers had less than 10 h/week of intense exercise. Their entry plasma total cholesterol levels were below 8 mmol/l and plasma activities of L-γ-glutamyltransferase (*EC* 2.3.2.2), alanine aminotransferase (*EC* 2.6.1.2), aspartate aminotransferase (*EC* 2.6.1.1) and alkaline phosphatase (*EC* 3.1.3.1) were all within the normal ranges. Leucocyte, erythrocyte and platelet count, haemoglobin level and packed cell volume were all within the normal ranges. The volunteers were not participating in any other biomedical trial and had not donated blood within 1 month before the start of the study. Female volunteers were non-lactating and not pregnant. The study set-up was approved by our local Medical-Ethical Committee and written informed consent was obtained from all subjects.

Study design

The study had a balanced, double-blind, crossover design with a run-in period of 1 week with the control spread so that the volunteers could become accustomed to the use of the spreads. Immediately thereafter the experimental period of 9 weeks started in which each spread was given in random

Table 1. Descriptive characteristics of the volunteers participating in the plant-sterol-enriched spread study
(Mean values and standard deviations)

	Total (*n* 76)		Males (*n* 39)		Females (*n* 37)	
Characteristic	Mean	SD	Mean	SD	Mean	SD
Age (years)	44	11	45	12	43	11
Body weight (kg)	75·2	10·7	81·7	9·6	68·4	7·1
Body height (m)	1·75	0·09	1·82	0·07	1·69	0·05
BMI (kg/m^2)	24·4	2·4	24·7	2·2	24·0	2·5
Sporting activities (h/week)	1	2	1	2	1	1
Alcoholic beverage consumption (units/week)	5	6	6	6	3	5

weeks for 3 weeks at an intake level of 25 g/d. Subjects were randomly allocated over the six different period × group sequences. Groups were stratified according to sex. After each experimental period of 3 weeks, a fasting venous blood sample was taken from an antecubital vein with the subject seated in a chair. Body weight was measured on a digital balance (Mettler Instruments AG, Greifensee, Switzerland), with the subjects wearing light indoor clothing, without shoes and after voiding.

Experimental spreads

The test articles were specially prepared spreads fortified with non-esterified plant sterols derived from either edible vegetable oil (mainly soyabean oil) distillates (Henkel Corporation, LaGrange, IL, USA), or a sheanut oil concentrate in which the plant sterols were esterified, mainly with cinnamate (69 %) and acetate (25 %) (Loders Croklaan, Wormerveer, The Netherlands). The distillate or concentrate was used in spread production together with other edible oils and fats in order to achieve an acceptable product. The final plant sterol concentration was 31 g/kg for the soyabean-oil sterol product and 133 g/kg for the sheanut-oil sterol product, when expressed as free sterol equivalents. Flora (VandenBergh Foods, Purfleet, Surrey, UK), a spread not fortified with plant sterols, was used as the control. Table 2 shows the composition characteristics of the spreads. Tubs containing the individual daily amounts of spread were labelled with a blind product code. The spreads were intended for personal use and meant to replace all or part of the volunteers' habitual spread or butter used for spreading, but not to be used for baking or frying. Compliance was assessed by asking the volunteers about spread consumption and by weighing left-overs from the tubs.

Lifestyle and dietary intake

The volunteers were instructed to maintain their normal lifestyle and dietary and activity patterns during the entire study. The dietary intake during each period, i.e. over 21 d, was assessed at the end of each period using a validated food-frequency questionnaire (Grootenhuis *et al.* 1995), with special emphasis on intakes of total energy, macronutrients, fatty acids, cholesterol and fibre. This questionnaire was modified for this study to give more detailed information on the intake of specific brands of spreads and dietary fats in the habitual diet in order to better evaluate fat composition of the habitual diet during each period. The answers to the questionnaire were checked for completeness by dietitians. Throughout the study volunteers recorded illnesses, medicine use, and any important deviations from their lifestyle or dietary and activity patterns.

Measurements

Blood samples. Fasting venous blood samples were taken on the last day of each period. Plasma total- and HDL-cholesterol levels were determined using test kits from Boehringer (Mannheim, Germany). Plasma LDL-cholesterol level was calculated by the Friedewald formula (Friedewald *et al.* 1972). Plasma total and free glycerol concentrations were analysed using a total glycerol test kit (Roche, Basel, Switzerland) and a free glycerol test kit (Sigma, St Louis, MO, USA). Plasma triacylglycerol level was calculated as plasma total glycerol level (mmol/l) minus plasma free glycerol level (mmol/l). In blood, activities of L-γ-glutamyltransferase and alkaline phosphatase and levels of bilirubin, creatinine, albumin and glucose were determined using test kits from Boehringer. The activities of aspartate aminotransferase and alanine aminotransferase and plasma levels of urea were determined using test kits from Roche. All determinations were done on a Cobas Mira S automated analyser (Roche). Plasma α- plus β-carotene and lycopene levels were only assessed in the blood samples which were taken after consumption of the soyabean-oil-sterol spread, which caused a statistically significant reduction in plasma total- and LDL-cholesterol concentrations, and in the blood samples which were taken

Table 2. Analysed composition of the spreads (per kg product) used in the plant-sterol-enriched spread study

Spread...	Flora*	Soyabean†	Sheanut‡
Total fat (g)§	710	710	720
Water (g)	290	260	110
Fatty acid composition (g):			
Saturated fatty acids	160	140	190
Monounsaturated fatty acids	210	220	190
Polyunsaturated fatty acids	340	350	340
Free sterols plus sterol esters (g)	3	33	167
Sterol equivalents (g)	3	31	133
Sterol composition (g):			
4-Desmethylsterols	2·7	30·5	4·6
4-Monomethylsterols	0·3	0·4	1·2
4,4-Dimethylsterols	0·2	0·2	127
Total vitamin E (mg)‖	430	460	300
$\alpha+\beta$-Carotene (mg)	4·6	4·9	4·7

* Control spread, not enriched with plant sterols.
† Spread enriched with 31 g soyabean-oil sterols/kg.
‡ Spread enriched with 133 g sheanut-oil sterols/kg.
§ Total *trans* fatty acid content of spreads was less than 10 g/kg fat, sterols excluded.
‖ Includes α-, β-, and γ-tocopherol.

after consumption of Flora. This was done to assess the potential impact of the sterol-containing spread on the absorption of lipophilic dietary compounds. The plasma carotenoids were separated by HPLC on a Nucleosil 5-N$(CH_3)_2$ column (Marchery & Nagel, Düren, Germany) using *n*-heptane as eluent as described previously (Weststrate & van het Hof, 1995). Plasma carotenoids were determined from the absorption at 450 nm. Plasma carotenoid concentrations were standardized for plasma lipid (total cholesterol and triacylglycerol) levels as previously described (Weststrate & van het Hof, 1995), because the sterol-enriched spreads did affect blood lipoprotein (plasma carotenoid carriers) concentrations. Haemoglobin concentrations, leucocyte count, platelet count and calculation of the packed cell volume were all performed on a Sysmex F-800 blood cell counter (Toa Medical Electronics Co. Ltd, Kobe, Japan). Staff conducting the laboratory analyses were blind to the group assignments.

Spreads. Directly after production and in each experimental period the spreads were analysed. Mean values for the composition are given in Table 2. During the study the stability of the spreads was checked once. The homogeneity of the sterols in the spreads was good, CV were generally less than 10 % (results not shown). For fat analysis the sample was mixed with celite, freeze-dried and extracted with dichloromethane. After evaporation, the residue (fat) was weighed. The triacylglycerol content was calculated by subtracting the total sterol and/or sterol ester content from the total fat content. Fatty acid composition was analysed by methanolysis of the fatty acids. The methyl esters were extracted with heptane and analysed by GC (Hewlett Packard, Amstelveen, The Netherlands) using a CP-Sil 88 column (Chrompack, Middelburg, The Netherlands). Analysis of sterol types was performed according to previously described methods (Grob *et al.* 1989; Artho *et al.* 1993; Plank & Lorbeer, 1993; Gordon & Miller, 1997). The spreads were saponified with KOH before extracting the unsaponifiable part into cyclohexane. Thin-film chromatography (with as TLC-eluent a mix of toluene with ethyl acetate) was used for separating the 4-desmethylsterol, the 4-monomethylsterol and the 4,4-dimethylsterol fractions (10 g ultraphor/l water as detection reagent) with β-cholestanol as internal standard. The fractions were extracted with a mixture of chloroform, diethyl ether and ethanol. After silylizing with pyridine and *N,O*-bis(trimethylsilyl)trifluoroacetamide this fraction was analysed by GC (Fisions Instruments, Milan, Italy) using a CP-Sil 19 column (Chrompack). Detection was by flame ionization detector (Fisions Instruments). For determination of the amounts of free and esterified sterols a portion of the isolated fat phase was silylated in order to convert the free sterols into trimethylsilyl ether derivatives. After isolation of the free sterols and steryl fatty acid esters from the triacylglycerol matrix by means of normal-phase HPLC, the free sterols and steryl fatty acids were quantified by GC using internal standards. Sterol equivalents were calculated by multiplying the weight percentages of steryl fatty acid esters by a factor 0·608 (calculated from the average molecular mass of sterols and fatty acids in soyabean oil). The total sterol equivalents were obtained by adding the concentration of free sterols and the sterol equivalents from the steryl fatty acid esters. Vitamin E was analysed by a straight-phase HPLC method, based on international standard operation procedures (American Oil Chemists' Society, 1992). Carotenoid content was assessed for Flora and the spread containing free soyabean-oil sterols. For the carotenoid analysis the sample was mixed with diethyl ether. After vortex-mixing the extract was dried under N_2 and dissolved in *n*-heptane containing the internal standard, ethyl-β-apo-8-carotenoate. The analysis was performed by straight-phase HPLC using an ET 200/8/4 nucleosil 5-N$(CH_3)_2$ column (Marchery & Nagel) and *n*-heptane–isopropanol (1000 : 24, v/v) as mobile phase at a flow rate of 1 ml/min and a column temperature of 20°. Carotenoids were detected by u.v.-visible detection at 450 nm for α- plus β-carotene and at 470 nm for lycopene.

Statistical analysis

Differences in plasma variables and dietary intake between spread groups were initially evaluated using ANOVA using as factors subject, period, spread, carry-over, sex and sex × spread. The carry-over effect was subsequently removed from the model as it was not statistically significant. Blood lipid and carotenoid data were log normalized before analysis. Geometric means are given with 95 % CI and have been back transformed (Table 4). Differences between spread groups were assessed using the Tukey multiple comparison test (Miller, 1981). A P value of 0·05 was considered statistically significant. The Statistical Analysis Systems program version 6.12 (1987; SAS Institute Inc., Cary, NC, USA) was used for analysis. All the statistical significance levels are given two-sided.

Results

Thirty-nine men and thirty-seven women completed the study. During the study two women dropped out because of medicine use and their data were not used in final analysis. Two people missed one period because of diarrhoea and one person did not give a blood sample in one period.

Compliance

The mean intakes of Flora, the vegetable-oil-sterol spread and the spread enriched with sheanut-oil sterols, estimated by weighing the left-over spread in the tubs after each period, were respectively 24·2 g/d, 24·8 g/d and 24·4 g/d with CV 1·1 %, 1·9 % and 4·0 % (results not shown). The mean daily total plant sterol intakes were respectively 0·08 g, 0·8 g and 3·2 g. The habitual dietary intake (including the test spread consumption) is given in Table 3. Except for the statistically significant but very small difference in intake of saturated fatty acids (in % energy) between the soyabean-oil-sterol spread and the sheanut-oil-sterol spread, no statistically significant differences were observed among the groups in dietary intake. The average body weight in the group with the spread containing free soyabean oil sterols was slightly higher than the average body weight in the Flora group (75·5 kg *v.* 75·2 kg). However, an analysis for males and females separately revealed no statistically

Table 3. Energy and dietary nutrient intakes (as percentage of total energy unless otherwise indicated) of the volunteers participating in the plant-sterol-enriched spread study

(Mean values and 95% confidence intervals)

Spread...	Flora†		Soyabean‡		Sheanut§	
Nutrient	Mean	95% CI	Mean	95% CI	Mean	95% CI
Energy (kJ/d)	9977	9732, 10221	10060	9816, 10304	10019	9775, 10264
Total fat	38·3	37·8, 38·8	38·3	37·8, 38·8	38·4	37·9, 38·9
Saturated fatty acids	13·9	13·7, 14·2	13·8	13·6, 14·1	14·3*	14·0, 14·6
Monounsaturated fatty acids	12·8	12·6, 13·0	12·9	12·6, 13·1	12·5	12·3, 12·7
Polyunsaturated fatty acids	9·2	9·1, 9·4	9·2	9·1, 9·4	9·2	9·1, 9·4
Protein	15·6	15·4, 15·9	15·4	15·2, 15·6	15·6	15·4, 15·9
Carbohydrates	43·2	42·7, 43·8	43·3	42·7, 43·8	43·1	42·6, 43·7
Cholesterol (mg/d)	246	236, 256	247	238, 257	242	232, 252
Fibre (g/d)	26·0	25·3, 26·8	26·7	26·0, 27·5	26·3	25·5, 27·0

Mean value was significantly different from that for soyabean spread: * $P < 0.05$.
† Control spread, not enriched with plant sterols.
‡ Enriched with 31 g soyabean-oil sterols/kg.
§ Enriched with 133 g sheanut-oil sterols/kg.

significant differences in average body weight between the groups. No important deviations from lifestyle, dietary or activity pattern occurred during the study.

Blood variables

Table 4 shows the plasma lipid, α- plus β-carotene, and lycopene concentrations based on the 3-week sampling result. There was no interaction effect between spread group and sex. The soyabean-oil-sterol spread lowered plasma total- and LDL-cholesterol levels significantly compared with Flora without an effect on plasma HDL-cholesterol levels. Consequently the ratio LDL : HDL-cholesterol was also statistically significantly reduced. The sheanut-oil-sterol spread did not statistically significantly change blood lipid levels compared with Flora. Fig. 1 shows the individual changes in plasma LDL-cholesterol concentrations after consumption of the soyabean-oil-sterol spread and the sheanut-oil-sterol spread compared with the control spread (Flora). In most subjects the soyabean-oil-sterol spread caused a decrease in plasma LDL-cholesterol concentrations, however, some subjects showed a small increase. Two subjects showed large increases in plasma total- and LDL-cholesterol levels after consumption of the soyabean-oil-sterol spread, probably due to chance. The sheanut-oil-sterol spread caused an increase in plasma LDL-cholesterol concentrations in more subjects. Plasma triacylglycerol levels were not affected by the spreads. The soyabean-oil-sterol spread caused a statistically significant reduction in plasma lycopene concentration but not in plasma α- plus β-carotene level. Standardization of the plasma lycopene concentration for decreased lipid did diminish the lowering effect of soyabean-oil-sterol spread on blood lycopene level, but the effect still remained statistically significant. All values of the blood variables during the study were within the normal ranges.

Discussion

This double-blind crossover study in healthy adults showed that daily consumption of 25 g of a spread enriched with free soyabean-oil sterols (31 g sterol equivalents/kg; 0·8 g/d),

Table 4. Plasma lipid, α- plus β-carotene and lycopene concentrations in volunteers consuming a control spread (Flora) or plant-sterol-enriched spreads (25 g/d) for 3 weeks†

(Mean values and 95% confidence intervals for seventy-five subjects)

	Flora		Soyabean‡		Sheanut§	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Total cholesterol (TC) (mmol/l)	5·06	4·99, 5·13	4·87*	4·80, 4·94	5·01	4·94, 5·08
LDL-cholesterol (mmol/l)	3·14	3·07, 3·20	2·95*	2·89, 3·01	3·08‖	3·01, 3·14
HDL-cholesterol (mmol/l)	1·28	1·26, 1·30	1·30	1·27, 1·32	1·29‖	1·27, 1·31
LDL- : HDL-cholesterol	2·45	2·39, 2·51	2·28*	2·23, 2·33	2·39‖	2·33, 2·44
Triacylglycerol (TAG) (mmol/l)	1·20	1·15, 1·25	1·14	1·09, 1·20	1·20	1·15, 1·25
α-+β-Carotene (μg/l)	226	219, 234	217	210, 224	ND	
Lycopene (μg/l)	130	122, 138	113*	106, 120	ND	
α-+β-Carotene/(TC+TAG) (μg/mmol)	35·8	34·8, 36·8	35·7	34·7, 36·8	ND	
Lycopene/(TC+TAG) (μg/mmol)	20·6	19·3, 21·9	18·6*	17·5, 19·8	ND	

ND, not determined.
Mean values were significantly different from those for Flora: * $P < 0.05$.
† For details of diets and procedures, see Table 2 and pp. 274–276.
‡ Spread enriched with 31 g soyabean-oil sterols/kg.
§ Spread enriched with 133 g sheanut-oil sterols/kg.
‖ *n* 74.

1997). This suggests that a difference of more than 1 mg/d lycopene intake will correspond to a difference of plasma lycopene levels of about 60 %. In the present study we observed a reduction of 13 % in unstandardized plasma lycopene levels. We hypothesize that this difference in plasma lycopene levels may be expected to correspond to a difference in dietary lycopene intake of about 0·2 mg/d. The study of Giovanucci *et al.* (1995) showed that a difference in intake of about 9 mg/lycopene/d was associated with a change in relative risk for prostate cancer of about 20 %, or about a 2 % change in relative risk per 1 mg/d change in dietary lycopene intake. Even when we assume that the results of this study indicate a causal relationship between reduced lycopene intake and increased risk of prostate cancer (which is not proven) the decrease in plasma lycopene levels observed in our present study translates to a negligible change in relative risk for prostate cancer.

Because of the within-person variations in plasma total- and LDL-cholesterol levels, which are up to about 10 % (analytical variation excluded) (Smith *et al.* 1993), not all individuals showed a reduction in plasma total- and LDL-cholesterol concentrations after consumption of the spread enriched with free soyabean-oil sterols (which caused average reductions in plasma total- and LDL-cholesterol levels of respectively 3·8 % and 6 %, smaller than the within-person variations for plasma total- and LDL-cholesterol levels). These increases in plasma total- and LDL-cholesterol concentrations are thus related to chance. If more measurements had been done per individual to establish his or her 'true' blood lipid levels, the chance of observing increases would be minimal and more accurate individual values of plasma total- and LDL-cholesterol concentrations would have been obtained. Plant sterols have been part of the human diet for a very long time and for vegetarians, for example, plant sterol intake can be substantial, e.g. 500 mg/d (Ling & Jones, 1995), which is in contrast to the intake of plant stanols. The introduction of plant-sterol-enriched products would in itself not introduce new compounds into the diet: it might, however, cause an increase in the exposure to plant sterols in consumers of such products. The possible health risks associated with such an introduction need to be carefully assessed. Current evidence indicates that plant sterols are unlikely to raise severe concerns regarding possible human health risks. Many human studies with dose levels up to 25 g/plant sterols/d for up to several months have been performed without adverse effects (reviewed by Pollak & Kritschevsky, 1981). The only undesirable effect observed have been occasional gastrointestinal disturbances, e.g. constipation (Lees *et al.* 1977; Kane & Malloy, 1982) and diarrhoea, which are more related to the vehicle (large volumes of vegetable oils) used to administer the plant sterols. The only reported adverse effects of plant sterols have been in individuals with phytosterolaemia. Phytosterolaemia is a vary rare inherited lipid storage disease, affecting a few individuals per million, in which a high proportion of plant sterols is absorbed from the intestine (Salen *et al.* 1992). The condition has a high risk of premature atherosclerosis. Individuals with this condition should avoid the consumption of plant sterols. However, for the general population this increased absorption of plant sterols does not occur, rather the plant sterols are poorly absorbed and, if absorbed, rapidly cleared (Ling & Jones, 1995). This is well illustrated by the results of a previous study from our group where an intake of about 3·3 g/d of esterified vegetable oil plant sterols from a spread *v.* an intake of about 100 mg/d of plant sterols from a control spread raised plasma levels of sitosterol from 3·3 mg/l to 4·6 mg/l and of campesterol from 7 mg/l to 12·1 mg/l (Weststrate & Meijer, 1998). Thus, in spite of significant increases in plant sterol intake i.e. of more than a factor of 30, the increase in plant sterol plasma levels was less than a factor of 2. We have, in addition to the present study, performed a number of studies with high dose levels of plant sterols addressing specific toxicological and physiological issues. These studies have not shown evidence of oestrogenic, toxic or adverse effects of plant sterols on bile acid and neutral sterol excretion or the gut microflora (Ayesh *et al.* 1999; Baker *et al.* 1999; Hepburn *et al.* 1999; Weststrate *et al.* 1999).

We estimate, based on a meta-analysis by Law *et al.* (1994) that the observed reductions in total and LDL-cholesterol concentrations for the soyabean-oil sterol spread could help in reducing CHD risk by about 15 % at age 40 and 6 % at age 70. According to a study of Stampfer *et al.* (1991) the predicted reduction in risk for CHD would be about 10 %.

We conclude that this study shows that consumption of a spread enriched with a low concentration of soyabean-oil sterols in unesterified form is effective in reducing mean total- and HDL-cholesterol levels in free-living subjects by about 4 and 6 % respectively. These decreases are similar to what may be expected from free-living subjects adhering to prudent diets (Tang *et al.* 1998). We have also shown that these decreases occur with limited effects on plasma levels of carotenoids. It would be of public health importance if part of the fat or water in edible fat-containing products could be replaced by plant sterols. This would be predicted to help in reducing the risk of cardiovascular disease in the population.

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hypogonadism can be clearly shown, treatment with sildenafil citrate (with appropriate warnings about cardiovascular risks and drug interactions with nitrites) is likely to be safer and more efficacious than injections of testosterone esters.

To conclude, I really do not find the analogy of the female menopause helpful in understanding or trying to manage the problems of senescence in men. Moreover, the endocrinology of ageing is much broader than that the term suggests. As Lamberts, van den Beld, and van der Lely have pointed out,[16] while the fragility of elderly people might be related to a gonadopause, an adrenopause (the age related fall of dehydroepiandrosterone sulphate concentrations), or a somatopause (the decline in secretion of growth hormone and insulin like growth factor), actually in old people the commonest endocrine disorders are diabetes mellitus and hypothyroidism. These conditions are definitely treatable.

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Plant sterol and stanol margarines and health

Malcolm Law

Department of Environmental and Preventive Medicine, Wolfson Institute of Preventive Medicine, St Bartholomew's and the Royal London School of Medicine and Dentistry, London EC1M 6BQ
Malcom Law *reader*
M.R.Law@mds.qmw.ac.uk

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A new polyunsaturated margarine with added plant stanols, Benecol, was introduced in several European countries last year, and a similar margarine with added plant sterols will be introduced under the Flora label later this year. These products lower serum concentrations of cholesterol, but they are expensive.[1-14] In Great Britain the cost is about £2.50 ($4.00) for a 250 g tub compared with 60p for ordinary polyunsaturated margarine and 90p for butter. This article considers quantitatively the health aspects of adding plant sterols and stanols to margarines and other foods.

Summary points

Plant sterols and stanols reduce the absorption of cholesterol from the gut and so lower serum concentrations of cholesterol

Plant sterols or stanols that have been esterified to increase their lipid solubility can be incorporated into foods

The 2 g of plant sterol or stanol added to an average daily portion of margarine reduces serum concentrations of low density lipoprotein cholesterol by an average of 0·54 mmol/l in people aged 50-59, 0·43 mmol/l in those aged 40-49, and 0·33 mmol/l in those aged 30-39

A reduction in the risk of heart disease of about 25% would be expected for this reduction in low density lipoprotein cholesterol; this is larger than the effect that could be expected to be achieved by people reducing their intake of saturated fat

The added costs of £70 per person per year will limit consumption; however, if stanols and sterols become cheaper, their introduction into the food chain will make them an important innovation in the primary prevention of heart disease

Methods

Randomised trials included in this review were identified by a Medline search using the term "plant sterols." Additional trials were identified from citations in these papers and in review articles. Other trials in children with familial hypercholesterolaemia were not included.

Plant sterols and stanols

Sterols are an essential component of cell membranes, and both animals and plants produce them. The sterol ring is common to all sterols; the differences are in the side chain. Cholesterol is exclusively an animal sterol. Over 40 plant sterols (or phytosterols) have been identified but β-sitosterol (especially), campesterol, and stigmasterol are the most abundant. These three sterols are structurally similar to cholesterol: they are all 4-desmethyl sterols (containing no methyl groups at carbon atom 4).

Stanols are saturated sterols (they have no double bonds in the sterol ring). Stanols are less abundant in nature than sterols. Plant stanols are produced by hydrogenating sterols. The term sterol is sometimes used as a generic term to include unsaturated sterols

and saturated stanols, but it is used here to refer specifically to the unsaturated compounds.

It was recognised in the 1950s that plant sterols lower serum concentrations of cholesterol[15]; they do this by reducing the absorption of cholesterol from the gut by competing for the limited space for cholesterol in mixed micelles (the "packages" in the intestinal lumen that deliver mixtures of lipids for absorption into the mucosal cells).[6 11 16-18] In Europe the average consumption of butter or margarine is 25 g per person each day, and the fortified margarines contain 2 g of plant sterols or stanols per daily portion. About 0.25 g of plant sterols and 0.3 g of cholesterol occur naturally in the daily diet; the amount of plant sterols consumed daily is twice as high in a vegetarian diet. The added plant sterols or stanols in fortified margarine reduce the absorption of cholesterol in the gut—both dietary and endogenous (that is, excreted in bile)—by about half, from the normal proportion of about half the total cholesterol to one quarter. This reduced absorption lowers serum cholesterol despite the compensatory increase in cholesterol synthesis which occurs in the liver and other tissues.[6 11] Plant sterols are potentially atherogenic like cholesterol[19] but atherogenesis does not occur because so little of the plant sterols are absorbed (for example, about 5% of β-sitosterol, 15% of campesterol, and less than 1% of dietary stanols are absorbed).[16] The use of plant sterols as cholesterol lowering drugs has been limited: initially the market was small and later the greater efficacy of statins was evident. By the 1980s, however, it was recognised that as naturally occurring substances plant sterols and stanols could be added to foods. Because fats are needed to solubilise sterols, margarines are an ideal vehicle for them, although cream cheese, salad dressing, and yoghurt are also used. Esterification of the plant sterols and stanols with long chain fatty acids increases their lipid solubility and facilitates their incorporation into these foods. Benecol was the first fortified margarine, and stanols were added because the evidence suggested that they had greater potential to lower cholesterol than sterols and the amount absorbed from the gut is negligible.[16 18 20 21]

Benefits of plant sterols and stanols

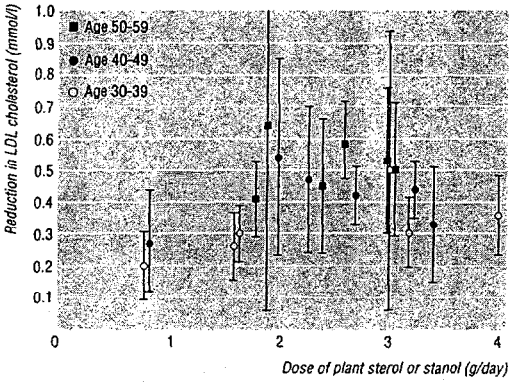
The table summarises the results of randomised double blind trials in adults that compared the ability of polyunsaturated margarines with and without added plant sterols to lower cholesterol. The effect of selection for comparatively high concentrations of serum cholesterol in some trials was modest and, with the exception of one small trial,[13] mean serum concentrations of low density lipoprotein (LDL) cholesterol in the control groups ranged from 3.0 mmol/l to 4.5 mmol/l (median 3.8 mmol/l), close to the age-specific mean found in the Western world. The randomised comparisons in three trials suggested that there was little difference in the extent to which sterols or stanols lower cholesterol concentrations (although the confidence intervals are consistent with the evidence above that stanols are better).[1 12 14] The table shows the reduction in LDL cholesterol in each trial; the reductions in total cholesterol concentrations were similar and there was little change in serum concentrations of high density lipoprotein cholesterol or triglyceride.

The figure shows the reduction in concentration of LDL cholesterol achieved in each trial and the dose of plant sterol or stanol. The reduction in the concentration of LDL cholesterol at each dose is significantly greater in older people than in younger people. The dose-response relation is continuous up to a dose of about 2 g of plant sterol or stanol per day. At higher doses no further reduction in LDL cholesterol is apparent, confirming the evidence of a plateau identified by earlier non-randomised studies.[17] At doses of ≥2 g per day the average reduction in serum LDL cholesterol was 0.54 mmol/l (14%; 95% confidence

Randomised double blind trials that reported the difference in serum cholesterol obtained from using polyunsaturated margarines with and without added plant sterols or stanols. Trials were parallel group trials unless indicated otherwise

Trial (place)	No of participants in treatment group/placebo group	Mean age (years)	Duration of trial (weeks)	Type	Average daily dose (g)	Placebo adjusted reduction in serum low density lipoprotein cholesterol (mmol/l)
Westrate et al[1] (Netherlands)	80*	45	3.5	Stanol	2.7	0.42 (0.33 to 0.51)
				Sterol	3.2	0.44 (0.35 to 0.53)
Hendriks et al[2] (Netherlands)	80*	37	3.5	Sterol	0.8	0.20 (0.10 to 0.31)
				Sterol	1.6	0.26 (0.15 to 0.36)
				Sterol	3.2	0.30 (0.20 to 0.41)
Miettinen et al[3] (Helsinki)	51/51	50	52	Stanol	1.8	0.41 (0.29 to 0.53)
	51/51			Stanol	2.6	0.59 (0.47 to 0.71)
Hallikainen et al[4] (Finland)	38/17	43	8	Stanol	2.3	0.47 (0.24 to 0.70)
Vanhanen et al[5] (Helsinki)	34/33	46	6	Stanol	3.4†	0.33 (0.15 to 0.51)
Gylling et al[6] (Helsinki)	22*	51	7	Stanol	3.0	0.53 (0.30 to 0.76)
Jones et al[7] (Montreal)	22*	35	1.4	Sterol	1.7‡	0.30 (0.21 to 0.39)
Gylling et al[8] (Helsinki)	21*	53	5	Stanol	2.4§	0.45 (0.24 to 0.66)
Jones et al[9] (Montreal)	16/16	about 50	4	Stanol	1.9	0.64 (0.06 to 1.22)
Niinikoski et al[10] (Finland)	12/12	37	5	Stanol	3.0	0.50 (0.06 to 0.94)
Gylling et al[11] (Helsinki)	11*	58	6	Stanol	3.0	0.50 (0.29 to 0.71)
Miettinen et al[12] (Helsinki)	9/8	45	9	Stanol	1.0†	0.28 (0.01 to 0.55)¶
	7/8			Sterol	0.8†	0.26 (−0.05 to 0.57)¶
Vanhanen et al[13] (Helsinki)	7/8	47	6	Stanol	0.8†	0.28 (0.0 to 0.56)¶
	7/8			Stanol	2.0†	0.54 (0.23 to 0.85)
Plat et al[14] (Netherlands)	70/42	33	8	Stanol	4.0	0.36 (0.23 to 0.49)

*Crossover trial. †In mayonnaise. ‡In olive oil. §In butter. ¶Data from these small trials which tested low doses are combined in the figure.



Results of randomised double blind trials of margarines with and without added plant sterols or stanols showing the reduction in serum concentrations of low density lipoprotein (LDL) cholesterol (95% confidence intervals) plotted against the daily dose. The data are from the table

interval 0.46 to 0.63 mmol/l) for participants aged 50-59,[5 6 8 11] 0.43 mmol/l (9%; 0.37 to 0.47 mmol/l) in participants aged 40-49,[1 4 5 13] and 0.33 mmol/l (11%; 0.25 to 0.40 mmol/l) for those aged 30-39[1 2 5 10]; this trend was statistically significant (P = 0.005). At a dose of 2 g per day (the amount added to an average daily portion of fortified margarine) the reduction in LDL cholesterol is likely to be at least 0.5 mmol/l for those aged 50-59 and 0.4 mmol/l for those aged 40-49.

Data from observational studies and randomised trials indicate that in people aged 50-59 the reduction in LDL cholesterol of about 0.5 mmol/l would reduce the risk of heart disease by about 25% after about two years.[22] In younger people the proportionate reduction in risk would be similar (the reduction in cholesterol concentrations is smaller but the association between cholesterol and heart disease is stronger).[22] Trials of six different interventions to lower serum cholesterol have all found a reduction in the incidence of heart disease (these interventions include four pharmacologically unrelated drugs, a reduction in dietary saturated fat, and ileal bypass surgery).[22 23] Nothing except a reduction in cholesterol is common to the six interventions, and for each intervention the proportionate reduction in mortality from heart disease is commensurate with the reduction in cholesterol concentration.[17 18] Margarines with plant sterols or stanols thus reduce the risk of heart disease by one quarter: this is the reduction expected from the decrease in serum cholesterol.

This is an impressive result for a dietary change that, price apart, is modest. It is larger than the effect that could be expected to occur if people ate less animal fat, and it is all the more impressive in light of the fact that despite the extensive promotion of healthy eating there has been little reduction in average serum cholesterol concentrations in many countries. Recent surveys in England found that mean cholesterol concentrations are only 1-2% lower than those of 25 years ago.[24 25] For a person replacing butter with a plant sterol margarine the reduction in cholesterol would be even greater. Replacing butter with ordinary polyunsaturated margarines lowers total serum and LDL cholesterol by about 0.3 mmol/l,[26 27] so the overall reduction would be about 0.7 mmol/l, or as much as any cholesterol lowering drug except statins.

Efficacy in combination with low fat diets

One non-randomised study found only a small average reduction in LDL cholesterol concentrations (0.16 mmol/l) despite participants taking 3 g of plant stanols daily.[28] The participants were on a low fat and low cholesterol diet, and the result was interpreted as suggesting that plant sterols are ineffective when dietary fat, dietary cholesterol, or LDL cholesterol concentrations are low. This is unlikely. In two recent randomised trials of stanol margarines in which participants were on low fat, low cholesterol diets, the reductions in serum concentrations of LDL cholesterol were similar to those found in other trials in which the intake of dietary fat was higher.[4 9] Plant stanols were equally effective in patients taking statins who had mean LDL cholesterol concentrations of only 2.9 mmol/l.[6] Other explanations for the discrepancy are more plausible: chance (at the upper confidence interval of the result, an LDL cholesterol reduction of 0.43 mmol/l is what might be expected) or the fact that the stanol was administered in capsules and not esterified and blended into the fat of a meal. (Sterols administered in capsules may not disperse fully or dissolve in the gut, limiting their ability to reduce the absorption of cholesterol.[9])

Safety

The most important concern about plant sterols is that they reduce the absorption of some fat soluble vitamins. Randomised trials have shown that plant sterols and stanols lower blood concentrations of β carotene by about 25%, concentrations of α carotene by 10%, and concentrations of vitamin E by 8%.[1 2 8 29] Since these vitamins protect LDL cholesterol from oxidation, and sterols and stanols reduce the amount of LDL cholesterol, the changes in blood concentrations of the vitamins were adjusted in the trials for the lower concentrations of LDL cholesterol. With this adjustment concentrations of vitamin E were not lower but concentrations of β carotene were reduced by between 8% and 19%.[1 2 8 29] There was no benefit to increasing the blood concentrations of β carotene and vitamin E by greater proportions than these,[30 31] although we do not know whether this is the case for other carotenes. Eating more fruit and vegetables would counter the decrease in absorption. The blood concentration of vitamin D is unaffected.[2 8] No other side effects or biochemical anomalies were evident in the randomised trials of plant sterol or stanol margarines (one of which lasted a year[5]), in earlier studies testing doses as high as 3 g/day for three years, or in animal studies testing proportionately higher doses.[16 17 32] Stanol margarines have been sold in Finland for three years without evidence of hazard, and a tenth of the amount of plant sterols found in these margarines occurs naturally in a normal diet. Plant sterols or stanols do not adversely affect the taste or consistency of margarines.[5 10]

The place of sterol and stanol margarines in the diet

The excess cost per person of margarines containing added plant sterols or stanols is about 20p per day or £70 per year. Affluent people may willingly pay this to reduce their risk of death from heart disease by a quarter but poorer people, who are at higher risk of heart disease, will tend to be dissuaded from buying the product. The cost reflects the large amount of raw material needed (about 2500 parts to extract one part sterol). Moreover, supplies are limited. The present sources—extracted from a byproduct in the refining of vegetable oils or from the oil obtained from pinewood pulp in papermaking—can supply only about 10% of the people in the West. In the foreseeable future, the product will be used only by a minority of people. However, in many countries there is also a legal obstacle: no health claim can be made in the advertising of these margarines because they are a food, not a drug. More people might buy the product if they were aware of the size of the health benefit.

Plant sterol and stanol margarines may appeal to patients with ischaemic heart disease but they should not replace statins because the reduction in the concentration of LDL cholesterol is greater with statins. Both could be taken together however, since the cholesterol lowering effects of the two are additive.[6] The overall costs of the two are equivalent: statins cost about three times as much as plant sterol margarines but they lower serum cholesterol by three times as much.

In the longer term, the addition of plant sterols and stanols to foods could be an important public health policy if new technology and economies of scale can lower the cost and enable a greater demand to be met. The serum cholesterol of the average older adult in Western countries is high (6.0 to 6.5 mmol/l),[24] with a correspondingly high lifetime risk of death from heart disease (about 25%). Introducing plant sterols into the food chain would lower the average serum cholesterol concentration in Western countries, with the added advantage of "demedicalising" the reduction (that is, one would not have to become a patient to benefit). There is a precedent for such fortification: in the United States folic acid has been added to flour since 1997. In addition to the expected reduction in the incidence of neural tube defects, there has also been a significant reduction in the average serum concentration of homocysteine,[33] which is likely to reduce mortality from heart disease.

The launch of margarines containing plant sterols and stanols is a welcome first step in what may become an important innovation in the primary prevention of ischaemic heart disease. It is to be hoped that in the longer term plant sterols and stanols will become cheap and plentiful and so will be able to be added to foods eaten by the majority of the population.

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LB198

Self-Help Weight Loss Versus A Structured Commercial Program After One Year: A Randomized Controlled Study S. Heshka, J. W. Anderson, R.L. Atkinson, S.D. Phinney, F. Greenway, J.O. Hill, K. Miller-Kovach, F.X. Pi-Sunyer. NY Obesity Research Center, St. Luke's/Roosevelt Hospital, NY 10025; VA Medical Center, Lexington, KY 40511; Nutritional Sciences, U. Wisconsin, Madison WI 53706; Dep't. of Medicine, UC Davis, Sacramento CA 95817; Pennington Biomedical Ctr, Baton Rouge, LA 70808; Health Sciences, U Colorado, Denver CO 80262; Weight Watchers Int., Woodbury, NY 11797.

We report 1-year results of a multicenter, randomized, parallel groups study of a popular commercial program (Weight Watchers, WW). Four hundred and twenty three overweight men and women, BMI (kg/m^2) 27 – 40, 18–65 yrs old, were randomized into either a self-help (SH) condition consisting of two 20-min. sessions with a nutritionist and provision of printed educational material and other publicly available self-help resources, or to attendance at local WW meetings. At 6 months 347 subjects (82%) and at 1 year 341 subjects (81%) returned to the study center for measurements. Body weight of these subjects decreased at 6 months from baseline 93.7±13.0 kg (mean±SD) by 5.7±5.7 kg in the WW group and from 93.4±14.8 by 1.7±5.0 kg in SH ($p<.001$). At one year, mean body weight was 5.0±8.3 kg below starting weight in WW group vs 1.7±6.8 kg below in SH ($p<.001$). 22% of WW vs 11% of SH remain 10% or more below initial body weight ($p<.05$). A structured commercial weight loss program is more effective for achieving and maintaining modest weight loss over a 1 year period than brief counseling and self-help.
Research supported by Weight Watchers Foundation

LB199

EFFECT OF DIETARY STEROL ESTERS IN SALAD DRESSINGS ON BLOOD LIPIDS, LIPOPROTEINS AND CAROTENOIDS. J. Judd, D. Baer, B. Clevidence, S. Chen, and G. Meijer. Beltsville Human Nutrition Research Center, ARS, USDA, Beltsville, MD 20705; and Lipton, Baltimore, MD 21229.

The plasma lipid modifying effects of feeding vegetable oil sterol esters (VOSE) in salad dressings were determined in 26 men and 27 women fed controlled, isocaloric diets for 6 wk. Diets contained typical American foods that provided 32.5 percent of energy from fat with a P:M:S ratio of 1.3:1.4:1.0. To determine blood lipid effects of fat level and VOSE in salad dressings, 3.6 g VOSE/d (2.2 g sterol equivalent), was divided into 2 servings/d of either "Ranch" dressing (8 g fat per serving), or "Italian" dressing (4 g fat per serving), added to the same basal diet, and compared in a parallel design. Within each type of dressing, diets having dressing without (control) and with VOSE (test) were fed for 3 wk/diet and crossed over randomly. Control and test dressings had similar fat and fatty acid concentrations. The type of salad dressing or gender did not affect plasma LDLC, HDLC, or TG ($p>0.05$). With data for dressings and gender combined, switching from a self-selected baseline diet to the control diet with no VOSE resulted in reduction in LDLC of 8.4% ($p<0.0001$), HDLC of 3.1% ($p=0.009$), and TG of 9.2% ($p<0.0001$). Daily consumption of 3.6 g VOSE resulted in further decreases in LDLC of 9.7% ($p<0.0001$) and TG of 7.3% ($p=0.005$). There was no further decrease in HDLC due to VOSE consumption ($p>0.4$). Total plasma carotenoids, uncorrected for plasma lipids, decreased by 11.6% ($p<0.0001$) with VOSE consumption compared to control. However, plasma carotenoids on all diets remained within normal ranges. We conclude that low fat foods, such as reduced fat salad dressings, are effective carriers for VOSE and, with their low fat content, can readily be included in a moderately low fat, low saturated fat (prudent) diet to lower blood cholesterol.

LB200

EFFECTS OF SOYOLIGOSACCHARIDES ON HUMAN FECAL MICROFLORA AND PLASMA TRIGLYCERIDE CONCENTRATION.
Sook He Kim, Da Hee Lee, Eun Jung Gu, Woo Kyung Kim, Hye Young P. Kim, Ewha Womans University, Dankook University, Yongin University, Korea

We reported previously (J. Korean Nutr. 32(3):221-229, 1999) that dietary soybean oligosaccharides were beneficial for increasing numbers of intestinal bifidobacteria and lowering plasma triglyceride(TG) concentrations of the experimental rats. The purpose of this study was to elucidate the effects of soybean oligosaccharides on human fecal microflora proliferation, evacuation habits and plasma lipid concentration of the normal female college students. The thirty-two female students were randomly assigned to either a soyoligosaccharide or a control group. The subjects were supplied with soyoligosaccharides(10g) or sucrose(5g) dissolved in a cup of warm water daily for 5 weeks. The soyoligosaccharide supplementation increased the number of fecal bifidobacteria of the subjects. The frequency of stool evacuation and the color of stool were also improved by oligosaccharide supplementation. Dark brown color was diminished and yellow color increased. However, the required times to evacuate, the hardness of stool were not changed much by either supplementation. The plasma TG concentration of the subjects, which was low in the beginning(66.7 ± 6.8mg/dl), was not affected much by oligosaccharide supplementation, with increasing tendency by supplying sugar. Therefore, the results of this study suggest that supplementation of soybean oligosaccharides benefits gastrointestinal tract via proliferation of bifidobacteria and plasma TG concentration of female college students. (Supported in part by Ministry of Health and Welfare of Korea #HMP-97-F-5-0030)

LB201

OXIDATIVE STRESS AND ANTIOXIDANT SUPPLEMENTATION IN YOUNG CHILDREN. [1]R. Stewart, [1]E.W. Askew, [2]C.M. McDonald, [2]J. Metos, [2]W.D. Jackson, [3]T. Balon and [4]R.L. Prior. [1]University of Utah and [2]Primary Children's Medical Center, Salt Lake City, UT 84112; [3]City of Hope, Duarte, CA; [4]Tufts Human Nutrition Research Center, Boston, MA.
Little information exists concerning oxidative stress in healthy young children. A placebo controlled clinical trial with 39 healthy mixed gender children between the ages of 5-10 years old was designed to study oxidative stress indicators and their response to a commercially available fruit and vegetable based antioxidant supplement. The subjects were randomly assigned to a placebo group (n=20) and a treatment (antioxidant supplement, commercially produced from dried fruit and vegetable extracts and fortified with antioxidants, resembled a gummy type candy) group (n=19). The placebo and the supplement were taken in two doses per day for 21 days. Breath and urine samples were collected on days 1 and 21 and assayed for breath pentane and urine 8-hydroxydeoxyguanosine, malondialdehyde, nitrite, and 8-isoprostane as non-invasive indicators of oxidative stress. Urine Oxygen Radical Absorbance Capacity (ORAC) was measured at days 1 and 21 as an indirect indicator of the antioxidant capacity of the body. Three-day food records, collected at the beginning and end of the study, determined mean fruit and vegetable intakes to be similar to the national average intake for American children; vitamins A and C exceeded, but vitamin E was 60% of, the RDA. There was no significant difference for any of the markers of oxidative stress between the placebo and treatment groups at day 1 or day 21. Oxidative stress indicators of healthy children appear to be similar to those of healthy adults and were not changed by antioxidant supplementation. The results of this study may be useful as normative data for future research with children in disease states who are subject to elevated levels of oxidative stress.

BIOCHEMISTRY OF VITAMINS AND MINERALS

LB202

^{13}C- INTRINSICALLY LABELLED FOLATES IN SPINACH FOR BIOAVAILABILITY STUDIES. P. M. Finglas, D. Hart, C. Wolfe, A. Wright, R. Faulks, S. Southon and J. F. Gregory. Institute of Food Research, Norwich, GB and University of Florida, Gainesville, FL 32611-0370, USA.

Folates have been intrinsically labelled in spinach for bioavailability studies. Spinach plants were grown in greenhouses and gassed with $^{13}CO_2$ on 9 occasions every 3-4 days, starting when the plants were at the two major leaf stage. The spinach was harvested at 10 weeks yielding 6 kg leaves, washed with water, microwaved for 6 min, and frozen in 150 g portions. Folate content, determined by HPLC with fluorimetric detection following FBP-affinty column clean-up, was 110µg/100g, mainly as 5-methytetrahydrofolate (5-CH_3H_4-$PteGlu_n$). Isotopic enrichment was determined using a combined LC-MS system. Eluant from the affinity columns was injected onto a LiChrosphere RP18 column (250x4.6 mm) with a volatile mobile phase of 0.1M acetic acid:acetonitrile (90:10; v:v; 0.6 ml/min), adjusted to pH 3.3 using 1M ammonium acetate (retention time for 5-CH_3H_4-PteGlu = 9.1 min). The eluant was passed directly into the mass spectrometer using a post-column splitter (0.33:0.67; MS:LC). Mass spectrometry was carried out using negative ion electrospray with selective ion monitoring at m/z 458-461. The atom enrichment of 5-CH_3H_4-PteGlu was +67% (m+1), +268% (m+2); total enrichment was 2.2%. The material is being fed to human volunteers, and plasma collected for enrichment measurements. The work was funded by MAFF.

LB203

HOMOCYSTEINE AND VITAMIN B12 IN AN INDIAN POPULATION.
H Refsum, CS Yajnik, M Gadhari, J Schneede, A Joglekar, MG Sayyad, AB Guttormsen, A-L Bjørke-Monsen, A Ulvik and PM Ueland (Spon: CH Halsted).
Dept. of Pharmacology, Univ. of Bergen, Norway and Diabetes Unit, King Edward Memorial Hospital and Research Centre, Pune, India.

In India, most subjects adhere to a vegetarian diet which may lead to a vitamin B12 deficiency. In the present study, we investigated total homocysteine (tHcy) and vitamin B12 status in 204 subjects (35 women) aged 27-55 y, recruited in Pune, Mahrashtra, India.

Plasma tHcy was markedly elevated (median tHcy=19.8 µM) whereas serum B12 was low (median B12=154 pM). Plasma tHcy was not associated to age, sex, creatinine or folate, but showed strong correlation to vitamin B12 (r=-0.60) and MMA (r=0.48). The diet did not explain this finding since both vegetarians (n=78) and non-vegetarians (n=126) frequently had hyperhomocysteinemia (79 vs 76%) and B12 deficiency (60 vs 39%). Neither hyperhomocysteinemia nor B12 deficiency was associated with anemia or macrocytosis.

In conclusion, the majority of subjects in this population have elevated tHcy related to a vitamin B12 deficiency. If impaired vitamin B12 status is equally common in the general Indian population, this may have major health implications.

Experimental Biology

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