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By courier

November 8, 2001

Food and Drug Administration (FDA)
Dockets Management Branch (HFA-350)
5630 Fishers Lane, rm. 1061
ROCKVILLE MD 20852
U.S.A.

Ref. Reopening of Comment Period for the interim final rule authorizing a health claim on the association between plant sterol/stanol and reduced risk of Coronary Heart Disease (CHD); publication date October 5, 2001; docket Nos. 00P-1275 and 00P-1276.

COMMENT BY MB MULTI BENE HEALTH OY LTD ON UNESTERIFIED PLANT STEROLS/STANOLS

With regard to the subject Comment Period, as described in the title above, we would like, on behalf of the Finnish registered limited company MB Multi Bene Health Oy Ltd, to file the following comments on the beneficial effects of unesterified plant sterols/stanols, which is the main business of our company. Please note that this letter is signed by Mrs. Anu Mehtonen, legal representative of the said company, and its Legal Counsel, Mrs. Maria Parker of Ernst & Young in Helsinki, Finland. Any communications in this matter should be made to one of both of them (details below).

1. Background

To estimate the eligibility of the unesterified forms of plant sterols/stanols for the health claim, the Food and Drug Administration has requested additional information and comments concerning the effectiveness of unesterified plant sterols/stanols in reducing the elevated serum cholesterol level and the risk of coronary heart disease (CHD), particularly at lower intake levels from various food products.

After studying the effectiveness and safety of unesterified plant sterols for years, MB MultiBene Health Oy Ltd submits the following documents to support its opinion, that there should be enough scientific studies and human trials to justify the health claim for unesterified plant sterols and stanols.

00P-1275 C 15

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2. Multibene ® patent

The use of natural, unesterified plant sterols/stanols in food products with one or more of the minerals calcium, magnesium and potassium is protected by the United States Patent 6,136,349. This invention is called MultiBene® for its multiple beneficial effects. A Novel Food Application has been submitted to place plant sterols containing the MultiBene®-ingredient on the European market. Also to ANZFA has been submitted the Novel Food Application to place MultiBene® on the Australian- New Zealand novel food market. (GRAS notification may be submitted by the end of the year 2001).

3. Equivalence

In the human gastrointestinal (gut) track, plant sterol esters are hydrolysed to fatty acids and free sterols by pancreatic carboxyl ester lipase (Miettinen et al. 1999; European Commission, 2000; Federal Register FDA, 2000; Normen et al. 2000). Therefore, sterol esters are biologically and physiologically equivalent to free sterols (Federal Register FDA, 2000).

FDA has also concluded that the studies of the effectiveness of free plant stanols in blood cholesterol reduction are relevant to the evaluation of the relationship between plant stanol esters and reduced risk of coronary heart disease when such studies met the study selection criteria specified (Federal register FDA, 2000). Irrespective of the ingested form (free *vs.* esterified), the free (unesterified) plant sterol is the active component also in safety related phenomenon. Thus, it can be concluded that the data relating to plant sterol and stanol esters can be directly applied to free plant sterols and stanols.

4. Studies and trials

Several extensive animal and human studies on the effects of plant sterols containing MultiBene® have shown a very effective lowering of elevated blood pressure and cholesterol concentrations. A wide range of food products, such as meat products, yoghurt and other dairy products, bread and other bakery products have been used in these studies.

In a clinical trial (Korpela et al. 2001) the vegetable oil derived plant sterol enriched yoghurt, low-fat fresh cheese and low-fat hard cheese decreased serum total and LDL-cholesterol levels in mildly hypercholesterolemic subjects. A randomised, placebo-controlled, double-blind parallel study design with six-week intervention period was used. 164 subjects participated in

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the study. The subjects were randomised into six groups, which were sterol yoghurt, placebo yoghurt, sterol fresh cheese, placebo fresh cheese, sterol hard cheese and placebo hard cheese groups. The subjects consumed either 150 g of yoghurt, 50 g of fresh cheese or 50 g of hard cheese per day. Daily doses of test products provided circa 2 grams of plant sterols.

Total cholesterol concentrations decreased by 4.0-8.3 % in the plant sterol groups. The plant sterol enriched fresh cheese and hard cheese reduced serum total cholesterol concentrations statistically significantly compared to their placebos. LDL-cholesterol concentrations decreased by 8.7-11.2 % in the plant sterol groups. In each plant sterol group, the reduction was statistically significant compared to their placebo groups.

In a recently published study made with plant sterol enriched low-fat yoghurt (Volpe et al. 2001), thirty moderate hypercholesterolemic subjects participated in study. The study started with an 8-week low-fat low-cholesterol diet. After that, the subjects were randomly assigned to one of two treatment groups for a period of 4 weeks: a low fat yoghurt (100 ml) enriched with a 1 g plant sterol extract versus 100 ml of traditional low-fat yoghurt. After a 2-week wash-out period, subjects were crossed over for an additional 4-week period. After a 4-week wash-out period, eleven of the thirty subject were treated with 2 g plant sterols per day for a period of 8 weeks. The consumption of yoghurt with 1 gram daily dose of plant sterols significantly reduced serum total cholesterol and LDL-cholesterol levels and LDL-cholesterol: HDL-cholesterol. The effect of plant sterols on serum total and LDL cholesterol seemed to be dose-dependent. No changes were observed in HDL-cholesterol and triacylglycerol levels.

In a clinical trial (Sarkkinen et al. 1999) tall oil derived plant sterols enriched frankfurters and cold cuts reduced serum total cholesterol concentrations significantly in hypercholesterolemic subjects. A randomised, placebo-controlled, single-blind repeated measure design with three test periods was used. Altogether 21 subjects participated in the study. The subjects consumed a total of 75 grams of frankfurters and cold cuts per day, which provided 2.1 grams of plant sterols.

As a result, serum total cholesterol concentration decreased by 4.9 % and LDL-cholesterol by 4.6 %. No effect on serum HDL-cholesterol and triglyceride concentrations was found.

In a clinical trial (Tikkanen et al. 2001; a manuscript) three different food items were enriched with tall oil derived plant sterols. Daily consumption 100 g of bread (wheat and rye bread), 8-16 g of jam (ingested mixed with

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natural non-flavoured yoghurt) and 70 g of meat products (meatballs and sausage products), significantly reduced both serum total and LDL-cholesterol concentrations and improved the HDL/LDL-ratio. A randomised, double-blind, placebo-controlled parallel study with two groups and three test periods was used. The study comprised of 71 hypercholesterolemic subjects. Half of the subjects consumed plant sterol enriched food items and half of the subjects placebo products. During the first intervention period the plant sterol enriched food items were calculated to provide 1.25 g of plant sterols, during the second period 2.5 g and during the third period 5 g.

According to the food diaries, the actual intake of plant sterols were: during the first period 0.9 g, the second period 1.9 g and the third period 4,2 g. During the three periods (0.9 g; 1.9 g; 4.2 g, respectively) both total and LDL-cholesterol levels decreased significantly more in the intervention group than in the placebo group: total cholesterol by 6 %, 6 %, 8 % (intervention) vs. 0-3 % (placebo) and LDL-cholesterol by 10 %, 10 %, 13 % (intervention) vs. 3-5 % (placebo). The HDL/LDL-ratio improved significantly in the intervention group compared with the placebo group.

5. References and study reports to demonstrate effects of plant sterols/stanols in various food products.

(The reports attached not hereto are market with *).

ATTACHMENT 1: European Commission. Opinion of the Scientific committee on food on a request for the safety assessment of the use of phytosterol esters in yellow fat spreads, 2000, European Commission, Health & Consumer Protection Directorate-General.

ATTACHMENT 2 (front cover only): Federal Register: September 8, 2000. Volume 65, Number 175, Pages 54685-54739
http://vm.cfsan.fda.gov:80/rd/fr000908.html

ATTACHMENT 3: H. Karppanen, T. Vaskonen and E. Mervaala: Novel "MultiBene" food composition lowers serum cholesterol and decreases obesity. XIII International Symposium on Drugs Affecting Lipid Metabolism. Florence, Italy, 1998. Abstract Book, page 32.

ATTACHMENT 4: Korpela Riitta, Högström Pia et al. Plant sterols reduce serum lipids incorporated in low fat dairy products. 2001.

* Miettinen TA. Stanol esters in the treatment of hypercholesterolemia. European Heart Journal Supplement 1999;1:S50-S57.

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* Normen L, Dutta P, Lia Å, Andersson H. Soy sterol esters and b-sitostanol ester as inhibitors of cholesterol absorption in human small bowel. American Journal of Clinical Nutrition 2000;71:908-913.

ATTACHMENT 5: Sarkkinen Essi, Tapola Niina, Uusitupa Matti. Effect on low fat and low salted meat products enriched with Multibene® on serum total, lipoprotein lipids and blood pressure in subjects with mild to moderate hypercholestrolemia. Study report. 1999.

ATTACHMENT 6: Sumuvuori V., Vaskonen T., Helenius H., Mervaala E. and Karppanen H.: Cholesterol lowering effect of sitostanol ester margarine and plant sterols is enhanced by co-administration of calcium, magnesium and potassium. Abstract Book, A-club meeting, April 16-17, 1999, Helsinki.

ATTACHMENT 7: Vaskonen T., Mervaala E. and Karppanen H.: Incorporation of mineral nutrients and plant sterols in a high-salt, high-cholesterol, and high-fat diet lowers blood pressure and serum cholesterol, and decreases obesity. Abstract Book, A-club meeting, April 16-17, 1999, Helsinki.

ATTACHMENT 8: Tikkanen Matti J., Högström Pia et al. Effect of diet based on low-fat foods enriched with no-esterified plant sterols and mineral nutrients on serum cholesterol. Manuscript.

ATTACHMENT 9: Tuomilehto J., Högström P., Keinänen-Kiukaanniemi S., Tikkanen M., Karppanen H.: Reduction of serum cholesterol concentration with a diet consisting of common foods enriched with plant sterols. European Heart Journal 1999; 20 (Abstract Supplement), XXI Congress of the European Society of Cardiology, Barcelona, Spain. Page 176.

ATTACHMENT 10: Vaskonen T., Mervaala E. and Karppanen H.: Novel food composition prevents the increase of blood pressure and serum cholesterol during a high-fat, high-salt diet, and decreases obesity. Scandinavian Cardiovascular Journal 1999; 33 (Suppl. 51), P 62. Page 45.

ATTACHMENT 11: Vaskonen T., Mervaala E. and Karppanen H.: Incorporation of mineral nutrients and plant sterols in a high-salt, high-cholesterol, and high-fat diet lowers blood pressure and serum cholesterol, and decreases obesity. 5th International Symposium on Multiple Risk Factors in Cardiovascular Disease: Global Assessment and Intervention, Venice (Italy), October 28-31, 1999. Abstract Book.

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ATTACHMENT 12: Vaskonen T., Mervaala E. and Karppanen H.: Incorporation of mineral nutrients and plant sterols in a high-salt and high-fat diet lowers blood pressure and serum cholesterol, and decreases obesity. Circulation 1999 (Supplement), Abstracts of the 72nd Scientific Sessions, November 7-10, 1999, Atlanta, Georgia, USA.

ATTACHMENT 13: Volpe Roberto, Niittynen Leena, et al. Effects of yoghurt enriched with plant sterols on serum lipids in patients with moderate hypercholesterolemia. *British Journal of Nutrition* (2001), 86, 233-239

ATTACHMENT 14: Sumuvuori et al. Appendix 31. Scientific abstact on animal studies.

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Should you have any queries or require additional information, as well as for any other communication, please contact the signatories.

Yours truly,

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APPENDICES

ATTACHMENT 1

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

SCF/CS/NF/DOS/1 FINAL

OPINION OF THE

SCIENTIFIC COMMITTEE ON FOOD

ON

A REQUEST FOR THE SAFETY ASSESSMENT OF

THE USE OF PHYTOSTEROL ESTERS IN YELLOW FAT SPREADS

6 APRIL 2000

1. TERMS OF REFERENCE

With reference to the initial assessment carried out by the Dutch authorities, in the light of the relevant comments/objections presented by member states and pursuant to the article 11 of Regulation (EC) 258/97, the Committee is asked to assess the safety from the point of view of consumer health, of phytosterol esters in yellow fat spreads as a novel food.

2. BACKGROUND

The Commission has received the initial evaluation of a petition for an approval for the use of phytosterol esters in yellow fat spreads as a novel food. Phytosterol esters, used at levels proposed in the application, will lead to a new product with enhanced cholesterol lowering activity.
The application was made under the Novel Foods and Novel Food Ingredients Regulation 258/97/EC. The product is classified under Class1, Subclass1: a pure chemical substance or simple mixture obtained from sources already in use for food purposes in the European Community.

The petitioner first submitted an application to the Dutch competent authority for initial assessment. The Dutch Preliminary Advisory Committee on the Safety of Novel Foods performed the initial safety assessment of this new product, concluding that "...the product.....is safe for human use at the levels indicated below. The committee has assessed the full dossier and with current knowledge sees no human health concern occurring from a nutritional or toxicological point of view. However, ...the committee advises to restrict the dosage of the phytosterols to a maximum of 8% w/w. At this level serum cholesterol levels drop practically at the same rate as with higher doses of phytosterols, but there is less or no drop in serum carotenoid levels".

During the consultation procedure following the initial assessment by the Netherlands, comments/objections were made by a number of Member States' Authorities. The European Commission therefore decided to submit this dossier to the Scientific Committee on Food (SCF) for evaluation of the safety of this product. Major questions, concerns and recommendations made by Member States were:

- It was questioned whether the product is actually free of rDNA,
- Appropriate labelling of the phytosterol esters ingredient is needed to reach the proper target groups,
- The level of phytosterols permitted to be used in the margarine should be limited to a maximum of 8% (w/w),
- Long term clinical studies are recommended to clarify whether the reduction of cholesterol absorption will be compensated by increased endogenous cholesterol synthesis,

- The assessment of the ß-carotene lowering effect should be based on the 97.5th percentile of intakes rather than the mean,
- Approval of the product will include validation of health claims,
- Time limited (1 year) permission and post-marketing information should be recommended,
- Oxidation products of cholesterol and phytosterols should be addressed,
- Health risk posed by the product to phytosterolaemic individuals should be sufficiently taken into consideration.

These questions, concerns and recommendations are addressed by the SCF in this opinion if considered relevant for the safety evaluation.

3. EVALUATION

The application submitted by the petitioner follows the Commission Recommendations (1). The product falls into Class 1, Subclass 1: a pure chemical substance or a simple mixture obtained from sources already in use for food purposes in the European Community. According to Regulation 258/97 phytosterols would fall under article 1, Paragraph 2, Section e.

The present evaluation is based on the structured schemes of the SCF as a guide to identify the different aspects required to establish the safety of the novel food (1), on the information submitted by the petitioner (2), and on the comments of the member states on the initial assessment report made by the Dutch Competent Authority.

3.1 Specification of phytosterol esters

With phytosterol esters as a novel food ingredient added to margarine, the applicant developed a yellow fat spread that enhances cholesterol-lowering activity in humans. This novel food ingredient is the esterification product of phytosterols, mainly with polyunsaturated and, in part, monoenoic fatty acids. The common or usual name of phytosterol esters is proposed to be "vegetable oil sterol esters". Phytosterols are extracted from the edible oils (soya, maize, rapeseed, sunflower) and esterified with sunflower oil fatty acids. The product tested in genotoxicity studies had the following specification (results of the analysis of five different batches) (2):

- Free fatty acids <0.2% (w/w)
- Total fatty acids 37.0 – 37.7% (w/w), mean 37.4% (w/w)
- Fatty acid composition (of total fatty acids):
  - C16:0 7.5 – 7.6%
  - C18:0 4.8 – 4.9%
  - C18:1 22.6 – 22.7%
  - C18:2 65.0 – 65.1%
- Free sterols 10.5 – 11.8% (w/w), mean 10.9% (w/w)
- Total sterols 62.3 – 63.0% (w/w), mean 62.6% (w/w)
- Sterol profile (of total sterols):

Cholesterol	0.2 – 0.3%
Brassicasterol	2.7 – 3.1%
Campesterol	26.5 – 27.0%
Stigmasterol	17.4 – 18.0%
ß-sitosterol	50.8 – 51.2%
unknown	1.2 – 1.7%

- Total volatiles <0.5% (w/w)
- Peroxide values 1.5 – 2.1 meq/kg, mean 1.9 meq/kg

Based on the variability in sourcing/seasonal variation of the plant sterols, the applicant expects the following sterol profile (2):

Cholesterol	0.0 – 2.0% of total sterols
Brassicasterol	0.0 – 9.0%
Campesterol	10.0 – 40.0%
Campestanol	0.0 – 6.0%
Stigmasterol	6.0 – 30.0%
ß-sitosterol	30.0 – 65.0%
Sitostanol	0.0 – 10.0%
D5-Avenasterol	0.0 – 4.0%
D7-Avenasterol	0.0 – 2.0%
D7-Stigmastenol	0.0 – 2.0%
Other	0.0 – 5.0%

Phytosterols occur naturally in food as free alcohol, esterified with long chain fatty acids (25 – 80% of total phytosterols) and conjugated as glucosides (usually in small amounts). The majority of plant oils contains 0.1 – 0.5%, while some germ oils (rice bran, wheat germ, oats) contain up to 4% total phytosterols (3). Reduced and low fat spreads on the market contain approximately 0.3-0.4% phytosterols.

The application for this novel food ingredient covers yellow fat spreads with increased levels of phytosterols (up to 12%, on average 8%).

3.2 Effects of production processes

Phytosterols are by-products of vegetable oil refining. Phytosterols are isolated from conventional edible oils (soya, maize, sunflower, rapeseed). The conventional caustic refining procedure comprises degumming, neutralisation, bleaching and deodorization. The last step, a mass-transfer process, by which substances are evaporated from the oil under reduced pressure (2 – 10 mbar) and elevated temperature (230 – 270°C), leads to a distillate making around 0.1 – 0.3 % of oil mass and contains 8 – 20 % sterols (details in 5).

Formulation and process rules currently used to ensure safety of conventional spreads have been used for the new products. All materials are produced according to Good Manufacturing Practices. All processing materials are of food-grade or equivalent.

Storage and distribution temperatures used are the same as conventional spreads and the same Hazard Analysis and Critical Control Point (HACCP) schemes are used to control product safety and quality.

Phytosterols from oil from already approved genetically modified plant strains might be present in the mixture. The isolated phytosterols are re-esterified with fatty acids from sunflower oil.

The production process of the spread has a history of safe use for ingredients for the food industry and it does not affect the composition and structure of the components.

3.3 History of source organism

Commercially available and used plant oils from different plants, but mainly from soya, sunflower, maize, and rapeseed, are the sources for production of the novel food ingredient. These source oils are not derived from plants especially grown for this purpose and they are generally not genetically modified, although it cannot be excluded that some mixtures contain oils or isolated sterols from GMOs. In this case these can be expected to be approved organisms and derived products like the Round-up-ready soybean and its products. Thus, whenever labelling requirements exist for the use of already approved ingredients derived from GMOs, these requirements will also apply to this novel food.

3.4 Ingredients use in food

The applicant has applied to use vegetable sterol esters in new vegetable oil-based spreads at levels up to 20% (≈ 12% free sterols). The use of the new product is intended to help maintain healthy cholesterol levels as part of a diet low in saturated fat and cholesterol. The spread base contains around 40% fat, composed of edible vegetable oils which are high in polyunsaturated (PUFA), low in saturated and very low in *trans* fatty acids. The product is not intended for use in cooking.

3.5 Anticipated intake/extent and consequences of use

With normal consumption of this kind of spreads being 20 – 30 g/d, the intake of phytosterols when using the novel product will increase to about 1.6 – 2.4 g/d, amounting to an 8-12 fold increase of the current daily intake from traditional products. In the USA plant sterol esters in plant oil-based spreads at levels up to 20% are generally recognised as safe (GRAS) (4).

The main sources of phytosterols in the basic diet are cooking oils and margarines. Bread and cereals can also contribute significantly to total phytosterol intake (6). Reduced-fat health spreads contain 0.3 – 0.4% phytosterols, corn oil margarines are highest in phytosterols (0.5%). Vegetables and fruits contain <0.05% (based on the edible portions), except seedlings of barley, beans, peas which contain 0.1 – 0.2% phytosterols. Some seeds are also rich sources: sunflower and sesame seeds contain 0.5 – 0.7%, and legumes can contain 0.22% phytosterols.

A typical U.S. diet provides approximately 250 mg of phytosterols per day (≈ 4 mg/kg bw/d) (7). In the UK and the Netherlands phytosterol intake was estimated to be about 200 mg/d. In the adult Finnish population average intakes amounted to 300 mg/d (≈ 5 mg/kg bw/d) with an upper limit of 680 mg/d (≈ 10 mg/kg bw/d). Generally the intake of adult vegetarians and their children is higher (up to 40%) than the average for the population as a whole (7-9).

Infant formulae based on cow's milk contain 0.08 – 0.20 mmol/l ß-sitosterol, 0.03 – 0.10 mmol/l campesterol and around 0.02 mmol/l stigmasterol, while the phytosterol content in human milk is negligible and can not be detected using current methods (10).

Oral phytosterol intake of about 3g/d inhibits the intestinal cholesterol absorption, probably by blocking the receptors (11). Aiming at a similar blood cholesterol modulating effect, studies with hypercholesteraemic subjects have employed dose levels of many grams (up to 25 g/d) of phytosterols per day for up to three years (8). Such limited total- and LDL-cholesterol lowering effect can be compensated by an increased endogenous cholesterol synthesis, unlike the use of drugs suppressing synthesis or perturbing the enterohepatic cycle of cholesterol and bile acids, which might lead to very low cholesterol levels. In addition, studies have shown that drug treatment with statins to reduce cholesterol levels increased the phytosterol/cholesterol ratio by increased absorption. For example, the ratio of sitosterol/cholesterol was shown to increase by 200 % (9, 13). This means, that high intake of phytosterols could be a potential problem when ingested together with cholesterol lowering/inhibiting drugs.

From marketing data on users and usage of a similar yellow fat spread containing phytostanols in the EU, the petitioner concludes that the product is used predominantly by people who are more than 50 years old and of relatively high socio-economic standing, and that the majority of households using the product consist of one or two people. The petitioner's current sales data for yellow fat spreads containing phytosterol esters in USA and Australia indicate a comparable consumer profile.

Typical daily consumption of yellow fat spreads in Europe is between 20 and 30 g/d. In order to achieve the required cholesterol lowering effects the intake of phytosterols - according to petitioner - should amount to 1.6 – 3.6 g/d (expressed as esters 2.2 – 5.0 g/d). That means that phytosterol concentrations in the yellow fat spread should be 6 – 12% (w/w). Estimates of fat spread use of males above 50 years of age in the UK and the Netherlands show that the 95th percentile of use is approximately 57 g/d and 70 g/d respectively.

The marketing of the product is focussed on the particular section of the population that is trying to control its blood cholesterol levels.

As indicated by the applicant there is only a small number of people at risk of adverse reaction. These comprise individuals with an inborn error of phytosterol metabolism (autosomal, recessive); worldwide 50 cases are known (2). Appropriate

labelling should assure that phytosterolaemic patients can avoid consuming the product.

Although it is not intended to recommend this product to healthy young adults, or for children, these individuals may consume the product when it is available in a family home. The initial assessment came to the conclusion that children in Europe are not expected to experience negative effects from possible lowered cholesterol levels.

The new product is intended to replace other yellow fat spreads. Based on the product specification the novel food differs from other fat spreads only in the phytosterol ester content at the cost of corresponding amounts of non-fat compounds (water). Thus there will be no change in intake of nutrients and/or other compounds. The new product contains a similar amount of polyunsaturated fatty acids as other so called "heart health" products.

3.6 Nutritional information

Phytosterol esters are hydrolysed by pancreatic carboxyl ester lipase. Absorption of free phytosterols in humans and experimental animals is low: 4 – 5% for β-sitosterol and stigmasterol, 9 – 10% for campesterol and brassicasterol (12). At higher dietary intake (2000 mg/d), absorption of sitosterol by humans is reduced. In healthy subjects campestanol is better absorbed than campesterol (12.5% vs. 9.6% of intake). Phytosterol absorption in women was found to be slightly higher than in men (13) and higher in children than in adults (14).

Absorbed phytosterols are transported in the triglyceride-rich lipoprotein (VLDL) and chylomicrons, taken up by the liver and then excreted into the bile (15). Circulating phytosterols are transported in the blood mainly in LDL and HDL fractions. Tissues with LDL receptors such as the liver, adrenals and testes may then take up phytosterols and convert them into steroid hormones (16). Since their concentration in these tissues is much lower than that of cholesterol, this will not significantly contribute to hormone synthesis. However, no information on the relative potency of hormones derived from either cholesterol or phytosterols is available. Unabsorbed sitosterol and campesterol is converted by the human colonic microflora to sitostanol/stigmastanone and campestanol/campestanone, respectively. Among a group of 31 normal North Americans, 23 high converters have been found converting a mean of 83 ± 9% sitosterol (17).

After ingestion of 8.6g phytosterols/d in healthy human adults, faecal concentrations of sterols and sterol metabolites increased from about 40 to 190 mg/g dry weight and 30 to 50 mg/g dry weight, respectively. The major sterol metabolites excreted were metabolites saturated at the 5,6-position in β-configuration or metabolites formed by oxidation at the 3-position. The faecal concentration of 4-cholesten-3-one was slightly but significantly increased (about 2 mg/g). Faecal secondary bile acid concentration was reduced. The formation of small amounts of oxysterols could not be excluded, but considered to be unlikely (18). Phytosterols in food can be oxidised, particularly at higher temperatures (>180 °C) (19). Oxidation products (7-hydroxy-

and 7-keto-components) are formed at very low levels which are similar to other plant oil products containing phytosterols and are also poorly absorbed (2).

If the product is consumed on regular basis (20 – 25 g phytosterol-enriched spread daily, equivalent to 1.6 – 2.0 g phytosterols/d) then the average lowering of plasma LDL-cholesterol will be 8 to 10%, relative to initial plasma levels (4.16 ± 0.5 to 6.54 ± 0.61 mM). The reduction in blood cholesterol levels of the magnitude anticipated from consumption of phytosterol-enriched spreads is safe in those individuals who do not have elevated plasma cholesterol levels. This was confirmed by the results of the three-year Dietary Intervention Study in Children (DISC). In 8 – 11 year old children a diet low in fat, saturated fatty acids and cholesterol lead to a modest decrease in LDL-cholesterol, while maintaining adequate growth, iron status, nutritional quality and psychological well-being during the critical growth period of adolescence (20). Children and adults would not be expected to experience any adverse effect on metabolism when their blood cholesterol is lowered. The novel food is intended to be used by population groups above 50 years of age, who try to control their elevated blood cholesterol.

At levels of phytosterols in spreads of 3.4, 6.5 and 11 – 13% (w/w) in short term (21) and 8% (w/w) in a one-year follow-up study (2) the new product is equally effective in lowering LDL cholesterol by 8 – 10%, relative to the initial plasma level. At 11 – 13% of phytosterols in the fat spreads no appreciable effect on the fat-soluble vitamins calciferol, tocopherols and phylloquinone was noticed, but a 10% reduction of α- and ß-carotene as well as lycopene was observed. This reduction of 10% itself seems not to be of physiological relevance, but, considering a long term exposure and taking into account the 97.5 percentile of intake, the decline of ß-carotene levels might be higher.

In the initial assessment by the Dutch competent authority it was expected that a maximum of 8% (w/w) phytosterols will cause little or no drop in serum carotenoid levels.

The applicant considered the points raised by some member states after the initial assessment and commissioned a one-year follow up study (2) with healthy subjects using fat spread containing 8% (w/w) phytosterols (the preparation contained 38g fat in 100 g spread). A report with the results of this study was forwarded to the SCF. The results can be summarised as follows:

- When adjusted for total lipids no statistically significant changes after week 26 and week 52 were found for lutein, zeaxanthin, ß-cryptoxanthin, lycopene and α-carotene. Only ß-carotene was significantly ($p=0.037$) decreased at week 52; the level dropped by 24% (21% when lipid adjusted) as compared with the initial level at time point 0. This significant drop of ß-carotene level occurred after 52 weeks despite the fact that the tested new fat spread contained a maximum of 50 mg carotenoids per kg fat (mainly ß-carotene and lycopene added for coloration). The reduction was twice that observed in the short-term studies with 12% phytosterols. Nevertheless, it can be seen from the week 0 results that there is a large variation within the normal range for

plasma ß-carotene levels, that there are also seasonal variations and that this reduction is within normal range (Table 1).

Table 1: Changes in plasma ß-carotene levels in one-year follow-up study (data from ref.2, doc. ref. D99/047)

n=64 healthy adults	ß- carotene (nmol)	ß-carotene/lipid adjust. (nmol/mmol)
Week 0	410.4±216.8	58.3±33.1
Week 26	321.9±175.6	48.0±28.1
Week 52	310.1±190.3	46.1±28.6

The reduced plasma ß-carotene levels might become more relevant when the vitamin A status is not optimal. This is the case for pregnant and lactating women as well as younger children.

- There were no significant differences in the plasma levels of retinol, 25-OH-cholecalciferol or α-tocopherol (total lipid adjusted) during 52 weeks of study. Phylloquinone status was not statistically different between test and control groups after 26 weeks of study.

- There was a significant cholesterol-lowering effect of phytosterol esters enriched margarine throughout the one-year duration of the study (2).

- There were no side effects seen in the individuals during the study.

3.7 Microbiological information

Spreads containing phytosterol esters have been tested for their microbiological stability and have been found to be similar to conventional spreads. The production process and the inherent properties of the novel products give no rise to concerns of microbiological risk. No data indicate an effect on the intestinal flora in terms of bacterial profile or metabolic activity beyond natural variations (2).

3.8 Toxicological information

The toxicological information available on phytosterols and phytosterol esters comprises data from studies on absorption, distribution, metabolism, and excretion and on (a) subchronic toxicity, (b) genotoxicity, (c) reproductive toxicity, (d) potential estrogenic activity and from (e) human studies:

(a) In a 13-week feeding study with rats, a mixture of phytosterol esters, obtained mainly from soya bean oil and re-esterified with fatty acids from sunflower oil, was tested at dosages of 0.16, 1.6, 3.2 and 8.1% in the diet. The mixture contained 62% total sterols consisting of mainly β-sitosterol (48.7%), campesterol (25.8%) and stigmasterol (21.6%) with only 1.1% brassicasterol.

Neither D5- and D7-avenasterol nor D7-stigmastenol were present. Apart from some minor changes in haematology and clinical-chemical parameters, no relevant toxic effects up to the highest dose of 6.6 g/kg bw/day (corresponding to 4.1 g phytosterols/kg bw/day) were found (22).

(b) Phytosterols (47.9% β-sitosterol, 28.8% campesterol, 23.3% stigmasterol) and phytosterol-esters (47.3% β-sitosterol, 28.1% campesterol, 24.1% stigmasterol) show neither evidence of mutagenic activity in the bacterial mutation assay with Salmonella typhimurium (strains TA 1535, 1537, 98 and 100) nor clastogenic activity in tests on chromosomal aberrations with human peripheral blood lymphocytes *in vitro* both in the presence and absence of S-9 mix derived from rat livers.

In addition, two *in vivo* genotoxicity studies were conducted using a phytosterol ester mixture containing 0.3% cholesterol, 3.0% brassicasterol, 28.1% campesterol, 0.8% campestanol, 18.7% stigmasterol, 45.5% ß-sitosterol, 2.6% ß-sitostanol, 1.1% D5-avenasterol and 1.9% others. Using an *in vivo/in vitro* procedure the mixture did not induce unscheduled DNA synthesis (UDS) in the livers of orally dosed male rats (once with 2000 mg/kg). In another study in rats the plant sterol mixture did not induce micronuclei in the polychromatic erythrocytes of bone marrow of male rats treated up to 2000 mg/kg/d (2, doc. ref. D00/004).

Neither 4-cholesten-3-one, an oxidation product of cholesterol increased in volunteers fed phytosterols, nor 5β-cholestan-3-one showed mutagenic activity when tested in the bacterial mutation assay with five strains of *Salmonella typhimurium*. Furthermore, none of the substances showed a clastogenic potential in the *in vitro* chromosome aberration assay with human lymphocytes (23,24).

(c) A variety of effects on the reproductive system such as antiandrogenic action in rabbits and decrease in testicular weight and sperm concentration in rats have been reported for ß-sitosterol and phytosterol-rich extracts (25, 26, 27, 28). These observations have been made after administration by the subcutaneous route and/or with phytosterol preparations, the purity of which was not specified.

However, in a two-generation reproduction study in rats, phytosterol esters of the same composition as in the 13-week study had no effect on the reproduction of the F0 and F1-generations, nor on the development of the F1- and F2-pups, nor on the sexual maturation of the F1-weanlings nor on oestrous cycles. A dietary phytosterol ester concentration of 8.1% was shown to be the no-observed-adverse-effect level (NOAEL). This was equivalent to a dose of 2.5-9.1 g/kg bw/day depending on the period during the study (29).

(d) Orally administered ß-sitosterol of unknown purity increased uterine weight in rats receiving a low dose for 30 days (6.2 µg/dl in drinking water). This weak oestrogenic response was not observed at higher doses (12.4 µg and 18.6 µg/dl) (30).

In contrast to these studies, uterotrophic assays with immature female rats orally gavaged with phytosterols (47.9% β-sitosterol, 28.8% campesterol,

23.3% stigmasterol) and phytosterol esters (47.3% β-sitosterol, 28.1% campesterol, 24.1% stigmasterol) in doses of 5, 50 and 500 mg/kg bw/day for 3 days did not reveal any oestrogenic response using uterine weights as the end point. In addition, phytosterols of the same composition did not display oestrogenic activity in a recombinant yeast assay for oestrogenic potential, nor did they show binding in a rat uterine cytosol oestrogen receptor binding assay (31). These studies, together with the two-generation reproduction study, provide sufficient reassurance of absence of endocrine effects via the oral route.

(e) In a 3-week study with 12 men and 12 women who consumed 5.8 g phytosterols (in 40 g margarine) per day no changes in the sex hormone levels in females was shown (18). Two double-blind placebo-controlled 14-week tests did not provide evidence for any adverse effects on haematological and clinical parameters (21,32). These trials and the one-year follow-up study using phytosterol esters (8% w/w expressed as phytosterols) in the fat spreads were carried out primarily with the view to assessing the cholesterol lowering effect of phytosterol esters. These tests have not reported any toxic effects relating to the phytosterols.

Phytosterol preparations are used for the medical treatment of benign prostatic hyperplasia. A number of placebo-controlled, double-blind clinical trials was conducted with preparations of uncertain compositions said to be mainly β-sitosterol. With doses of 20 mg β -sitosterol three times per day (33) and 130 mg ß-sitosterol daily (34), significant improvements in symptoms and urinary flow parameters were reported (35). The mechanism of this effect and the active ingredient remains to be determined. Side effects have not been reported.

4. CONCLUSIONS

- The Committee considers that the dossier and the additional information submitted during 1999 and 2000 is complete and follows the SCF recommendations. The novel food, phytosterol esters in yellow fat spreads, has been correctly classified as Class1, Subclass1, a pure chemical substance or simple mixture obtained from sources already in use for food in the European Community.

- The new yellow fat spread differs from conventional fat spreads/margarine by its phytosterol origin (obtained from edible vegetable oils), their chemical structure (esters with long chain unsaturated fatty acids of sunflower oil) and concentration (about 16 – 24-fold higher than the conventional product). This concentration will increase the total intake of phytosterols by 8 – 12 times, compared with traditional products.

- Based on extensive toxicological testing of phytosterol preparations in a 13-week feeding study with rats, in a two-generation feeding study with rats, in studies on

oestrogenic potential and in tests on genotoxicity, no safety concerns were apparent.
The safety in use of phytosterols has been demonstrated for mixtures of predominantly ß-sitosterol, campesterol and stigmasterol and/or their esters with fatty acids, to which the specification of the new product should be restricted. The phytosterol profile of 30-65 % ß-sitosterol, 10-40 % campesterol, 6-30 % stigmasterol and a total of 5% other phytosterols, based on total sterol content (w/w), is considered acceptable by the Committee.

- The Committee considers that the very small number of people with inborn error of phytosterol metabolism (phytosterolaemia) should be made aware of the presence of higher levels of phytosterols in this product and that patients on cholesterol-lowering medication should only consume the product under medical supervision.

- Ingestion of 20g per day for one year of products containing 8 % free phytosterols reduced plasma ß-carotene concentrations by 20%. Although the ß-carotene concentration was still within the normal range and within normal seasonal variations, such a reduction in plasma ß-carotene levels might become more relevant for persons whose vitamin A status is not optimal. The Committee is therefore of the opinion that this ß-carotene lowering effect should be communicated to the consumer, together with appropriate dietary advice regarding the regular consumption of fruits and vegetables.

- Given the overall evaluation of the submitted information the Committee concludes that the use of phytosterol esters in yellow fat spreads at a maximum level corresponding to 8% free phytosterols is safe for human use.

- The Committee is of the opinion that the applicant should perform, in accordance with Chapter XI in the Annex of Commission Recommendation 97/618/EC (1), a post-marketing surveillance study to obtain data on consumption and further investigation of possible health effects, among others the effects on plasma ß-carotene levels. The Committee will wish to review this information when it becomes available.

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ATTACHMENT 2

Federal Register: September 8, 2000 (Volume 65, Number 175)]
[Rules and Regulations]
[Page 54685-54739]
From the Federal Register Online via GPO Access [wais.access.gpo.gov]
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Part III

Department of Health and Human Services

Food and Drug Administration

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21 CFR Part 101

Food Labeling: Health Claims; Plant Sterol/Stanol Esters and Coronary Heart Disease; Interim Final Rule

[[Page 54686]]

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 101

[Docket Nos. 00P-1275 and 00P-1276]

Food Labeling: Health Claims; Plant Sterol/Stanol Esters and Coronary Heart Disease

AGENCY: Food and Drug Administration, HHS.

ACTION: Interim final rule.

SUMMARY: The Food and Drug Administration (FDA) is authorizing the use, on food labels and in food labeling, of health claims on the association between plant sterol/stanol esters and reduced risk of coronary heart disease (CHD). FDA is taking this action in response to a petition filed by Lipton (plant sterol esters petitioner) and a petition filed by McNeil Consumer Healthcare (plant stanol esters petitioner). Based on the totality of publicly available evidence, the

agency has concluded that plant sterol/stanol esters may reduce the risk of CHD.

DATES: This rule is effective September 8, 2000. Submit written comments by November 22, 2000. The Director of the Office of the Federal Register approves the incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51 of certain publications in 21 CFR 101.83(c)(2)(ii)(A)(2) and (c)(2)(ii)(B)(2), as of September 8, 2000.

ADDRESSES: Submit written comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852.

FOR FURTHER INFORMATION CONTACT: Sharon A. Ross, Center for Food Safety and Applied Nutrition (HFS-832), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-205-5343.

ATTACHMENT 3

XIII International Symposium on
DRUGS AFFECTING LIPID METABOLISM

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MODERATE INTAKE OF MYRISTIC ACID HAS BENEFICIAL EFFECTS ON LIPIDIC PARAMETERS: RESULTS OF A MONK COLLECTIVITY STUDY

H. Debadie, E. Peuchant, A. Cazanave, M. Bernard, V. Rigalleau, H. Gin, F. Mendy, M. Clerc, J. Paccalin. *Laboratoire de Thérapeutique, Université Victor Ségalen, Bordeaux, France*

Myristic acid (MA) is one of the most atherogenic fatty acids when consumed in large amounts, but little is known at moderate intake; this is the purpose of our study.

Twenty five male monks without dyslipidemia (mean age: 61 y, weight: 72 kg, and BMI: 25) were provided two isocaloric (2200 kcal) diets for 5 wk each. Diet 1 was 30 % fat (8 % SFA, 0.6 % MA, 12 % oleate, 6 % linoleate and 1 % linolenate), 55 % carbohydrate, 200 mg cholesterol. The diet 2 was 34 % fat (11 % SFA, 1.2 % MA), 51 % carbohydrate and no change in oleate, linoleate, linolenate and cholesterol (C). Baseline diet (35 % fat, 13 % SFA, 1.4 % MA, 52 % carbohydrate) was provided before each diet for 4 wk. Samples obtained at the end of each period were assessed for plasma lipids and fatty acids of phospholipids and cholesteryl esters.

Diets 1 and 2 caused a decrease in total C, LDL-C and triglycerides (TG) ($P<0.001$); HDL-C was not modified, apoA-I/apoB ratio was increased ($P<0.001$). Plasma TG was lower after diet 2 than after diet 1 whereas HDL-C was higher ($P<0.05$). Comparatively with baseline and diet 1, diet 2 was associated with an increase of MA ($P<0.01$), oleate, linoleate, EPA and DHA in phospholipids, and with an increase of linolenate in cholesteryl esters ($P<0.05$). MA intake in diet 2 were positively correlated with plasma total C, LDL-C, TG and apo B, and with phospholipid MA.

These data suggest that a diet 34 % fat with 11 % SFA and 1.2 % myristic acid has beneficial effects on plasma lipid and fatty acid profiles.

INHIBITION OF LOW-DENSITY LIPOPROTEIN OXIDATION IN RATS FED POLYUNSATURATED FATTY ACID ENRICHED DIETS SUPPLEMENTED WITH RED WINE EXTRACT.

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Laboratoire de Nutrition et Securite Alimentaire, INRA, 78352 Jouy-en-Josas Cedex, France.

Red wine consumption is thought to protect agains atherosclerosis by inhibiting lipid oxidation in low-densit lipoprotein (LDL) particles. In this study, we examined th effects of supplementation with red wine extract (WE) rich i polyphenolic antioxidants in rats fed high polyunsaturated fat acids (PUFA) diets with or without 0.8% cholestero Unexpectedly, the cholesterol contents of plasma were th lowest in rats fed cholesterol without effect of dietary WE. Th response was mainly due to the decrease of plasma HD levels (about 1.5-fold) without change in the distribution components and proportion of vitamin E. The generation conjugated dienes was followed for 26h in VLDL+LDL oxidize by copper. The kinetics showed that in rats fed WE with without cholesterol, the production reached a plateau from 1 up to 26h but at this time, the amounts were 2.2-fold lower wi cholesterol-depleted diets. In rats fed cholesterol, th supplementation with WE decreased the production by 1. fold. The differences between responses to dietary treatmen was apparently due to changes in the length of lag-time whic is an indicator of the antioxidant potential. These findin suggest that 1) diets containing cholesterol caused a reductic of the resistance of VLDL+LDL to lipid peroxidation decreasing the plasma concentration of HDL which a considered to protect LDL. 2) a fraction of polypheno antioxidant of red wine were associated with VLDL+LI particles and (or) were present in the aqueous phase there delaying the beginning of lipid peroxidation.

OMEGA-3 FATTY ACIDS : A METHODOLOGY TO EVALUATE THEIR CLINICAL PERTINENCE

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Omega-3 fatty acids (ω3) derived from fish oil are recognized for their health preserving effects. Their cardioprotective benefit was demonstrated from epidemiological studies in various populations.

The results of clinical studies on ω3 are however debatable. They depend on whether the trials were conducted *in primary prevention*, in patients at risk for coronary heart disease (CHD), presenting metabolic disorders such as hyperlipidemia/diabetes, or in *secondary prevention* in patients with previous CHD.

A method for evaluating the clinical pertinence of ω3 is proposed, using one decision analysis model, presenting all the clinical indications in cardiac and non cardiac diseases.

The pharmacological rationale, the design of the trial, the targeted population, the critical end-points and the duration of development, are scored with discrete variables and visualized in a matricial diagram.

1. The pharmacological rationale is classified in term of maturity of the concept : emerging/growing/mature and declining.
2. The trials are ranked according to six protocol designs : case-report, case-series, cross-sectional, cohort, case-control and controlled-trials, with a methodological assessment from standard guidelines for appraising medical research.
3. The targeted population is evaluated from the Oxman's consumer's guide to subgroup analysis.
4. The critical end-points are determined as follows : clinical or non clinical surrogate end-points, in relation with the clinical outcome.
5. The mean duration of development is calculated from current database of ω3 trials and from consensual/expert recommendations.

This overview would allow decision-makers to prepare future clinical trials, to give educational support for the medical community and to help for building the pharmacoeconomic analysis.

Ref. E. Varlan et R. Le Paillier, Thérapie 1996, 51:117-122 : Aide à la Décision : Théories, Méthodes en Recherche et Développement Clinique et Stratégie de Santé

NOVEL "MULTI-BENE" FOOD COMPOSITION LOWERS SERU CHOLESTEROL AND DECREASES OBESITY

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Dietary plant sterols (PS) slightly decrease serum cholesterol (T. Miettinen et al., NEJM 1995;333:1308-1312). Recently it has b claimed that the so-called Multi-Bene (M-B) foods, comprisi administration of PS combined with the mineral nutrients potassi (K), magnesium (Mg) and calcium (Ca) in food items, are able to bri about a much better control of serum cholesterol and, more important also to produce several other beneficial health effects, includ reduction of obesity (Pat. appl. FI 965251).

Using the animal model for the metabolic syndrome, the obese Zuc rats, we compared the effects of a 7-week administration of the M food composition with the effects of dietary administration of PS alo The following diets were used in groups of 10 rats: Group 1= Cont diet (C): commercial rat chow; Group 2= Hypercholesterolaemic c (HChol): 1 % cholesterol + 18 % butter + 6 % NaCl in C; Group M-B diet (M-B): 1 % of a mixture of β-sitosterol/β-sitostanol + 1.6 K + 0.12 % Mg + 2.5 % Ca in HChol; Group 4= Plant sterol c (PS): 1 % of a mixture of β-sitosterol/β-sitostanol in HChol.

The results are shown in the table (means ± SEM):

GROUP	S-cholesterol mmol/l	B-glucose mmol/l	S-insulin ng/ml	Body weigt g
1: C	3.6 ± 0.3	7.5 ± 0.2	41.2 ± 5.7	450 ± 13
2: HChol	7.4 ± 1.0 a)	8.2 ± 0.6	26.7 ± 3.1 a)	439 ± 10
3: M-B	3.2 ± 0.2 b)	6.9 ± 0.1	13.8 ± 1.7 c)	348 ± 6 c)
4: PS	5.7 ± 0.8 a)	7.4 ± 0.3	27.3 ± 2.7 a)	427 ± 8

a) $p<0.05$ *vs.* C; b) $p<0.01$ *vs.* HChol; c) $p<0.05$-0.001 *vs.* other grou

Conclusion: The beneficial effects produced by the Multi-Bene fo greatly exceeded those produced by plant sterols alone.

ATTACHMENT 4

APPENDIX 28:

Study report (Korpela et al. 2001): Plant sterols reduce serum lipids incorporated in low fat dairy products

CONFIDENTIAL INFORMATION

PLANT STEROLS REDUCE SERUM LIPIDS INCORPORATED IN LOW FAT DAIRY PRODUCTS

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Running head: Plant sterols in dairy products and serum cholesterol

ABSTRACT

Background: Plant sterols inhibit cholesterol absorption and therefore reduce serum total and LDL cholesterol and lower risk of atherosclerotic vascular diseases.

Objective: Our aim was to investigate whether plant sterol mixture reduce serum cholesterol added to low fat dairy products; yoghurt, low fat cheese or low fat fresh cheese, in subjects with moderately elevated serum cholesterol.

Design: Parallel, double-blind study, 164 mildly or moderately hypercholesterolemic subjects were randomly assigned into two groups: plant sterol containing dairy products (yoghurt, cheese or fresh cheese) and control group. The subject consumed products 6 weeks after three weeks screening period. The mainly plant sterol intake was around 2 g.

Results: During the treatment period the reduction in serum total cholesterol was 6.5% in the sterol group and 0% in the control group ($p<0.0005$). Low-density lipoprotein (LDL) cholesterol was reduced 10.4% and 0.6% ($P<0.00005$) respectively. HDL/LDL cholesterol ratio was increased by 16.1% in sterol group and 4.3% in control group ($P=0.0001$). HDL cholesterol and triacylglyserols did not change during trial.

Conclusions: We conclude that the yoghurt, low-fat cheese and low-fat cheese containing plant sterol mixture reduce serum cholesterol in hypercholesterolemic subjects.

KEY WORDS
Cholesterol, plant sterols, dairy products, humans

INTRODUCTION

It is previously shown that plant sterol can reduce serum cholesterol by inhibiting cholesterol absorption from small intestine (Heinemann et al. 1991). Plant sterols have been studied since 1950s (Pollak 1953, Lees et al. 1977) and theirs saturated derivatives stanols and theirs esters in margarines or mayonnaises (Miettinen et al. 1995, Gylling et al. 1995). Plant sterols and stanols are almost unabsortable (sitosterol and stigmasterol under 5% and campesterol under 10%) (Heinemann et al. 1993)

The purpose of the study was investigate that could plant sterol mixed in dairy products (yoghurt, low-fat cheese and low-fat fresh cheese) reduce serum total and LDL cholesterol concentrations.

SUBJECTS AND METHODS

Subjects

A total of 207 adult volunteers with mild to moderate hypercholesterolaemia were screened for the trial in four clinics in Finland, in Helsinki (yoghurt), in Tampere (cheese), in Ilomantsi (cheese) and in Oulu (fresh cheese). Inclusion criterias were serum total cholesterol concentration between 5.0 and 8.5 mmol/l during baseline period, serum triacylglycerols less than 4.0 mmol/l. During the study the patients were not allowed to use any lipid lowering drugs or any other lipid-lowering dietary regimen. Patients with severe diseases such as diabetes mellitus, history of myocardial infarction within the previous 3 months, history of malignancy, psychosis, malabsorption, chronic liver disease or homozygous familiar hypercholesterolaemia

were excluded. The use of any hypoglycemic agent, corticosteroids, oral anticoagulants, hormone or immunosuppressant treatment were also exclusion criteria. Post-menopausal women on stable non-intermittent hormone replacement therapy could enter this trial. 170 fulfilled the inclusion criteria and were randomly assigned either into plant sterol containing yoghurt/low-fat cheese/low-fat fresh cheese diet group and control group in each center separately. Of this subjects 164 (82 in the active group and 82 in the placebo group) completed the study. 5 of the randomized subjects voluntarily withdrew from the study and one died of cardiovascular disease during trial. No subjects (128 women and 36 men) had recent history of cholesterol lowering drug treatment. The subjects were requested to maintain their diet, weight and physical activity during the study. Baseline characteristics of the subjects are shown in Table 1.

The study protocol was approved by the Ethics Committee at the Department of Medicine, Helsinki University Central Hospital. All subjects received both written and oral information regarding the trial and gave a written consent.

Study design

The trial was carried out using a double-blind design. The screening (run-in) period before the random allocation of the study subjects into one of the two study groups lasted for three weeks (Figure 1). During the last week of run-in period the subjects received the placebo food items. During treatment period the subjects received either sterol containing study food items or placebo food items. Otherwise subjects had no restrictions with their diet.

Konelab Ltd, Espoo, Finland) using the Optima Clinical Chemistry Analyzer (Konelab Ltd, Espoo, Finland). The LDL cholesterol concentration was calculated with Friedewald's formula (Friedewald 1972).

Plant sterols

Phytosterols in serum were determined by using an application of the method published by Phillips *et al.* (1999). Serum samples were thawed overnight at 4 °C. The internal standard (epicholesterol) was added to a 0.9 mL sample at the beginning of the assay procedure. Sample was dissolved in 7 mL of ethanol containing 3% of pyrogallol and saponified with aqueous potassium hydroxide at 85 °C for 30 min. To extract unsaponifiable lipids, 20 mL of cyclohexane and 12 mL of deionized water were added to the sample tube. After vigorous shaking samples were centrifuged and the cyclohexane layer was transferred to a round-bottom flask. Cyclohexane was evaporated at 50 °C in a rotary evaporator. The residue was redissolved with 0.5 mL of hexane.

For the sample cleanup purposes, the sample solution was introduced to an silica solid phase extraction cartridge (Varian Bond Elut, 500 mg) that has been activated with hexane. The cartridge was washed with hexane and with hexane:diethyl ether (90:10, v/v). The sterols were eluted with hexane:diethyl ether (50:50, v/v).

After solvent evaporation, sterols were redissolved with anhydrous pyridine and derivatized with BSTFA containing 1% of TMCS. For the quantitative analysis of

phytosterols, capillary gas chromatography (5% diphenyl-95% dimethylpolysiloxane column) with an internal standard method was used.

The method application used was validated using phytosterol recovery tests. The average recovery obtained for the spiked sitostanol was 91.7% (n=6). The limit of quantitation was 0.1 mg/L for all the sterols quantitated.

For the daily quality control, an in-house serum reference sample was analyzed in each sample assay. Plant sterol contents of in-house serum reference sample were stable during the study. Plant sterol and lathosterol contents (n=55) were as follows: 5.6 ± 0.6 mg/L, 2.1 ± 0.2 mg/L, 0.2 ± 0.1 mg/L, 0.5 ± 0.04 and 2.3 ± 0.2 mg/L for campesterol, sitosterol, stigmasterol, Δ5-avenasterol and lathosterol, respectively.

Fat soluble vitamins

The plasma 25(OH)D concentration was measured by a radioimmunoassay (Incstar Corporation, Stillwater, Minnesota, USA). The intra- and inter-assay coefficients of variation were 10.1% and 14.9%, respectively. The reference range for 25(OH)D was 25-120 nmol/l.

Fat-soluble vitamins (α- and γ-tocopherol, β-carotene, all- *trans*- retinol and phylloquinone) of human plasma were determined by using HPLC applications of the methods published by Chuang *et al.* (1994) and by Koivu-Tikkanen *et al.* (2000). A portion of ethanol (4 ml) and internal standards (α-tocopherol acetate in ethanol and $K_{1(25)}$ in hexane) were added to the plasma sample (1 ml). Vitamins were extracted with *n*-hexane. Hexane layer was evaporated with nitrogen. The dried extract was dissolved in a 5 ml portion of *n*-hexane and the extract was divided into two portions:

1 ml for determination of α- and -tocopherol, β-carotene and all- *trans*- retinol and 4 ml for determination of phylloquinone (Koivu-Tikkanen *et al.*, 2001) .

For the determination of α-and γ-tocopherol, β-carotene and all- *trans*- retinol an aliquot of 1 ml was evaporated with nitrogen and dissolved in HPLC eluent (acetonitrile:methanol:dichlorometane, 7:2:1) and analysed with C_{18} column. An internal standard method with fluorescence detection was used for quantification of α- and γ-tocopherol. In the case of β-carotene and all- *trans*- retinol, UV detection and an external standard method was used for quantification. For phylloquinone determination an aliquot of 4 ml was purified with silica solid phase cartridges (Waters Silica, 1 g) and analysed with RP-HPLC (Koivu-Tikkanen *et al.* 2000).

Detection limits defined as a signal two times the height of the noise level were 10 ng/ml for β-carotene, 0.6 ng/ml for all- *trans*- retinol, 60 ng/ml for α- tocopherol, 30 ng/ml for γ-tocopherol and 20 pg/ml for phylloquinone. Determination limits (two times the detection limit) were 20 ng/ml for β-carotene, 1.2 ng/ml for all- *trans*- retinol, 120 ng/ml for α- tocopherol, 60 ng/ml for γ-tocopherol and 40 pg/ml for phylloquinone.

For quality control of the method a certified reference material (NIST SRM 986 c) was analysed. The results of all- *trans*- retinol, β-carotene, α- and γ-tocopherol analyses were in the certified ranges. For daily quality control an in-house reference plasma was analysed in every second sample assay. The day-to-day repeatabilities (CV %, n=38) for all- *trans*- retinol, β-carotene, α- tocopherol , γ-tocopherol and phylloquinone were 7.9 %, 9.6 %, 2.4 %, 4.8 % and 9.6 %, respectively.

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Apolipoproteins (ApoA1, ApoB, Lp(a), ApoE)

Plasma and lipoprotein cholesterol and triacylglycerols were determined by enzymatic colorimetric methods (Roche, Switzerland).

Apolipoprotein B and Apolipoprotein A1 were determined by immunochemical method (Orion Diagnostica, Finland).

The main lipoprotein fractions very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL2 and HDL3) were isolated by sequential ultracentrifugation (1).

The HDL fraction was obtained by the Mg+/dextran sulphate precipitation

Method.(2). LDL cholesterol levels were calculated by the Friedewald formula (3).

Lipoprotein (a) were determined by immunoturbidimetric method (WAK Chemie, Germany)

Apo E phenotyping was performed in serum by isoelectric focusing (5).

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Oxidation of LDL in vitro

A rapid method was employed to isolate LDL from EDTA plasma for oxidation experiments. VLDL (very low density lipoprotein) was separated by centrifugation in a Beckman Ti 50.4 rotor (269 000 g, 3.5 h, +15°C). Following the removal of the

VLDL fraction by slicing of the centrifuge tubes, LDL was separated, using the same rotor (155 000 g, 18 h, +15°C), and isolated by slicing.

EDTA was removed by gel-filtration of LDL on Sephadex G-25 in phosphate buffered saline solution (PBS), and thereafter the LDL sample was divided into three equal aliquots (a, b and c) for the oxiadtion experiments: 60mM EDTA was added to a final concentration of 1.2 mM to one aliquot part (part a, the native LDL). Copper mediated oxidation (Esterbauer et al, 1989) was begun in aliquots b and c. In sort, gel-filtered LDL (100 µg protein/ml) in PBS (pH 7.4) was supplemented with $CuSO_4$ to a final concentration of 10 µM. In part b (the totally oxidized LDL), the diene absorption at 234 nm was recorded at +20.5°C with a Shimadzu UV-1202 spectrophotometer (Shimadzu Corp, Kyoto, Japan) equipped with a Shimadzu cell temperatures CPS controller (Shimadzu Corp, Tokyo, Japan). When diene formation reached its maximum the oxidation of this aliquot was stopped by adding EDTA as above. For part c (the partially oxidized LDL), the same procedure was carried out but oxidation was stopped at 2.5 h by adding 60mM EDTA. Oxidation experiments were carried out twice at 48 h intervals, and mean values were used for calculations. The protein content of LDL was analysed by the method of Lowry et al. (1951).

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Other observations and measurements

Body weight (in 0.1 kilograms) in light indoor clothing without shoes and resting vital signs were recorded at every visit. Heart rate (60 sec) and blood pressure (2 mm Hg precision) were recorded twice in the seated position with mercury sphygmomanometer, using the standardised methods. Height (0.5cm precision) was

measured at the beginning of the study. Waist and hip circumferences were measured two times, at the beginning and in the end of this study. At the beging and in the end of the study the subjects were interviewed on their health status, smoking habits, medication. Physical activity and changes in their medication and health were asked every visit. The subjects were told to report the possible adverse effects an anonymous questionnary in the end of the study.

Statistical analyses

Normality of variables' distribution was confirmed using the Shapiro-Wilks test. Differences in changes of serum lipids before (randomisation visit) and after the trial periods were tested with t-tests between the sterol group and control group (Student's t-test) or with Wilcoxon test for non-normal variables. Test for homogeneity showed that there were no statistical significant differences between product groups (yoghurt, cheese and fresh cheese), therefore it was possible to pool the data from different product groups. P-values were established for the differences between the randomization groups. The p-value < 0.05 for the differences between the randomization groups was considered statistically significant. $?^2$-test was used to test differences between randomization groups for categorized variables (e.g. smoking habits, adverse effects). The results are expressed in tables as means ± SD and in figures means ± SE. Statistical analyses were performed according to the intention to treat. Statistical analyses were performed with SAS (Statistical Analysis System) version 6.12 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Baseline characteristics

There were no significant differences in baseline characteristics among the study groups (Table 1). Body weight changed during trial only slightly (–0.3±1.0 kg and –0.2±1.1 kg in sterol and control groups respectively). There was a significant difference between groups in the change of systolic blood pressure during trial (-4±5 mm Hg and -0±10 mm Hg in sterol and control groups respectively). Smoking habits did not change during trial. Physical activity did not change statistically significant between groups during the trial. There were no differences in adverse effects between groups but some had consipation or heartburn or some other separate symptoms.

Serum lipids

The serum lipid values during run-in, treatment and follow-up periods are shown in Figure 2. Serum total cholesterol and LDL cholesterol concentrations decreased within all study food item groups (Table 3). Total cholesterol were reduced in sterol diet group -4%, -8%, -7% and -7% in yoghurt, cheese and fresh cheese groups and all groups together respectively and LDL cholesterol –9%, -11%, -11%and -10% respectively. In all these lipid parameters, except the total cholesterol in the yoghurt, the differences between sterol and control groups were statistically significant. There were no significant differences in changes in HDL cholesterol and triacylglyserols between sterol and control groups.

In oder to determine the predictors for serum lipid changes (total cholesterol, LDL cholesterol and HDL/LDL ratio), the backward regression analysis was carried out. Analyzed variables were randomisation group, age, sex, change in weight and physical activity, body mass index at the baseline, study product, intake of total fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and cholesterol at the baseline, change in intake of total fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and cholesterol. In backward regression model only three variables left (randomisation group, change in intake of fat and change in intake of polyunsaturated fatty acids) were significant at the 10% level. The randomization group was statistically significant predictor for serum total cholesterol, LDL cholesterol and HDL/LDL ratio (all p-value<0.0001), even after change in the intake of total fat and polyunsaturated fatty acids were taken into account.

When the subjects were divided into two groups (baseline LDL below 4.1 mmol/L and above 4.1 mmol/L) by their baseline LDL cholesterol values (Table XX), the changes in the high baseline LDL cholesterol group in total cholesterol, LDL cholesterol were greater in the sterol group (change -0.8 mmol/l) than in the control group (-0.1mmol/l), but also in low baseline LDL group the difference in total and LDL cholesterol between two randomisation groups were statistically significant.

Diet

There were no statistically significant baseline (home diet) differences in nutrient intake between groups (data not shown).

There were few statistical significant differences in changes in changes of nutrient intake between run-in and treatment periods between randomisation groups (Table 4). Energy intake of alcohol was increased more in sterol group than in control group (p-value 0.0046), intakes of retinol was decreased less in sterol group than in control group (p-value 0.0124) and vitamin K decreased more in sterol group than in control group (p-value 0.0229). Between home diet and run-in periods only significant difference was seen in change intake of fiber per energy between randomisation groups (p-value (0.0180), in sterol group intake decreased less than in control group (x and y, respectively).

Serum plant sterols and lathosterol

Serum total amount of plant sterols more in sterol group from 11.5±4.4 mg/l to 12.3±4.2 mg/l, 10% and where as in control group decreased from 10.6±4.7 mg/l to 10.3±4.6 mg/l, –2% (Table 5). Also sitosterol concentrations changed significantly more in sterol diet group than in control group (62%, -2%, respectively). Avenasterol concentrations decreased in both groups, but statistically significantly more in sterol diet group (-15%). Also campesterol and stigmasterol concentrations decreaced in both groups, but there were not significantly differences between groups. Also no differences in changes in lathosterol, metabolite of cholesterol synthesis, between groups.

Plasma fat soluble vitamins

There were no significant differences between groups in plasma gamma-tocopherol, retinol, vitamin K1, vitamin D concentrations during trial (Table 6). Plasma alpha-tocopherol decreased more in sterol diet group than in control group. Also beta-carotene was decreased in sterol diet group and was increased in control group. However, there were no significant changes between groups when alpha-tocopherol and beta-carotene were related to serum total cholesterol. The ratios of plasma retinol to total cholesterol and D-vitamin to total cholesterol concentrations increased significant more in sterol diet group than in control group.

Apolipoproteins

The distribution among study subjects of apoE polymorphism is presented in Table 7. There were no differences in the lipid changes (total cholesterol, LDL-colesterol and HDL/LDL ratio) between randomisation groups when the subjects were divided into ApoE 33+34 and ApoE 43+44 groups (Table 8). But in both ApoE groups the total and LDL cholesterol values decreased statistically significant more in the sterol group than in control group. Also no differences in the serum plant sterols changes were seen in two groups when the subjects were divided these two ApoE groups (data not shown).

Apolipoproteins are shown in table 9.

LDL oxidation

No statistically significant differences were seen in the rate of LDL oxidation, maximal oxidation, lagpahse or diene formation and tbars between the sterol and control group (Table 10).

DISCUSSION

Plant sterols

The added plant sterol powder contains mainly beta-sitosterol, it is probable that serum concentrations would some degree increase. Serum plant sterol levels are however in normal ranges. Plant stanol concentration were so low, that it was not meaningful to determine serum plant stanols levels.

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Table 1. Baseline characteristics of subjects [1]

Variables	Sterol group [2] (n=82) mean±SD	Control group [3] (n=82) mean±SD
Age (y)	57.6±9.1	57.0±8.4
Women/men	65/17	64/18
Weight (kg)		
men	83.4±11.5	83.9±12.5
women	70.2±10.6	69.3±10.7
Body mass index (kg/m^2)	27.1±3.9	26.8±4.2
Waist/hip ratio		
men	0.96±0.05	0.93±0.06
women	0.83±0.05	0.84±0.05
Serum lipids (mmol/l)		
Total cholesterol	6.4±0.7	6.4±0.6
LDL cholesterol	4.1±0.6	4.0±0.7
HDL cholesterol	1.7±0.4	1.7±0.4
HDL/LDL cholesterol ratio	0.4±0.1	0.4±0.2
Triacylglyserols [4]	1.3±0.6	1.4±0.6
Blood pressure (mm Hg)		
Systolic	132±14	126±14
Diastolic	82±7	81±8

[1] Weight, BMI, lipids and blood pressure measurements are from visit 3 (randomisation)
There is no statistical significant differences between groups
[2] n=82
[3] n=82
[4] n=78 in the sterol group

Table 2. Composition of plant sterol-containing yoghurt, low-fat plant sterol-containing cheese and fresh cheese and control (standard) food items. Values are per daily amount of the product.

Nutrients	Yoghurt (150 g)	Control yoghurt (150 g)	Low-fat hard cheese (50 g)	Control hard cheese (50 g)	Low-fat fresh cheese (50 g)	Control fresh cheese (50 g)
Energy (kJ)	536	540	475	475	375	375
Protein (g)	5.25	5.25	15.5	15.5	3.25	3.25
Carbohydrates (g)	19.5	19.5	1.4	1.4	3.95	4
Total fat (g)	3.0	3.0	5	5	7	7
Fatty acids						
Saturated (g)	1.95	1.95	3	3	4.0	4.0
Monoun-saturated (g)	0.6	0.6	1	1	1.35	1.35
Polyun-saturated (g)	0	0	0.1	0.1	0.15	0.15
Cholesterol	7.8	7.8	13	13	18	18
Plant sterols (g)	1.65	0	2	0	2	0
Beta-sitosterol (g) [1]	1.24		1.5		1.5	
Beta-sitostanol (g) [1]	0.16		0.2		0.2	
Campesterol (g) [1]	0.16		0.2		0.2	
Campestanol (g) [1]	0.03		0.04		0.04	
Other plant sterols (g) [1]	0.03		0.04		0.04	
Milk fat (g)	0.75	3.0	4.5	7.5	4-5	6-7
Vegetable fat (g)	0.6	0	0.6		0.6	
Calcium (mg)						
Magnesium (mg)						
Potassium (mg)						
NaCl (g)			0.35	0.7	0.1-0.45	0.1-0.45

[1] plant sterol amounts are calculated from manufacturers information of plant sterol powder (75% beta-sitosterol, 10% beta-sitostanol, 10% campesterol, 2% campestanol, 2% others)

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Analyysi tulokset:
Koskivatko sekä sterolilla lisättyä tuotetta että kontrollituotetta?
Viikin analyysit: Jogurtissa sitosterolin osuus (48%) todella erilainen kuin juustoissa (n.78%) , valmistaja ilmoitti 75%

Table 3. Changes (%) in serum lipids between sterol and control group in different food items groups [1]

	Yoghurt	Cheese	Fresh cheese	All groups together
Total cholesterol				
sterol	-4.0±8.9	-8.3±10.1	-6.6±7.0	-6.5±9.0
control	-0.9±8.1	-1.4±7.7	2.3±8.2	-0.0±8.1
p-value [2]	0.3417 [3]	0.0038	0.0001	<0.00005
LDL cholesterol				
sterol	-8.7±11.7	-11.2±13.6	-11.2±8.3	-10.4±11.6
control	-1.0±11.5	0.03±11.3	2.6±13.4	0.6±12.0
p-value [2]	0.0313 [3]	0.0009	0.0001 [3]	<0.00005
HDL cholesterol				
sterol	4.6±10.4	0.0±11.4	4.8±10.7	2.8±11.0
control	3.4±10.8	3.5±9.0	4.6±12.6	3.8±10.8
p-value [2]	0.6834	0.2900 [3]	0.9339	0.5593
HDL/LDL ratio				
sterol	16.1±16.6	14.2±16.5	18.7±14.0	16.1±15.8
control	5.4±13.0	4.6±13.6	3.1±15.1	4.3±13.9
p-value [2]	0.0142 [3]	0.0156	0.0003	0.0001 [3]
Triacylglyserols [2]				
sterol	3.4±34.3	-7.0±31.3	-4.8±23.7	-3.4±30.0
control	-5.6±30.8	-10.7±25.9	1.9±31.3	-4.8±29.5
p-value [4]	0.3511	0.6224	0.3946	0.9823 [3]

[1] means±SD, in sterol group, n=25 (yoghurt), n=33(cheese), n=24 (fresh cheese), n=82 (all together), in control group, n=25 (yoghurt), n=29(cheese), n=28 (fresh cheese), n=82 (all together)
[2] t-test for parametric variables and Wilcoxon test [3] for non-parametric variables
[4] three from yoghurt sterol group and one from cheese sterol group are excluded from analysis

Table XX. Serum total, LDL cholesterol and HDL/LDL ratio and changes in serum total, LDL cholesterol and HDL/LDL ratio during the 6 weeks trial adjusted for changes in the intake of fat and unsaturated fatty acids, when patients are divided into two groups based on the LDL cholesterol median at randomisation (baseline)

Variable	Group	Baseline LDL<4.08 (n=82)			Baseline LDL>4.08 (n=81)			
		n	baseline	change	n	baseline	change	p-value [2]
Total cholesterol (mmol/l)	Sterol	44	6.0	-0.2	37	6.9	-0.8	0.0001
	Control	38	5.9	0.1	44	6.8	-0.1	0.2271
				p-value [1] 0.0180			p-value [1] 0.0001	
LDL cholesterol (mmol/l)	Sterol	77	3.6	-0.2	37	4.6	-0.8	0.0001
	Control	38	3.5	0.1	44	4.6	-0.1	0.0828
				p-value [1] 0.0018			p-value [1] 0.0001	
HDL/LDL ratio (mmol/l)	Sterol	44	0.5	0.1	37	0.4	0.1	0.5689
	Control	38	0.5	-0.0	44	0.4	0.0	0.1053
				p-value [1] 0.0005			p-value [1] 0.0002	

[1] between randomisation groups
[2] between different LDL cholesterol groups
yksi puuttuu, koska ei ole ruokapäiväkirjaa

Table 4. Nutrient intake during trial (calculated from 3-day recalls) (mean±SD)

Nutrients	Group [1]	Run-in period	Experiment period	Change between run-in and treatment periods	t-test or Wilcoxon test[2] for change between groups p-value
Energy (MJ/day)	sterol [3]	7.7±2.0	7.6±2.1	-0.05±1.7	
	control	7.6±2.3	7.5±2.2	-0.07±1.5	0.9305
Fat (% of energy)	sterol [3]	33.8±5.6	33.7±5.8	-0.08±4.7	
	control	33.3±4.8	34.1±4.8	0.8±5.9	0.3088
fatty acids (% of energy)					
-saturated	sterol [3]	15.6±3.3	15.9±3.7	0.4±3.2	
	control	15.2±3.4	16.1±3.1	0.9±3.0	0.2908
-monoun-saturated	sterol [3]	12.3±2.4	12.3±2.6	0.04±2.3	
	control	12.1±2.3	12.4±2.0	0.2±2.9	0.6420
-polyun-saturated	sterol [3]	5.2±1.7	4.8±1.2	-0.5±1.5	
	control	5.2±1.7	4.9±1.3	-0.3±2.1	0.3720
Protein (% of energy)	sterol [3]	17.8±3.3	17.3±3.4	-0.5±3.5	
	control	18.6±3.3	17.9±2.8	-0.7±3.4	0.6465
Carbohydrate (% of energy)	sterol [3]	46.9±6.4	46.2±6.0	-0.9±5.4	
	control	45.9±5.9	45.3±6.6	-0.5±6.5	0.7232
Alcohol (% of energy)	sterol [3]	1.4±3.1	2.8±4.7	1.4±3.9	
	control	2.2±4.2	2.6±4.7	0.5±4.7	0.0046 [2]
Cholesterol (mg/MJ)	sterol [3]	36.9±12.1	36.9±12.9	0.2±14.4	
	control	36.1±12.3	37.1±13.2	1.0±14.1	0.7152
Fiber (g/MJ)	sterol [3]	2.5±0.7	2.3±0.8	-0.2±0.7	
	control	2.4±0.9	2.2±0.7	-0.2±0.8	0.6386 [2]
Vitamin A (RE, µg)	sterol [3]	947±894	793±905	-154±1223	
	control	998±1717	791±605	-206±1741	0.0624 [2]
Retinol (µg)	sterol [3]	506±853	431±823	-73±1173	
	control	577±1652	430±506	-147±1661	0.0124 [2]

Carotenoids (µg)	sterol [3]	5357±3398	5488±3362	105±3277	0.2244
	control	5083±3464	5859±3013	776±3729	
Beta-carotene (µg)	sterol [3]	2224±1990	1743±1488	-493±2148	0.3675 [2]
	control	2203±2187	1841±1489	-362±2119	
Vitamin D (µg)	sterol [3]	5.4±5.0	5.5±5.2	0.2±6.5	0.4683 [2]
	control	6.4±7.1	5.6±4.9	-0.8±7.6	
Vitamin E (TE, mg)	sterol [3]	9.4±3.8	8.0±2.6	-1.4±3.4	0.4912
	control	8.6±3.5	8.0±2.7	-0.5±3.7	
Vitamin K (µg)	sterol [3]	79±35	62±24	-17.5±31.0	0.0229 [2]
	control	68±34	64±33	-3.2±36.7	

[1] in the sterol group n=82 and in the control group n=82
[2] Wilcoxon test for non-parametric variables
[3] in the sterol group, one does not return diary record at the treatment period (n=81)

Table 5. Serum plant sterols and lathosterol levels during trial

	Baseline (0 wk) means±sd	Treatment period (6 wk) means±sd	Change means±sd	T-test or Wilcoxon test [1] for change p-value
Campesterol (mg/l)				
sterol (n=82)	7.5±3.0	6.9±2.5	-0.6±1.3	
control (n=82)	6.8±3.2	6.7±3.1	-0.2±1.2	0.0669 [1]
Sitosterol (mg/l)				
sterol (n=82)	2.9±1.2	4.4±1.7	1.6±1.1	
control (n=82)	2.6±1.3	2.6±1.2	-0.1±0.5	0.0001 [1]
Stigmasterol (mg/l)				
sterol (n=82)	0.3±0.2	0.3±0.2	-0.0±0.1	
control (n=82)	0.3±0.2	0.3±0.2	-0.0±0.1	0.2765 [1]
Avenasterol (mg/l)				
sterol (n=82)	0.9±0.3	0.7±0.2	-0.1±0.2	
control (n=82)	0.8±0.3	0.8±0.3	-0.0±0.2	<0.00005
Sum of plant sterols (mg/l)				
sterol (n=82)	11.5±4.4	12.3±4.2	0.8±2.1	
control (n=82)	10.6±4.7	10.3±4.6	-0.3±1.7	0.0001 [1]
Lathosterol (mg/l)				
sterol (n=82)	3.0±1.2	3.1±1.4	0.1±0.8	
control (n=82)	3.4±1.5	3.4±1.5	0.0±0.7	0.6427 [1]

Pitoisuudet pitäisi kait ilmoittaa mol/l → seuraava taulukko

Table 5. Serum plant sterols and lathosterol levels during trial

	Baseline (0 wk) means±sd	Treatment period (6 wk) means±sd	Change means±sd	T-test or Wilcoxon test [1] for change p-value
Campesterol (µmol/l)				
sterol (n=82)	18.7±7.5	17.2±6.3	-1.5±3.2	
control (n=82)	17.1±8.1	16.6±3.1	-0.5±2.9	0.0655 [1]
Sitosterol (µmol/l)				
sterol (n=82)	7.0±1.2	10.7±4.0	3.8±2.6	
control (n=82)	6.4±3.0	6.2±3.0	-0.2±1.1	0.0001 [1]
Stigmasterol (µmol/l)				
sterol (n=82)	0.7±0.4	0.6±0.4	-0.05±0.2	
control (n=82)	0.7±0.5	0.7±0.4	-0.02±0.3	0.2745 [1]
Avenasterol (µmol/l)				
sterol (n=82)	2.1±0.6	1.7±0.5	-0.4±0.4	
control (n=82)	2.0±0.7	1.9±0.8	-0.01±0.4	<0.00005
Sum of plant sterols (µmol/l)				
sterol (n=82)	28.4±10.8	30.2±10.4	1.9±5.2	
control (n=82)	26.2±11.6	25.4±11.5	-0.7±4.1	0.0001 [1]
Lathosterol (µmol/l)				
sterol (n=82)	7.8±3.2	8.1±3.6	0.3±2.0	
control (n=82)	8.7±4.0	8.8±3.9	0.02±1.8	0.6701 [1]

Table 6. Plasma fat soluble vitamins and ratios to serum total cholesterol during drial

	Baseline (0 wk) means±SD	Treatment period (6 wk) means±SD	Change means±SD	T-test or Wilcoxon test [1] for change p-value
Gamma-tocopherol (µg/l)				
sterol (n=82)	852±337	815±328	-37±244	
control (n=81)	798±355	775±332	-23±246	0.7387 [1]
Gamma-tocopherol/TC [1] (µg/mmol)				
sterol (n=82)	135±57	137±53	2.3±42.1	
control (n=81)	127±59	122±50	-4.8±43.2	0.2019 [1]
Alpha-tocopherol (mg/l)				
sterol (n=82)	14.2±2.9	13.1±2.9	-1.1±2.4	
control (n=81)	14.4±2.9	14.4±3.2	0.0±1.7	0.0004 [1]
Alpha-tocopherol/TC [1] (mg/mmol)				
sterol (n=82)	2.2±0.4	2.2±0.4	-0.01±0.3	
control (n=81)	2.3±0.4	2.3±0.4	0.003±0.2	0.6104 [1]
Retinol (µg/l)				
sterol (n=82)	432±89	420±79	-11±57	
control (n=81)	428±84	429±97	0.9±57	0.1809
Retinol/TC [1] (µg/mmol)				
sterol (n=82)	68±13	71±13	3.3±9.1	
control (n=81)	67±14	68±15	0.2±8.6	0.0276
Beta-carotene (µg/l)				
sterol (n=82)	316±172	307±152	-9±74	
control (n=81)	352±228	385±205	33±104	0.0002 [1]
Beta-carotene/ TC [1] (µg/mmol)				
sterol (n=82)	50±27	52±28	2.6±13.0	
control (n=81)	55±35	60±31	5.1±16.2	0.0823 [1]
K1-vitamin (µg/l)				
sterol (n=82)	0.49±0.45	0.41±0.36	-0.08±0.37	
control (n=81)	0.37±0.27	0.36±0.30	-0.02±0.34	0.7089 [1]
K1-vitamin/TC [1] (µg/mmol)				
sterol (n=82)	0.08±0.07	0.07±0.05	-0.01±0.06	
control (n=81)	0.06±0.05	0.06±0.05	-0.00±0.05	0.8826 [1]
D-vitamin (µg/l)				
sterol (n=82)	13.7±6.3	17.5±6.2	3.8±5.0	
control (n=82)	13.4±4.7	16.7±5.2	3.3±4.1	0.2933 [1]
D-vitamin/TC [1] (µg/mmol)				
sterol (n=82)	2.2±1.0	3.0±1.1	0.8±0.9	
control (n=82)	2.1±0.8	2.7±0.9	0.5±0.7	0.0103 [1]

TC= Total cholesterol

Pitoisuudet mooleina → seuraava taulukko

Table 6. Plasma fat soluble vitamins and ratios to serum total cholesterol during drial

	Baseline (0 wk) means±SD	Treatment period (6 wk) means±SD	Change means±SD	T-test or Wilcoxon test [1] for change p-value
Gamma-tocopherol (nmol/l)				
sterol (n=82)	2046±808	1956±786	-90±586	
control (n=81)	1915±851	1860±796	-55±590	0.7375 [1]
Gamma-tocopherol/TC [2] (nmol/mmol)				
sterol (n=82)	323±136	329±127	5.4±101.0	
control (n=81)	304±142	292±121	-11.4±103.7	0.2019 [1]
Alpha-tocopherol (µmol/l)				
sterol (n=82)	33.0±6.6	30.5±6.7	-2.5±5.5	
control (n=81)	33.5±6.7	33.5±7.5	0.0±4.0	0.0004 [1]
Alpha-tocopherol/TC [2] (µmol/mmol)				
sterol (n=82)	5.2±0.8	5.1±1.0	-0.00±0.8	
control (n=81)	5.3±0.9	5.3±1.0	0.00±0.6	0.6104 [1]
Retinol (nmol/l)				
sterol (n=82)	1506±309	1468±277	-39±201	
control (n=81)	1495±293	1499±340	3.3±200	0.1809
Retinol/TC [2] (nmol/mmol)				
sterol (n=82)	237±46	248±46	11±32	
control (n=81)	236±48	236±51	0.6±30	0.0276
Beta-carotene (nmol/l)				
sterol (n=82)	588±321	571±283	-16±139	
control (n=81)	656±424	718±382	62±193	0.0002 [1]
Beta-carotene/ TC [2] (nmol/mmol)				
sterol (n=82)	93±50	97±51	4.9±24.2	
control (n=81)	103±65	112±58	9.6±30.3	0.0823 [1]
K1-vitamin (nmol/l)				
sterol (n=82)	1.09±1.01	0.91±0.79	-0.18±0.83	
control (n=81)	0.83±0.61	0.79±0.67	-0.03±0.74	0.7002 [1]
K1-vitamin/TC [2] (nmol/mmol)				
sterol (n=82)	0.17±0.15	0.15±0.12	-0.02±0.13	
control (n=81)	0.13±0.10	0.12±0.11	-0.01±0.12	0.8826 [1]
D-vitamin (nmol/l)				
sterol (n=82)	34.2±15.6	43.7±15.5	9.5±12.5	
control (n=82)	33.5±11.7	41.8±13.1	8.2±10.2	0.2948 [1]
D-vitamin/TC [2] (nmol/mmol)				
sterol (n=82)	5.4±2.5	7.5±2.9	2.0±2.2	
control (n=82)	5.3±1.9	6.6±2.3	1.4±1.8	0.0103 [1]

[2] TC= Total cholesterol

Table AA. Baseline serum total, LDL cholesterol and HDL/LDL ratio and changes between baseline and end of treatment during trial in different randomization and ApoE-groups

Variable	Group	ApoE 32 and 33		ApoE 43 and 44		t-test between different apoE-groups
		baseline	change	baseline	change	p-value
cholesterol (mmol/l)	Sterol	6.4±0.6	-0.5±0.6	6.4±0.8	-0.4±0.4	0.5386
	Control	6.4±0.6	-0.0±0.6	6.3±0.6	0.0±0.4	0.8039
			p-value [1]		p-value [1]	
			<0.00005		0.0092	
LDL cholesterol (mmol/l)	Sterol	4.1±0.7	-0.5±0.5	4.0±0.6	-0.4±0.5	0.5702
	Control	4.1±0.7	-0.0±0.5	4.0±0.7	0.0±0.4	0.7090
			p-value [1]		p-value [1]	
			<0.00005		0.0015	
HDL/LDL ratio (mmol/l)	Sterol	0.44±0.15	0.07±0.08	0.44±0.12	0.06±0.06	0.5837
	Control	0.44±0.16	0.01±0.07	0.42±0.17	0.01±0.07	0.5842
			p-value [1]		p-value [1]	
			<0.00005		0.0015	

[1] test between sterol and control group

Table BB. Serum baseline plant sterol and lathosterol levels and changes between baseline and end of treatment during trial in different randomization and ApoE-groups

Variable	Group	ApoE 32 and 33		ApoE 43 and 44		t-test or Wilcoxon test [1] between different apoE-groups
		baseline (wk 0)	change between weeks 0 and 6)	baseline wk 0	change between weeks 0 and 6	p-value
Campesterol (mg/l)	Sterol [2]	7.5±3.4	-0.7±1.3	7.4±2.5	-0.4±1.3	0.2271 [1]
	Control [3]	7.0±3.5	-0.4±1.1	6.4±2.5	0.4±1.3	0.0717 [1]
			p-value [4] 0.1210 [1]		p-value [4] 0.1321 [1]	
Sitosterol (mg/l)	Sterol [2]	2.9±1.2	1.5±1.0	2.9±1.2	1.7±1.2	0.3536 [1]
	Control [3]	2.7±1.3	-0.1±0.4	2.6±1.0	0.0±0.5	0.2380 [1]
			p-value [4] 0.0001 [1]		p-value [4] 0.0001 [1]	
Stigmasterol (mg/L)	Sterol [2]	0.3±0.1	-0.03±0.09	0.3±0.2	-0.01±0.07	0.3167 [1]
	Control [3]	0.3±0.2	0.00±0.11	0.4±0.2	-0.05±0.12	0.0042 [1]
			p-value [4] 0.0168 [1]		p-value [4] 0.00891 [1]	
Avenasterol (mg/L)	Sterol [2]	0.8±0.2	-0.14±0.13	0.9±0.3	-0.17±0.18	0.4065
	Control [3]	0.8±0.3	-0.01±0.15	0.8±0.3	0.01±0.16	0.8547
			p-value [4] 0.0001 [1]		p-value [4] 0.0012 [1]	
Sum of plant sterols (mg/L)	Sterol [2]	11.5±4.7	0.6±2.0	11.5±3.9	1.1±2.2	0.1486 [1]
	Control [3]	10.7±5.0	-0.5±1.6	10.2±3.8	0.4±1.8	0.1225 [1]
			p-value [4] 0.0036 [1]		p-value [4] 0.1771	
Lathosterol (mg/L)	Sterol [2]	3.0±1.3	0.2±0.7	3.1±1.2	0.1±0.9	0.4774 [1]
	Control [3]	3.2±1.2	-0.1±0.7	3.8±2.1	0.2±0.7	0.0757
			p-value [4] 0.2005 [1]		p-value [4] 0.2414 [1]	

[1] Wilcoxon test
[2] in the apoE 32 and 33 group n=49 and in the apoE 43 and 44 group n=32
[3] in the apoE 32 and 33 group n=60 and in the apoE 43 and 44 group n=22
[4] test between sterol and control group

SELITETÄÄN VAIN TEKSTINÄ

Table BB. Serum baseline plant sterol and lathosterol levels and changes between baseline and end of treatment during trial in different randomization and ApoE-groups

	ApoE 32 and 33		ApoE 43 and 44		t-test or Wilcoxon test [1] between different apoE-groups
	baseline (wk 0)	change between weeks 0 and 6)	baseline wk 0	change between weeks 0 and 6	p-value
Campesterol (µmol/L)					
Sterol [2]	18.8±8.4	-1.9±3.1	18.5±6.1	-0.9±3.2	0.2271 [1]
Control [3]	17.5±8.7	-1.0±2.7	16.0±6.2	0.9±3.2	0.0717 [1]
p-value [4]		0.1210 [1]		0.1321 [1]	
Sitosterol (µmol/L)					
Sterol [2]	6.9±2.9	3.5±2.5	7.0±2.9	4.1±2.8	0.3536 [1]
Control [3]	6.4±3.2	-0.3±1.1	6.3±2.5	0.1±1.2	0.2380 [1]
p-value [4]		0.0001 [1]		0.0001 [1]	
Stigmasterol (µmol/L)					
Sterol [2]	0.7±0.4	-0.07±0.22	0.7±0.4	-0.02±0.17	0.3167 [1]
Control [3]	0.7±0.4	0.01±0.26	0.9±0.6	-0.1±0.3	0.0042 [1]
p-value [4]		0.0168 [1]		0.00891 [1]	
Avenasterol (µmol/L)					
Sterol [2]	2.0±0.6	-0.3±0.3	2.2±0.7	-0.4±0.45	0.4065
Control [3]	1.9±0.7	-0.03±0.37	2.0±0.6	0.03±0.39	0.8547
p-value [4]		0.0001 [1]		0.0012 [1]	
Sum of plant sterols (µmol/L)					
Sterol [2]	28.4±11.8	1.2±5.0	28.4±9.5	2.7±5.4	0.1486 [1]
Control [3]	26.5±12.4	-1.3±3.9	25.2±9.3	0.9±4.4	0.1225 [1]
p-value [4]		0.0036 [1]		0.1771	
Lathosterol (µmol/L)					
Sterol [2]	7.7±3.3	0.4±1.8	8.0±3.2	0.1±2.4	0.4774 [1]
Control [3]	8.4±3.2	-0.2±1.8	9.8±5.5	0.6±1.8	0.0757
p-value [4]		0.2005 [1]		0.2414 [1]	

[1] Wilcoxon test
[2] in the apoE 32 and 33 group n=49 and in the apoE 43 and 44 group n=32
[3] in the apoE 32 and 33 group n=60 and in the apoE 43 and 44 group n=22
[4] test between sterol and control group

Table 9. Serum apolipoproteins during trial

	Baseline (0 wk) randomization	Treatment period (6 wk) (end of treatment)	change between weeks 0 and 6	P-value between randomation groups
ApoA1 (mg/mL)				
sterol (n=82)	1.47±0.20	1.48±0.24	0.01±0.20	
control (n=82)	1.47±0.21	1.49±0.24	0.02±0.21	0.6855 [1]
ApoB (mg/mL)				
sterol (n=82)	1.11±0.16	1.10±0.16	-0.01±0.14	
control (n=82)	1.12±0.15	1.17±0.17	0.05±0.14	0.0048 [1]
Lp(a) (mg/mL)				
sterol (n=82)	19.6±18.6	23.0±24.8	3.4±8.1	
control (n=82)	23.2±29.9	25.5±30.3	2.3±6.6	0.8230 [2]

[1] t-test between groups
[2] Wilxocon test between groups

Table 7. Apolipoprotein E polymorphism

type	sterol group n	%	control group n	%
32	3	(3.7)	4	(4.9)
33	46	(56.1)	56	(68.3)
42	1	(1.2)	0	(0)
43	26	(31.7)	20	(24.4)
44	6	(7.3)	2	(2.4)

Table CC. Serum apolipoproteins during trial

	run-in (wk –1)	wk 0 randomization	wk 3	wk 6 (end of treatment)	follow up (+2 wk)	change between weeks 0 and 6
ApoA1 (mg/ml)						
sterol [1]	1.47±0.20	1.47±0.20	1.49±0.24	1.48±0.24	1.54±0.27	0.01±0.20
control [2]	1.44±0.20	1.47±0.21	1.50±0.26	1.49±0.24	1.52±0.24	0.02±0.21
p-value [3]						0.6855
ApoB (mg/ml)						
sterol [1]	1.10±0.13	1.11±0.16	1.08±0.18	1.10±0.16	1.15±0.17	-0.01±0.14
control [2]	1.12±0.14	1.12±0.15	1.16±0.16	1.17±0.17	1.18±0.18	0.05±0.14
p-value [3]						0.0048
Lp(a) (mg/dl)						
sterol [4]	20.9±21.2	19.6±18.6	24.6±25.5	23.0±24.8	24.4±26.1	3.4±8.1
control [5]	22.9±27.3	23.2±29.9	27.1±32.4	25.5±30.3	28.0±33.4	2.3±6.6
p-value [6]						0.8230

[1] in sterol group n=82 (weeks –1,0,6) and n=81 (weeks 3,8)
[2] in control group n=82 (weeks –1,0,3,6) and n=81 (week 8)
[3] t-test between groups
[4] in sterol group n=82 (weeks –1,0,6,8) and n=81 (week 3)
[5] in control group n=82 (weeks –1,0,6) and n=81 (weeks 3,8)
[6] Wilxocon test between groups

Table 10. LDL oxidation (from 27 patients)

	wk 0 (at randomization)	wk 6 (end of treatment)	change	t-test p-value
rate of oxidation				
sterol group (n=12)	0.016±0.003	0.016±0.003	0.0004	
control group (n=15)	0.015±0.003	0.016±0.003	0.0006	0.8558
maximal oxidation				
sterol group (n=12)	1.43±0.19	1.53±0.17	0.0911	
control group (n=15)	1.49±0.13	1.50±0.13	0.0067	0.2915
lag phase (min)				
sterol group (n=12)	163.9±30.0	141.7±30.6	-22.22	
control group (n=15)	159.8±34.5	149.4±23.6	-10.42	0.3515
diene				
sterol group (n=12)	5.33±1.04	5.48±1.05	0.14	
control group (n=15)	5.13±0.90	5.34±0.89	0.21	0.8500
tbars				
sterol group (n=12)	38.9±16.3	49.6±22.7	10.75	
control group (n=15,14)	40.7±21.1	49.6±15.9	8.76	0.8573

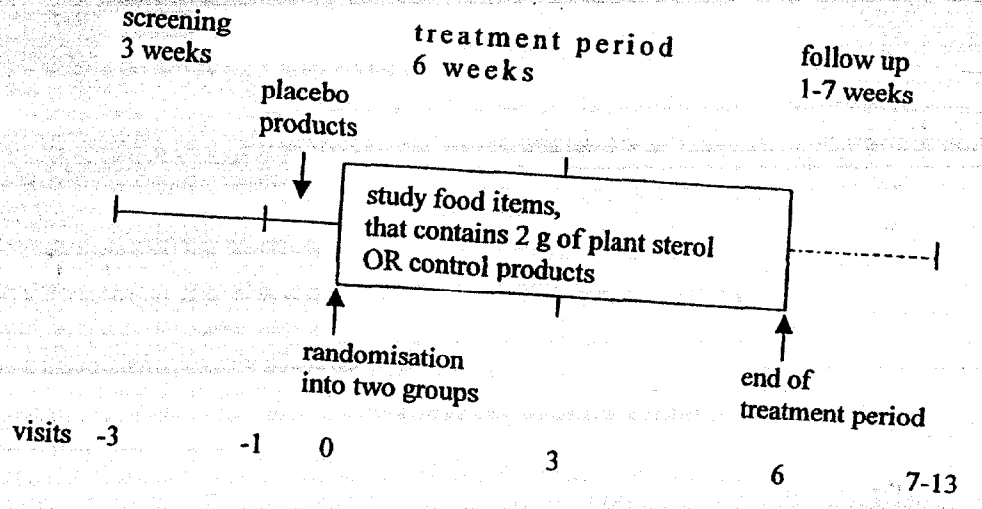

Figure 1. Study design

Total cholesterol, all groups together

	sterol grou	control gro	SE, sterol g	SE, control
-3	6,56	6,72	0,068	0,075
-1	6,44	6,41	0,067	0,074
0	6,4	6,39	0,076	0,071
3	6,23	6,63	0,074	0,081
6	5,95	6,38	0,065	0,078
8	6,4	6,41	0,063	0,079

LDL-cholesterol

	sterol grou	control gro	SE, sterol g	SE, control
-3	4,29	4,51	0,071	0,076
0	4,05	4,05	0,07	0,077
3	3,96	4,35	0,069	0,079
6	3,59	4,04	0,059	0,075
8	3,98	4,03	0,061	0,072

HDL-cholesterol

	sterol grou	control gro	SE, sterol g	SE, control
-3	1,63	1,61	0,044	0,042
0	1,72	1,69	0,044	0,044
3	1,68	1,63	0,043	0,045
6	1,76	1,75	0,047	0,045
8	1,78	1,71	0,047	0,045

HDL/LDL cholestrol ratio

	sterol grou	control gro	SE, sterol g	SE, control
-3	0,39	0,37	0,015	0,014
0	0,44	0,44	0,015	0,018
3	0,44	0,39	0,016	0,015
6	0,51	0,45	0,019	0,017
8	0,46	0,44	0,017	0,015

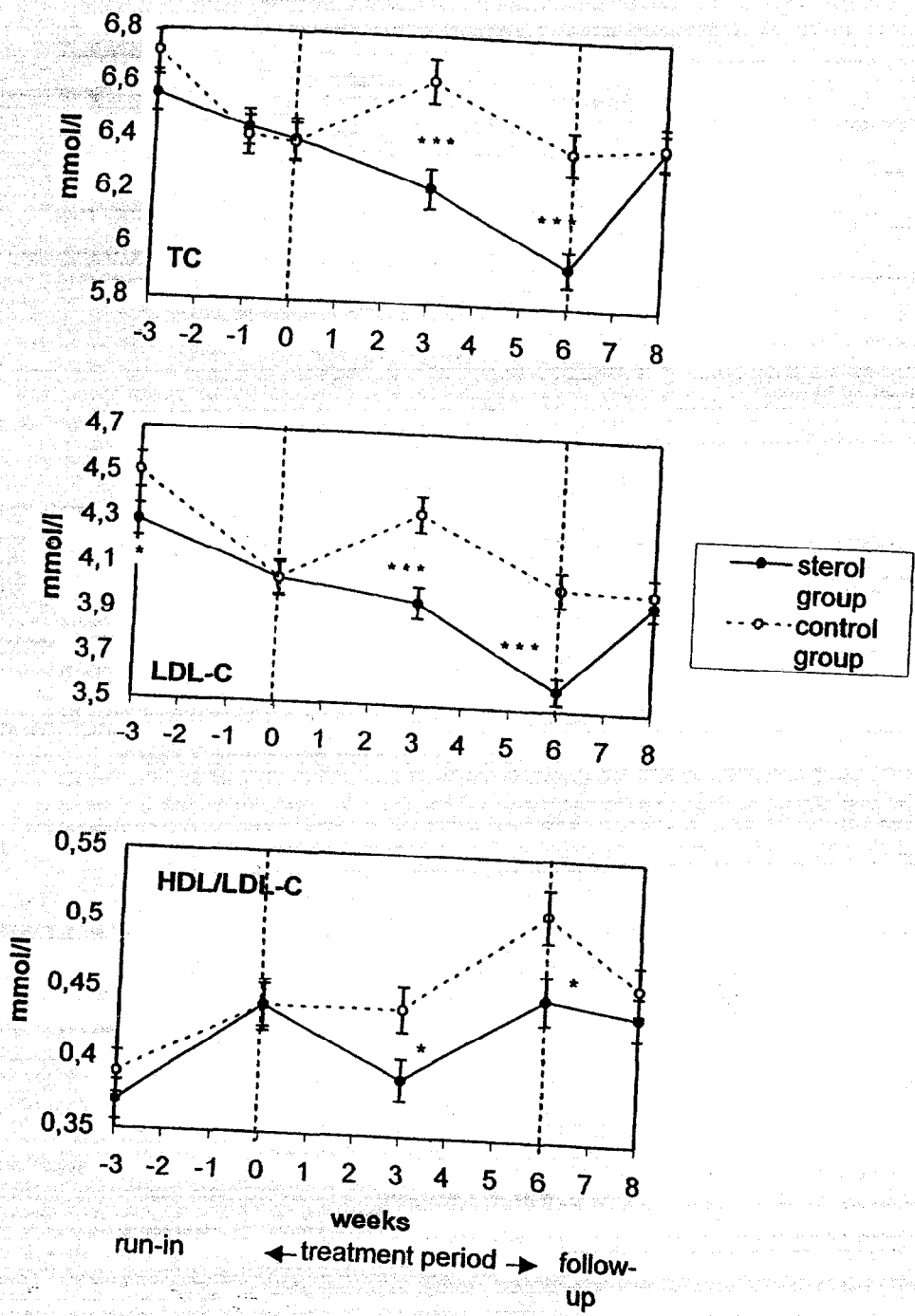

Figure 2. Sterol (n=82) and control (n=82) groups serum lipid concentrations during trial
TC= Total cholesterol, LDL-C= low-density lipoprotein cholesterol, HDL/LDL-C= high-density lipoprotein/low-density lipoprotein cholesterol ratio
* = p-value<0.05 and *** = p-value>0.001
values are expressed as mean±SE

APPENDIX 29:

Study report (Sarkkinen et al. 1999): Effect of low fat and low salted meat products enriched with MultiBene® on serum total, lipoprotein lipids and blood pressure in subjects with mild to moderate hypercholesterolemia

Oy Foodfiles Ltd

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FINAL REPORT:

Effect of low fat and low salted meat products enriched with Multibene® on serum total, lipoprotein lipids and blood pressure in subjects with mild to moderate hypercholesterolemia

Authors: Essi Sarkkinen, Ph.D., Niina Tapola M.Sc. and Matti Uusitupa M.D.

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Appendix 2	Main measurements by study visits
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Appendix 5	Instructions for consumption of test products
Appendix 6	Diary for recording of the use of test products and the possible changes of diet, health status, physical activity etc.
Appendix 7	Summary in Finnish

INTRODUCTION

Coronary heart disease is one of the leading causes of death in Western countries. Elevated serum total cholesterol and blood pressure are independent risk factors for coronary heart disease. Diet therapy is a primary mode of treatment for both hypercholesterolemic and hypertensive persons.

Plant sterols have been shown to have a hypocholesterolemic effect without having an effect on HDL cholesterol concentration (1-8). The cholesterol lowering property of plant sterols is based on the inhibition of absorption of biliary and dietary cholesterol from small intestine (4,9,10). Thus, plant sterol enriched products are offering a new avenue in the dietary management of elevated serum cholesterol concentrations. In this respect the effect of plant stanol ester fat spreads has been best documented (2,5,8). Daily use of plant sterol enriched food stuffs is necessary to sustain the cholesterol lowering effect. Previous studies have principally been made with saturated and esterified form of plant sterols and added to spreads or other fat products. Addition of crystalline, only physically processed, plant sterols is technologically possible to low-fat food matrix e.g. bread and meat. Therefore, it is important to study whether other food stuffs (like meat products in the present study) enriched with only physically processed plant sterols and used on daily basis would have significant cholesterol lowering properties.

The enrichment of a combination of crystalline plant sterols and calcium, magnesium and potassium minerals (Multibene®) has been shown to decrease serum total and LDL cholesterol concentration significantly in rats (Karppanen et al. published data). Unpublished data on humans indicate that Multibene® enriched food stuffs (combination of bread, meat and milk products) as a part of habitual Finnish diet reduce significantly serum total and LDL cholesterol concentration (Tikkanen et al, unpublished data). Therefore, there is need to examine the independent effect of meat products enriched with Multibene® on serum lipids.

AIM OF THE STUDY

The aim of this study was to find out the effect of plant sterol and mineral enrichment (Multibene®) in low fat and low salted meat products (frankfurters and cold cuts) compared to control meat products, on serum total and lipoprotein lipids and blood pressure.

SUBJECTS

Inclusion criteria of the study were serum total cholesterol between 5,0 and 8,5 mmol/l, serum total triglycerides below 3,0 mmol/l, age 30-65 years, normal liver, kidney and thyroid function, no lipid lowering medication, no unstable angina, no history of myocardial infraction, coronary artery bypass craft (CABG), percutaneous transluminal coronary angioplasty (PTCA) within the previous 6 months, no transient ischemic attack (TIA), no history of malignancy nor diagnosed diabetes.

Subjects for this study were recruited by an advertisement in a local newspaper. Thirty-three subjects volunteered to participate in the study. Four of them were excluded from the study during the pre-trial period. Twenty-eight subjects started to eat the test products. Eight subjects dropped out during the study. One subject dropped out during the pre-trial period due to personal reason and five during the first test period: three due to personal reason, one due to unpleasant taste of test frankfurters and one subject considered the daily amount of test products uncomfortable to eat and one person's data excluded from analysis due to lipid lowering medication that person have started during the study. The final number of subjects in this study was 21 (15 males and 6 females). Pre-trial characteristics of study subjects and drop outs is presented in tables 1 and 2, respectively. The purpose of the study was explained for the participants, and a written consent to the study was given by all of the subjects.

Lifestyle characteristics and medication of study subjects are presented in table 3 and pre-trial routine laboratory measurements are presented in table 4. Study subjects' physical activity, smoking habits and alcohol consumption remained stable.

STUDY DESIGN

A randomized, placebo-controlled, single-blind repeated measure design with three test periods was used. Subjects started with one to two week run in period. After run-in period each subject followed all three different test periods for three weeks in a randomized order. Randomization was made as a group. The randomized order of the periods was: sitosterol 1 period (daily amount of sterols was 1,2 g), control period (no added sterols) and sitosterol 2 period (daily amount of sterols was 2,1 g). The study was approved by the Ethics Committee of Kuopio University Hospital.

METHODS

Main examinations are presented in appendix 2.

Diets and test products

During the run-in period study subjects were on their habitual diet. During the test periods subjects' background diet was the same as their habitual diet and in addition they ate altogether 75 g meat products. Every study subject ate both frankfurters and cold cuts equal amounts of both types during each test period. Energy and nutrient compositions of test frankfurters and cold cuts are presented in table 5.

All subjects received individual oral and written instructions on the diet including a list of food items exchangeable for the test products to control for the intake of energy. Test products were ingested in 2-3 portions during the day. The use of test products was recorded daily and the records were checked by the nutritionist at each visit.

Health screening

Structured interview on previous and present diseases (including food allergies), current medication, alcohol and tobacco consumption, physical activity, use of vitamins, other nutrient supplements and functional foods was carried out at the beginning of the study (appendix 3). The subjects were requested to keep their alcohol consumption, smoking habits, physical activity and use of vitamins and other nutrient supplements that were allowed

to use constant during the study. Routine laboratory samples (blood count, s-TSH, s-GT, s-crea, s-urate) were collected and analyzed at the Kuopio University Hospital to ensure the health status and inclusion criteria of the subjects. Plasma glucose was analyzed at the Clinical Research Unit, University of Kuopio using commercial reagents (Granutest 100 Glucose, Merck,.Germany) by enzymatic photometric method with Kone Pro Analysator. In addition, subjects were recording changes in their health or life style daily during the study to the diary.

Dietary monitoring

The composition of the background diet was monitored with three 4-day (three week-day and one weekend-day) food records, once during each product period. Serving sizes were estimated with a portion size picture booklet. Oral instructions how to record food were given to all subjects personally and written instructions were also given. Nutrients were calculated using the Micro-Nutrica® dietary analysis software (developed by The Social Insurance Institution) which is based on Finnish nutrient databases.

Serum lipids

All blood samples were collected after an overnight fasting (12-h). Serum total and HDL-cholesterol and total triglycerides were analyzed at the Clinical Research Unit, University of Kuopio by enzymatic photometric method (Kone Pro Analysator, Kone Instruments, Finland) using commercial kits (CHOL CHOD-PAP, Peridochrom triglyserides GPO-PAP, Boehringer Mannheim GmbH, Germany). Serum LDL -cholesterol concentration was calculated by Friedewald formula.

Blood pressure, height and weight

Blood pressure was measured from the right arm after ten minutes rest in the sitting position with a mercury sphygmomanometer (Mercuro 300, Speidel & Keller GmpH & Co, Germany). Three measurements were done and the mean of the last two were used in analyses. Height was measured at first visit to the closest 0,5 cm. Weight with light clothing was measured twice (mean was used in analyses) at every visit with digital weighing scale (Scale Seca 707, Vogel & Falke GmpH & Co, Germany). The subjects were re-

quested to keep their weight constant during the study and therefore they recorded their weight once a week also at home besides the measurements at the study unit.

Statistical methods

The data were analyzed with the SPSS Base and Advanced models 9.0 statistics program (SPSS Inc. U.S.A). The results are given as means (SD). The main comparisons were made between the mean values at the end of each test period. The absolute and percentage changes were compared between the end measurements of both sterol periods and the end measurement of control period.

Normal distribution of variables was checked with Shapiro Wilks test before further analyses. Analysis of variance for repeated measurements (MANOVA) was used to compare the overall changes in continuous variables among different test periods. Two-tailed comparisons with paired t-test with Bonferroni correction was used in the further analyses. For dietary vitamin-A intake which was not normally distributed even after logarithmic transformation and for alcohol intake which was not normally distributed Friedman test was used. Sign test was used to compare the variables of smoking habits, alcohol consumption and physical activity.

RESULTS

Weight

Body weight and body mass index of the subjects did not change during the study (table 6).

Use of test products and composition of the background diet

The compliance with the use of test products was good and uneaten portions of test products were rare and occasional (table 7). The intake of nutrients from background diet during the different test periods are presented in table 8. Difference in the intake of fat was almost significant ($p = 0,05$). The intake of monounsaturated fatty acids was significantly lower and the intake of carbohydrates and magnesium were significantly higher during si-

tosterol 1 and 2 periods than during the control period. In addition, the intake of calcium was significantly higher during sitosterol 1 period than during the control period. The differences in nutrient intake between test periods are equal to differences in nutrient compositions of test products.

Serum total and lipoprotein lipids

The concentrations of serum lipids and lipoproteins during the study are shown in table 9. The absolute and percentage changes in serum lipids and lipoproteins at the end of sitosterol 1 and 2 period compared to the end of the control period are presented in table 10. At the end of sitosterol 2 period serum total cholesterol concentration was significantly lower than at the end of control period (difference 0,34 ± 0,53 mmol/l, 4,9 ± 7,5 %). Serum LDL-cholesterol concentration changed statistically significantly during the study ($p = 0,001$ for trend) and difference was almost significant between the end measurements of each test periods ($p = 0,056$). Both absolute and percentage changes of serum LDL-cholesterol concentration in reference to control were significantly greater during the sitosterol 2 period than the sitosterol 1 period. No differences in the HDL-cholesterol and total triglyceride concentrations were found during the study.

Blood pressure

No differences in systolic and diastolic blood pressure were found between the test periods (table 11).

DISCUSSION

The aim of this study was to find out the effect of Multibene® enrichment (crystalline plant sterols from tall oil, potassium, calcium and magnesium) in low fat and low salted meat products (frankfurters and cold cuts) compared to regular meat products (moderate in fat and salt), on serum total and lipoprotein lipids and blood pressure. A randomized, placebo-controlled , single-blind repeated measure design with three test periods was used. Opposite to previous studies crystalline form of plant sterols and low fat food matrix was used in the present study.

The differences in the background diet among the test periods were due to the differences of composition of the test products enriched with Multibene® compared to the control products. Test products were intentionally lower in fat and higher in minerals than regular meat products, which were used as a control.

Study subjects' weight, physical activity, smoking habits and alcohol consumption remained stable during the study. Therefore, the difference in serum total cholesterol concentration between the sitosterol 2 and control periods could be ascribed to be due to Multibene®.

Serum total cholesterol concentration was significantly lower at the end of sitosterol 2 period when daily intake of added sitosterols was 2,1 g than at the end of the control period. During the sitosterol 1 period, when daily amount of sitosterols was 1,2 g, serum total cholesterol concentration did not change significantly in reference to the control period. The earlier study using partly esterified form of plant sterols from vegetable oil origns has shown that sitosterol reduces total cholesterol concentration significantly, when the intake of total plant sterols has been as low as 0,83 g/d (11). In the studies with esterified or partly esterified form of sterols serum LDL-cholesterol concentration has decreased significantly, when the intake of sitosterols has been 1 g/d or greater (5, 11, 12). In the present study serum LDL-cholesterol concentration tended to be lower in the sitosterol 2 period than in the control period, but the difference was not statistically significant despite that the trens among periods was. Altogether the effect on total and LDL-cholesterol concentrations was about half of that achieved with esterified and saturated form of plant sterols given as part of spreads and whether tall oil or vegetable oil origins.

The supposed mechanism behind the cholesterol lowering effect of plant sterol is the competition of plant sterols with cholesterol for incorporation in mixed micelles (3, 6). It could be that the esterified form of plant sterol is more readily incorporated into the micelles and, therefore, it might be a better competitor for cholesterol than crystalline, only physically processed, plant sterol.

The mineral enrichment of test products seemed to have no additional or synergistic effects with plant sterols on serum cholesterol concentrations in the present study.

In agreement with the earlier studies, serum HDL-cholesterol and triglyceride concentrations did not change during the test periods in reference to the control period. And no differences in systolic and diastolic blood pressure were found among the test periods. Plant sterols have not been shown to have an effect on blood pressure. In the present study the blood pressure was a matter of interest because of mineral enrichment of test products.

In the earlier studies it was shown that plant sterols reduce serum cholesterol concentration within 2-3 weeks of initiation of treatment (6). Therefore, three weeks' duration of test period can be kept sufficient long to bring out the maximum effect on serum cholesterol concentration. In the present study the mean of serum cholesterol concentrations was the same already after one week consumption of test products than after three weeks consumption in the sitosterol 2 period. This indicates that the effect is reached already in one week.

CONCLUSIONS

In the present study frankfurters and cold cuts enriched with plant sterols from tall oil, potassium, calcium and magnesium, as part of habitual Finnish diet reduced serum total cholesterol concentration in hypercholesterolemic subjects when the intake of sitosterols was 2,1 g/d, but not with the lower dose and mineral and plant sterol enrichment of the test products did not affect the blood pressure.

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Table 1. Pre-trial characteristics of study subjects (n = 21). Mean (SD).

Men/Women	15/6
Age (years)	50 (10)
Body mass index (kg/m^2)	25,8 (2,8)
Serum total cholesterol (mmol/l)	6,7 (0,9)
Serum LDL-cholesterol (mmol/l)	4,7 (0,8) n = 18
Serum HDL-cholesterol (mmol/l)	1,2 (0,2) n = 18
Serum total triglycerides (mmol/l)	1,5 (0,6)
Education: Comprehensive school	1
Vocational school	10
Upper secondary school-leaving certificate	1
College-level institute	5
Polytechnic college	1
Academic degree	3

Table 2. Pre-trial characteristics of drop-outs (n = 8). Mean (SD).

Men/Women	4/4
Age (years)	52 (8)
Body mass index (kg/m^2)	26,0 (3,7)
Serum total cholesterol (mmol/l)	6,1 (0,9)
Education: Comprehensive school	1
Vocational school	6
Upper secondary school-leaving certificate	1

Table 3. Pre-trial lifestyle characteristics and medication of study subjects (n = 21). Mean (SD).

Number of smokers (regular/irregular)	4/2
Number of alcohol users	19
Number of water soluble vitamin supplement users	2
Number of fat soluble vitamin supplement users	1
Number of medication for the treatment of hypertension users	2
Number of postmenopausal hormone therapy or hormonal contraceptives users	2
Number of other medication users	3
Number of subjects having regular physical exercise*	19

* at least half an hour 2-3 times a week

Table 4. Pre-trial routine measurements (n = 21). Mean (SD).

B-Hemoglobin (g/l)	4,7 (0,8)
B-leukocytes (xE^9/l)	5,2 (1,4)
B-hematocrite	0,42 (0,03)
S-TSH (mU/l)	1,8 (1,0)
S-Gamma glutamyl transferase (U/l)	28,3 (16,9)
S-Creatinine (µmol/l)	92,1 (10,7)

Table 5. Energy and nutrient compositions of test products (/75 g).

	Control period		Sitosterol 1 period		Sitosterol 2 period	
	frankfurters	**cold cuts**	**frankfurters**	**cold cuts**	**frankfurters**	**cold cuts**
Energy (kJ)	793	852	576	681	649	737
Energy (kcal)	190	204	138	164	156	177
Fat (g) *	16,3	16,8	9,2	11,6	11,3	13,1
Saturated fatty acids (g)	6,3	6,1	3,3	4,7	4,1	5,3
Monounsaturated fatty acids (g)	6,9	7,2	4,1	4,9	4,9	5,6
Polyunsaturated fatty acids (g)	1,7	2,2	1,2	1,1	1,4	1,0
Total sitosterols (mg) *	-	-	1275	1200	2250	2025
Campesterol (mg)	-	-	75	75	150	150
Campestanol (mg)			15	15	30	23
Sitosterol (mg)			975	975	1800	1575
Sitostanol (mg)			150	150	300	225
Cholesterol (mg) *	44	46	29[a]	32[b]	29[a]	32[b]

* analyzed value

[a] cholesterol concentration of normal low fat frankfurters is used

[b] cholesterol concentration of normal low fat sausage cuts is used

Table will be continue in next page

Table 5 continues

	Control period		Sitosterol 1 period		Sitosterol 2 period	
	frankfurters	cold cuts	frankfurters	cold cuts	frankfurters	cold cuts
Potassium (mg) *	106	176	319	416	294	425
Calcium (mg) *	5	23	132	106	96	80
Magnesium (mg) *	8	12	85	75	59	58
Sodium (mg) *	545	569	376	377	352	386
Sodium chloride (%)	1,8	1,9	1,3	1,3	1,2	1,3

* analyzed value

Table 6. Body weight and body mass index during the study (n = 21). Mean (SD).

	Baseline (0 wk)	Control period (4 wk)	Control period (6 wk)	Sitosterol 1 period (1 wk)	Sitosterol 1 period (3 wk)	Sitosterol 2 period (7 wk)	Sitosterol 2 (9 wk)	p-values[a]
Weight (kg)	78,0 (9,3)	78,0 (9,4)	78,1 (9,6)	77,8 (9,4)	78,1 (9,6)	78,1 (9,5)	78,1 (9,5)	NS
Body mass index (kg/m^2)	25,8 (2,8)	25,8 (2,9)	25,9 (3,0)	25,8 (2,9)	25,8 (3,0)	25,9 (3,0)	25,9 (3,0)	NS

[a] indicates significance of differences for overall changes among the test periods analyzed with analysis of variance for repeated measurements (MANOVA)

Table 7. The number of subjects who recorded to have uneaten test products and number of uneaten portions.

	Control period	Sitosterol 1 period	Sitosterol 2 period
Number of subjects	3	2	5
Number of portions of test products	3 1/3	2 1/6	4

Table 8. Nutrient composition of the diets (n = 21). Mean (SD)

	Control period (6 wk)	Sitosterol 1 period (3 wk)	Sitosterol 2 period (9 wk)	p-values[a]
Energy (MJ/day)	8,6 (2,0)	8,5 (2,1)	8,3 (2,2)	NS
Energy (kcal/day)	2054 (490)	2035 (512)	1984 (518)	NS
Fat (E%)	35,4 (5,7)	33,1 (5,5)	33,8 (4,9)	0,05
Saturated fatty acids (E%)	13,6 (2,9)	13,4 (3,1)	13,7 (2,5)	NS
Monounsaturated fatty acids (E%)	12,7 (2,5)	11,3 (2,7)[b]	11,6 (2,3)[d]	0,001
Polyunsaturated fatty acids (E%)	5,7 (1,2)	5,2 (2,0)	4,9 (1,2)	NS
Protein (E%)	17,8 (2,0)	17,1 (2,5)	17,2 (2,5)	NS
Carbohydrates (E%)	42,3 (6,8)	45,4 (6,4)[c]	45,1 (6,4)[d]	0,004
Alcohol (E%)	3,0 (4,2)	3,1 (6,1)	2,6 (4,4)	NS*
Fiber (g/day)	24 (10)	26 (10)	24 (8)	NS
Cholesterol (mg/day)	268 (85)	248 (105)	250 (103)	NS

[a] indicates the significance of differences for overall changes among the test periods analyzed with analysis of variance for repeated measurements (MANOVA)

[b] significantly different from the control period analyzed with paired t-test with Bonferroni correction $p < 0,001$

[c] significantly different from the control period analyzed with paired t-test with Bonferroni correction $p < 0,01$

[d] significantly different from the control period analyzed with paired t-test with Bonferroni correction $p < 0,05$

* variables were analyzed with Friedman test

Table will be continue in next page

Table 8 continues

	Control period (6 wk) *	Sitosterol 1 period (3 wk)	Sitosterol 2 period (9 wk)	p-values[a]
Sodium (mg/day)	3453 (775)	3446 (855)	3377 (911)	NS
Potassium (mg/day)	3713 (873)	4067 (922)	3896 (940)	NS
Calcium (mg/day)	1028 (327)	1206 (428)[c]	1132 (344)	0,027
Magnesium (mg/day)	388 (100)	467 (100)[b]	425 (91)[d]	0,000
Vitamin C (mg/day)	98 (53)	110 (54)	102 (55)	NS
Vitamin E (mg/day)	8,9 (3,5)	8,5 (3,0)	7,9 (2,9)	NS
Vitamin A (µg/day)	1201 (2473)	1172 (1937)	892 (1008)	NS*

[a] indicates the significance of differences for overall changes among the test periods analyzed with analysis of variance for repeated measurements (MANOVA)

[b] significantly different from the control period analyzed with paired t-test with Bonferroni correction $p < 0,001$

[c] significantly different from the control period analyzed with paired t-test with Bonferroni correction $p < 0,01$

[d] significantly different from the control period analyzed with paired t-test with Bonferroni correction $p < 0,05$

* variables were analyzed with Friedman test

Table 9. Serum total, HDL-, LDL-cholesterol and total triglycerides during the study (n = 21). Mean (SD).

	Baseline (0 wk)	Control period (4 wk)	Control period (6 wk)	Sitosterol 1 period (1 wk)	Sitosterol 1 period (3 wk)	Sitosterol 2 period (7 wk)	Sitosterol 2 period (9 wk)	p-values[a]	p-values[b]
Total cholesterol (mmol/l)	6,8 (1,0)	6,5 (1,0)	6,7 (1,0)	6,7 (0,9)	6,6 (0,9)	6,4 (0,9)	6,4 (1,0)[c]	0,003	0,022
HDL-cholesterol (mmol/l)	1,2 (0,2)	1,3 (0,2)	1,3 (0,2)	1,3 (0,2)	1,3 (0,2)	1,2 (0,2)	1,3 (0,2)	NS	
LDL-cholesterol (mmol/l)	4,9 (0,9)	4,6 (0,9)	4,7 (0,9)	4,8 (0,9)	4,7 (0,9)	4,5 (0,8)	4,4 (0,8)	0,001	0,056
Triglycerides (mmol/l)	1,7 (0,5)	1,5 (0,7)	1,7 (0,7)	1,5 (0,4)	1,4 (0,6)	1,5 (0,6)	1,5 (0,8)	NS	

[a] indicates the significance of the differences for overall changes among the test periods analyzed with analysis of variance for repeated measurements (MANOVA)

[b] indicates the significance of the differences for overall changes among the end measurements of test periods analyzed with analysis of variance for repeated measurements (MANOVA)

[c] significantly different (p = 0,024) from the end of the control period analyzed with paired t-test with Bonferroni correction

Table 10. Absolute and percentage changes of serum total, HDL- and LDL-cholesterol and total triglycerides compared to the end of the control period (n = 21). Mean (SD)

	Sitosterol 1 period (3 wk)	Sitosterol 2 period (9wk)	p-values[a]
Total cholesterol (mmol/l)	-0,13 (0,50)	-0,34 (0,53)	NS
(%)	-1,4 (7,9)	-4,9 (7,5)	NS
HDL-cholesterol (mmol/l)	0,01 (0,13)	- 0,00 (0,15)	NS
(%)	1,0 (9,5)	0,3 (12,2)	NS
LDL-cholesterol (mmol/l)	0,00 (0,50)	-0,25 (0,59)	0,034
(%)	0,9 (11,4)	-4,6 (11,3)	0,043
Triglycerides (mmol/l)	-0,30 (0,36)	-0,20 (0,79)	NS
(%)	-15,8 (21,1)	-6,7 (37,8)	NS

[a] indicates the significance of the differences analyzed with paired t-test

Table 11. Systolic and diastolic blood pressure during the study (n = 21). Mean (SD).

	Baseline (0 wk)	Control period (4 wk)	Control period (6 wk)	Sitosterol 1 period (1 wk)	Sitosterol 1 period (3 wk)	Sitosterol 2 period (7 wk)	Sitosterol 2 period (9 wk)	p-values[a]
Systolic blood pressure (mmHg)	125,6 (8,0)	124,4 (8,0)	123,9 (8,8)	122,1 (8,4)	125,7 (11,6)	126,0 (8,2)	123,7 (8,3)	NS
Diastolic blood pressure (mmHg)	79,5 (7,9)	80,4 (7,4)	81,3 (9,3)	80,1 (8,9)	81,2 (10,5)	82,0 (7,0)	81,2 (7,4)	NS

[a] indicates the significance of the differences for overall changes among the test periods analyzed with analysis of variance for repeated measurements (MANOVA)

Appendix 1

Kuopion yliopiston ja Kuopion yliopistollisen sairaalan
TUTKIMUSEETTINEN TOIMIKUNTA
Lausuntohakemus tutkimustyön suorittamista varten

Hakemus 28/99 EJA

Saapunut 29. 1 1999

Tutkijat:
Essi Sarkkinen, Heikki Karppanen ja Matti Uusitupa

Laitos/klinikka: Oy Foodfiles Ltd

Tutkimuksen nimi (ja koodi):
Multibene®:llä rikastettujen, vähärasvaisten ja vähäsuolaisten lihavalmisteiden vaikutus kolesteroliaineenvaihduntaan ja verenpaineeseen kohtalaisessa sydän- ja verisuonitautiriskissä olevilla potilailla.

Tutkimuksen tarkoitus: Tutkimuksen tarkoituksena on selvittää vähärasvaisten ja vähäsuolaisten sekä Multibene®:ä (mineraaleja + fysikaalisesti käsiteltyjä mäntyöljyperäisiä kasvisteroleita) sisältävien lihavalmisteiden (leikkele- ja ruokamakkara) vaikutuksia seerumin kokonais- ja lipoproteiinien lipideihin ja verenpaineeseen tavanomaisiin lihavalmisteisiin verrattuna.

Yhteenveto tutkimussuunnitelmasta (tehtävät toimenpiteet/hoidot, millä muuttujilla mitataan vaikutuksia?):

Kasvisteroleiden on havaittu alentavan seerumin kolesterolipitoisuutta vaikuttamatta HDL-kolesterolipitoisuuteen. Kasvisteroleiden kolesterolipitoisuutta alentava vaikutus perustuu kasvisteroleiden kykyyn estää sappiperäisen ja ruokavalion kolesterolin imeytymistä ohutsuoleen. Päivittäinen kasvisteroleiden käyttö on kuitenkin välttämätöntä vaikutuksen säilyttämiseksi. Kasvisteroleiden toimivuutta muissa elintarvikeissa kuin rasvalevitteissä ei ole perusteellisesti tutkittu. Multibene®:llä eli kalsium, magnesium ja kalium mineraaleilla sekä kasvisteroleilla rikastettujen elintarvikkeiden on havaittu laskevan kokonais- ja LDL-kolesterolipitoisuutta ihmisillä. Tutkimuksen tarkoituksena on selvittää vähärasvaisten, vähäsuolaisten kasvisteroleilla sekä mineraaleilla (Multibene-konsepti) rikastettujen elintarvikkeiden vaikutuksia verenrasva-arvoihin ja verenpaineeseen. Asetelmana käytetään yksöissokkoa, satunnaistettua ja kontrolloitua toistettujen mittausten asetelmaa. Esivaiheen jälkeen koehenkilöt nauttivat kolmea eri tuotetta satunnaistetussa (satunnaistaminen ryhmänä) järjestyksessä kolmen viikon ajan kutakin. Kontrollituotteena toimii tavanomainen lihavalmiste (1.7 % suolaa, 22% rasvaa). Tutkittavat tuotteet sisältävät 1.0 tai 2.0 g kasvisterolia sekä täydennettyjä kalsium- (0.2g/pv) ja magnesium- (0.1g/pv) mineraaleja. Lisäksi koetuotteet ovat vähärasvaisia ja vähäsuolaisia.

Tutkimukseen rekrytoidaan 25 iältään 30-65-vuotiaita miehiä ja naisia, joilla on lievästi tai kohtalaisesti koholla oleva veren kolesterolipitoisuus (kokonaiskolesteroli 5,0-8,5 mmol/l ja triglyseridit alle 3 mmol/l), mutta ei lipidilääkitystä. Poissulkukriteereinä ovat diabetes, maksa- tai munuaissairaudet, hoitamaton kilpirauhasen toimintahäiriö, alle vuosi sitten sairastettu sydän- tai aivoinfarkti tai suoritettu ohitusleikkaus tai pallolaajennushoito, hoitamaton katkokävelyoireisto, TIA-kohtaukset tai syöpäsairaus.
Jokaisella käyntikerralla koehenkilöt punnitaan, mitataan verenpaine ja otetaan laskimoverinäyte 12 tunnin paaston jälkeen. Kokonais-, LDL-, HDL-kolesteroli ja triglyseridit määritetään jokaisen käyntikerran verinäytteestä. Ensimmäisestä verinäytteestä määritetään myös pieni verenkuva ja veren glukoosi. Tutkittavat pitävät ruokapäiväkirjaa kolme kertaa tutkimuksen aikana.

Tutkimuksen aloitus: /2 1999__ Arvioitu kesto _____ v _2.5_ kk

Tutkimusaineisto: N(Kuopiossa): 25 [x] paikallinen [ ] kotimainen
N(kokonaismäärä): 25 [ ] monikeskus [ ] kansainvälinen

Suomessa mukana myös: [ ] Helsinki [ ] Oulu [ ] Tampere [ ] Turku
[ ] Muu, mikä:

Tutkimustapa: [ ] avoin [x] satunnaistettu [ ] muu _____

Tutkittavat: [ ] täysivaltaisia potilaita [ ] vajaavaltaisia/lapsia [x] vapaaehtoisia koehenkilöitä

Informaatio annetaan: [x] kirjallisena [x] suullisena
Suostumus pyydetään: [x] tutkittavalta [ ] vanhemmilta/omaisilta
[x] kirjallisena [ ] suullisena

Toimikunta haluaa nähdä tutkittavalle toimitettavan informaation sisällön sekä suostumuslomakkeen.

Lääketutkimuksessa: Vaihe: 1[ ] 2[ ] 3[ ] 4[ ]
Geneerinen nimi:
Valmisteet(kauppanimet):
Valmistaja:

Kuopion yliopiston ja Kuopion yliopistollisen sairaalan
TUTKIMUSEETTINEN TOIMIKUNTA
Lausuntohakemus tutkimustyön suorittamista varten

Hakemus ____/____

Saapunut ____ . ____ 199

Ilmoitus Lääkelaitokseen: [ ] rekisteröimätön valmiste [ ] uusi indikaatio [ ] muu tutkimus

Tutkittaville aiheutuvat riskit (*tutkijan näkemys eettisistä ongelmista*):

Tutkimuksessa otetaan tavanomaisia laskimoverinäytteitä. Tutkijoilla on huomattava kokemus samantyyppisten ruokavaliointerventioiden toteuttamisesta. Kaikkia testattavien tuotteiden raaka-aineita on aiemmassa elintarvikekäytössä.

Selvitys tutkimuksen rahoituksesta (*lyhyesti kokonaisbudjetti, rahoituslähteet*):

Tutkimus on kokonaisuudessaan tilaajan rahoittama.

Mahdollisten vahinkojen korvaaminen:
[x] potilasvahinkovakuutus [ ] lääkevahinkovakuutus
[ ] lääketehtaan erillinen vakuutus[ ] muu _____

Allekirjoitukset ja nimenselvennykset (leima): **Päiväys:** 29 / 1 1999

Vastuuhenkilö: Essi Sarkkinen
Virka-asema: TutkimusjohtajaPuh: 2881261
Osoite/työyksikkö
Oy Foodfiles Ltd, PL 1450, 70501 Kuopio
Minne päätös toimitetaan: yllä olevaan osoitteeseen

Tutkija (ellei sama):
Virka-asema: Puh:
Osoite/työyksikkö:

Laitoksen/klinikan esimiehen suostumus (*kaikista yksiköistä, joissa tutkimusta suoritetaan*):
Olen antanut suostumukseni edellä esitetyn tutkimuksen suorittamiseen

29.1.99 Laura Jalkanen, toimitusjohtaja
Päiväys Laitoksen/klinikan esimies, allekirjoitus ja nimen selvennys (leima)

Päiväys Laitoksen/klinikan esimies, allekirjoitus ja nimen selvennys (leima)

Liitteet:
[x] Tutkimussuunnitelma kokonaisuudessaan
[ ] -laajasta vieraskielisestä suunnitelmasta suomenkielinen lyhennelmä
[ ] Selvitys tutkittavalle annettavasta informaatiosta (kuka informoi ja mitä?)
[x] Kopio tutkittavalle mahdollisesti lähetettävästä kirjeestä /annettavasta tiedotteesta

[x] Kopio suostumuslomakkeesta (yleensä suostumus kirjallisena)
[ ] Muu:

Lisäselvitysten jälkeen
Tutkimussuunnitelma lopullisesti hyväksytty 5 / 3 199 9

Kuopio 5 / 3 199 9

Eettisen toimikunnan sihteeri

Yhteydenotoissa eettiseen toimikuntaan mainittava lupahakemuksen numero (Ilmoitettu pöytäkirjanotteessa).

POJOIS-SAVON SAIRAANHOITOPIIRI
KUOPION YLIOPISTOLLINEN SAIRAALA
Tutkimuseettinen toimikunta

OTE PÖYTÄKIRJASTA 2
15.2.1999

KUOPION YLIOPISTON JA KYS:N TUTKIMUSEETTISEN TOIMIKUNNAN KOKOUS

Aika: tiistaina 09.02.1999 klo 13.15-16.10

Paikka: KYS, rakennus 3A, 3. kerroksen kokoushuone

Läsnä: Puheenjohtaja Matti Vapalahti
Varapj. Pertti Kirkinen
Sihteeri Sakari Nieminen
Leena Alhonen
Esa Jantunen
Esko Länsimies
Maija Mäntyjärvi
Ulrich Tacke
Eeva Töyry

Poissa: Janne Jauhiainen
Juha Kinnunen
Tarja Parkkonen

1. Todettiin kokous lailliseksi ja päätösvaltaiseksi.

3. Käsiteltiin toimikunnalle lähetetyt tutkimussuunnitelmat:

28/99 **Essi Sarkkinen:** Multibene^{R}:lla rikastettujen, vähärasvaisten ja vähäsuolaisten lihavalmisteiden vaikutus kolesteroliaineenvaihduntaan ja verenpaineeseen kohtalaisesa sydän- ja verisuonitautiriskissä olevilla potilailla.

Tutkimussuunnitelma muuten hyväksyttävissä, mutta yhteystyö yliopiston laitosten kanssa jäävät suunnitelmassa epäselviksi. Pitäisi selvittää mukana olevien laitosten osuus ja mahdollinen resurssien käyttö (laitosten esimiesten allekirjoitus riittää).

Päätös: Suunnitelma hyväksytään, kun edellä mainittu selvitys toimitettu eettisen toimikunnan sihteerille.

Pöytäkirjaotteen oikeaksi todistaa
Kuopiossa 18.2.1999

Matti Vapalahti
Tutkimuseettisen toimikunnan
puheenjohtaja

12.8.1997-> RESEARCH ETHICS COMMITTEE OF UNIVERSITY OF KUOPIO AND KUOPIO UNIVERSITY HOSPITAL

MEMBER NAME FIRST N.I. LAST	HIGHEST DEGREES EARNED	PRIMARY SCIENTIFIC SPECIALTY	AFFILIATION WITH INSTITUTION (If none, state)	ADDRESS AND PHONE NUMBER Chairperson only
Matti Vapalahti	Doctor of Medicine Chief Physician	Specialist's Degree in Neurosurgery	Professor of Neurosurgery, University of Kuopio/ KuopioUniversity Hospital, Chairman of the Committee	University Hospital, Dept. of Neurosurgery, 70211 Kuopio, P.O.Box 1777 Phone 358-17-172603
Leena Alhonen	Doctor of Philosophy Research Director	Animal Biotechnology	A.I.Virtanen Institute, BioTeknia	
Johanna Juntunen	Student of Medicine		Faculty of Medicine, University of Kuopio	
Juha Kinnunen	Doctor of Social Sciences	Health Policy and Management	Associate Professor, Dept. of Health Policy and and Management, University of Kuopio	
Pertti Kirkinen	Doctor of Medicine	Specialist's Degree in Obstetrics and Gynaecology	Associate Professor, Dept. of Obstetrics and Gynaecology, University of Kuopio/Kuopio University Hospital, Vice-Chairman of the Comittee	
Irma Koivula	Doctor of Medicine	Specialist's Degree in Internal Medicin	Dept. of Infectious Diseases, Kuopio University Hospital	
Esko Länsimies	Doctor of Medicine Chief Physician	Specialist's Degree in Clinical Physiology	Professor of Clinical Physiology, Department of Clinical Physiology, Kuopio University Hospital	
Maija Mäntyjärvi	Doctor of Medicine	Specialist's Degree in Ophthalmology	Professor of Ophthalmology, Dept. of Ophthalmology, University of Kuopio	
Sakari Nieminen	Doctor of Medicine	Researcher, Assistant	Dept. of Pharmacology and Toxicology, University of Kuopio	
Tarja Parkkonen	Highest degree in College of Business Administration		Head of the Finance Office, Lay member, Kuopio University Hospital	
Ulrich Tacke	Doctor of Medicine	Specialist's Degree in Pharmacology	Dept. of Psychiatry, University of Kuopio/ Kuopio University Hospital, Assistant Professor	
Eeva Töyry	Master of Nursing Science	Nursing Research Specialist	Researcher, Kuopio University Hospital	

Secretary of the Committee: 15.9.1998-01.08.1999 Sakari Nieminen

Tiedote LIPO -tutkimuksesta

Tutkimuksen tarkoitus:

Tutkimuksen tarkoituksena on selvittää mineraaleilla ja kasvisteroleilla rikastettujen, vähärasvaisten ja vähäsuolaisten lihavalmisteiden (nakki- ja/tai leikkelemakkara) vaikutus kolesteroliaineenvaihduntaan ja verenpaineeseen tavanomaisiin lihavalmisteisiin (nakki- ja/tai leikkelemakkara) verrattuna.

Tutkimuksen kulku:

Tutkimus alkaa 1-2 viikon esijaksolla, jonka aikana tutkittavat noudattavat normaalia ruokavaliotaan ja heidän soveltuvuus tutkimukseen tarkistetaan. Tämän jälkeen tutkittavat nauttivat kolme tuotejaksoa, kaksi erilaista tutkimuslihavalmistejaksoa ja yksi tavanomainen lihavalmistejakso. Tutkittavat syövät 75 grammaa nakki- ja/tai leikkelemakkaraa / päivä normaalin ruokavalion osana.

Tutkimus kestää yhteensä 10–11 viikkoa ja tutkimuskäyntejä tutkimusyksikköön (Oy Foodfiles Ltd, Sammonkatu 6) on kahdeksan (1-2 viikon välein). Ensimmäisellä käyntikerralla mitataan mm. verenpaine, paino, kolesteroli, verensokeri ja tehdään terveydentilahaastattelu. Tutkittavat pitävät ruokapäiväkirjaa syömisistään kolme kertaa tutkimuksen aikana, neljä päivää / kerta. Tutkimuskäynnit ovat aamuisin. Tutkittavien tulee olla motivoituneita noudattamaan ohjeistettua, tosin tavanomaista ruokavaliota. Painon tulee pysyä muuttumattomana koko tutkimuksen ajan.

Tutkimukseen voivat osallistua terveet 30 - 65–vuotiaat henkilöt. Poissulkevia sairauksia ovat diabetes, maksa- ja munuaissairaudet, hoitamaton kilpirauhasen toimintahäiriö, syöpäsairaus, alle vuosi sairastetusta sydän- tai aivoinfarktista, alle vuosi ohitusleikkauksesta tai pallolaajennushoidosta, hoitamaton katkokävelyoireisto tai TIA-kohtaukset.

Tutkimukseen osallistuminen on täysin vapaaehtoista ja osallistumisen voi keskeyttää milloin tahansa. Toivomme kuitenkin, että tutkimukseen osallistuva pysyisi mukana tutkimuksen loppuun asti. Laboratoriotutkimukset samoin kuin tutkimusvalmisteet ovat tutkittaville ilmaisia. Tutkittava saa verikokeiden ja ruokavalionsa koostumusta koskevat tulokset tutkimuksen päätyttyä. Mikäli verikokeissa havaitaan poikkeavia löydöksiä, ohjaamme tutkittavan asianmukaisiin jatkotutkimuksiin

Yhteystiedot:

Niina Tapola
Ravitsemusterapeutti
288 1262
Oy Foofiles Ltd

Jouni Hodju
Laboratoriohoitaja
288 1263
Oy Foodfiles Ltd

Suostumus LIPO -tutkimukseen osallistumiseen

Tutkimuksen johtaja Essi Sarkkinen, FT

Tutkimuspaikka: Oy Foodfiles Ltd, Sammonkatu 6, 70500 Kuopio

Koehenkilön nimi: _____

Syntymäaika: _____

Osoite: _____

_____

Puh: _____(koti)

_____ (työ)

Osallistun vapaaehtoisesti mineraaleilla ja kasvisteroleilla rikastettujen, vähärasvaisten ja vähäsuolaisten lihavalmisteiden vaikutusta veren rasvoihin ja verenpaineeseen käsittelevään tutkimukseen, joka kestää 10 viikkoa. Tiedän, että tutkimuskäyntejä on kahdeksan 1-2 viikon välein. Olen tietoinen, että tutkimus sisältää kahdeksan verinäytteenottokertaa. Tulen noudattamaan tutkimuksen kuluessa saamiani ohjeita, mutta olen vapaa keskeyttämään osallistumiseni tutkimukseen milloin tahansa.

Paikka ja aika: _____

Koehenkilön allekirjoitus: _____

Main measurements by study visits

Variable	-1 wk	0 wk	1 wk	3 wk	4 wk	6 wk	7 wk	9 wk
Medical history	X							
Interview of lifestyle	X							X
Food record				X		X		X
S-totchol	X	X	X	X	X	X	X	X
LDL-chol *	X	X	X	X	X	X	X	X
HDL-chol	X	X	X	X	X	X	X	X
Triglycerides	X	X	X	X	X	X	X	X
Height	X							
Weight	X	X	X	X	X	X	X	X
Blood pressure	X	X	X	X	X	X	X	X
P-glucose	X							
Blood count	X							
S-tsh	X							
S-crea	X							
S-ggt	X							
S-urate	X							
Serum plant sterols		X		X		X		X
Serum fat soluble vitamins		X		X		X		X
Extra samples **	X	X		X		X		X

*) calculated by Friedewald formula
**) extra samples of serum for optional analyses

Appendix 3

Oy Foodfiles Ltd/JH -99

LIPO / PERUSTIETOLOMAKE

Nimi: _____

Syntymäaika: _____

Osoite: _____

_____

Puh: koti:_____ työ:_____

muu:_____

Tutkimustunnus: I__I__I__I__I Tutkimusnumero: I__I__I__I Tutkimusjakso: I__I__I__I

Tutkimuskerta: 0 vko Pvm (pv,kk,vvvv): I__I__I__I__I__I__I__I__I__I

1. Sukupuoli (1= mies, 2= nainen) I__I

2. Ikä (vuosina)

I__I__I

3. Koulutus

0. vähemmän kuin kansakoulu
1. kansakoulu tai peruskoulu
2. alempi keskiaste (ammattikoulu, kansalaiskoulu, oppikoulu)
3. ylioppilas
4. ylempi keskiaste
5. ammattikorkeakoulu
6. akateeminen loppututkinto
7. muu mikä? _____

4. Lääkärin toteamat krooniset sairaudet (milloin todettu):

_____

_____

_____

Poissulkevia ovat: diabetes, maksa- ja munuaissairaudet, syöpä, epästabiili sepelvaltimosairaus (alle vuosi infarktista, ohitusleikkauksesta tai pallolaajennushoidosta), TIA-kohtaukset, hoitamaton kilpirauhasen vajaatoiminta tai muu lipidiaineenvaihduntaan vaikuttava sairaus. Myös ruoka-aineallergiat kirjataan tähän.

TUPAKOINTI

9. Tupakoitteko?

1 ei

2 kyllä, satunnaisesti

3 kyllä, säännöllisesti

10. Savukkeiden/piipullisten määrä päivässä

I__I__I

ALKOHOLIN KÄYTTÖ

11. Kuinka usein juotte olutta/siideriä/lonkeroa ?

0 ei lainkaan

1 päivittäin

2 3-5 kertaa viikossa

3 1-2 kertaa viikossa

4 pari kertaa kuukaudessa

5 harvemmin

12. Paljonko olutta/siideriä/lonkeroa nautitte yleensä kerralla?

I__I__I pulloa

13. Kuinka usein juotte viiniä?

0 ei lainkaan

1 päivittäin

2 3-5 kertaa viikossa

3 1-2 kertaa viikossa

4 pari kertaa kuukaudessa

5 harvemmin

14. Paljonko viiniä nautitte yleensä kerralla?

I__I__I lasia/pulloa (yliviivaa tarpeeton)

VAIN NAISILLE

18. Oletteko raskaana tai suunnitteletteko hankkiutumista raskaaksi ?

1. ei 2. kyllä

Raskaus on tutkimuksen poissulkukriteeri.

19. Kuinka pitkä on kuukautiskiertonne ?

1. I__I__I vrk, edellisten kuukautisten alkamispäivämäärä I__I__I.I__I__I
2. minulla ei ole enää kuukautisia

20. Käytättekö hormonaalista ehkäisyä?

1. Ei
2. Kyllä,mitä

Appendix 4a

CULTOR TECHNOLOGY

ANALYYSITODISTUS 990201

Antti Kaipainen/TRL 19.3.1999

Näytteet Lihajalosteet, Pouttu Oy
1: Kuorettomat nakit, Lipo 898
2: Kannuswurstiviipaleet, Lipo 898

Saapunut
4.3.1999

Tilaaja
Anneli Törrönen / CFS Technology Development

Projektinro
POU0001 (LIMS: SIT0002)

Tehtävä
Sterolien GC-analyysi

Tekijät
Antti Kaipainen / Tiina Laamanen

Menetelmä
β-Sitosterolin ja eräiden muiden sterolien GC-analyysi silyylijohdoksina kide- ja liuosnäytteistä, sovellus lihajalosteille.

Tulokset
Ks. Liite 1.

Näytteet sisälsivät β-sitosterolia n. 1,3 %/lp ja steroleita yhteensä 1,6 – 1,7 %/lp.

Kommentit
Edellinen vastaava analyysi, ks. An.tod. 980379, 27.4.1998.

Liitteet
1. Taulukko 1. Poutun makkarat, hydrolysoidut sterolit, %/lp.

Hyväksynyt
Antti Kaipainen

Jakelu
Tilaaja
Pirjo Aronen / Pouttu Oy, Turku
Liisa Niemi / Pouttu Oy, Kannus
Martti Marjamaa
Arkisto
Tekijät

19.3.1999

Nro	Projekti	Asiakas	Tilaaja
	990201 SIT0002T	FSD	Anneli Törrönen
Lisätiedot	Poutun makkarat		Saapumispvm
			4.3.1999
Näytekoodi	Näyte	Sitosterolin ja eräiden muiden sterolien	
	1 Kuorettomat nakit, Lipo 898		
	2 Kannuswurstiviipaleet, Lipo 898		

Taulukko 1. Poutun makkarat, hydrolysoidut sterolit, %/lp

Näyte	%/lp	K.ste.	K.sta.	S.ste.	S.sta.
1. Kuorettomat nakit, Lipo 898					
A	1,6	0,1	0,02	1,3	0,2
B	1,7	0,1	0,02	1,3	0,2
C	1,7	0,1	0,02	1,3	0,2
D (a	(2,3	0,2	0,03	1,8	0,3)
ka	1,7	-0,1	0,02	1,3	0,2
SD	0,04				
RSD%	3				
2. Kannuswurstiviipaleet, Lipo 898					
A	1,6	0,1	0,03	1,2	0,2
B	1,7	0,1	0,02	1,3	0,2
C	1,6	0,1	0,02	1,3	0,2
D	1,6	0,1	0,02	1,3	0,2
ka	1,6	0,1	0,02	1,3	0,2
SD	0,03				
RSD%	2				

a) Poistettu tulosten laskennasta Q-testin perusteella.

X:\SIT0002T\990201\[TULOSX.XLS]%lp

Appendix 4 b

CULTOR TECHNOLOGY

ANALYYSITODISTUS 990372

Antti Kaipainen/TRL 24.5.1999

Näytteet Lihajalosteet, Pouttu Oy
1. Kuorettomat nakit, valm.pv. 20.4.99/pakk.pv. 21.4.99
2. Kannuswurstiviipaleet, valm.pv. 20.4.99/pakk.pv. 21.4.99

Saapunut
22.4.1999

Tilaaja
Anneli Törrönen / CFS Technology Development

Projektinro
UMU0001 (LIMS: SIT0002T)

Tehtävä
Sterolien GC-analyysi

Tekijät
Antti Kaipainen / Tiina Laamanen

Menetelmä
β-Sitosterolin ja eräiden muiden sterolien GC-analyysi silyylijohdoksina kide-ja liuosnäytteistä, sovellus lihajalosteille.

Tulokset
Ks. Liite 1.

Näytteet 1 ja 2 sisälsivät β-sitosterolia 2,4 ja 2,1 %/lp ja steroleita yhteensä 3,0 ja 2,7 %

Kommentit
Edellinen vastaava analyysi, ks. Antod. 990201, AK, 19.3.1999.

Liitteet
1. Taulukko 1. Puotun lihajalosteet, sterolit hydrolyysin jälkeen, %/lp.

Hyväksynyt
Antti Kaipainen

Jakelu
Tilaaja
Pirjo Aronen / Pouttu Oy, Turku
Liisa Niemi / Pouttu Oy, Kannus
Martti Marjamaa
Arkisto
Tekijät

24.5.1999

Nro	Projekti	Asiakas	Tilaaja
990372	SIT0002T	FSD	Anneli Törrönen

Lisätiedot: Poutun makkarat II — Saapumispvm: 22.4.1999

Näytekoodi: Näyte — Sitosterolin ja eräiden muiden sterolien

1 Kuorettomat nakit: valm.pv. 20.4.99/pakk.pv. 21.4.99

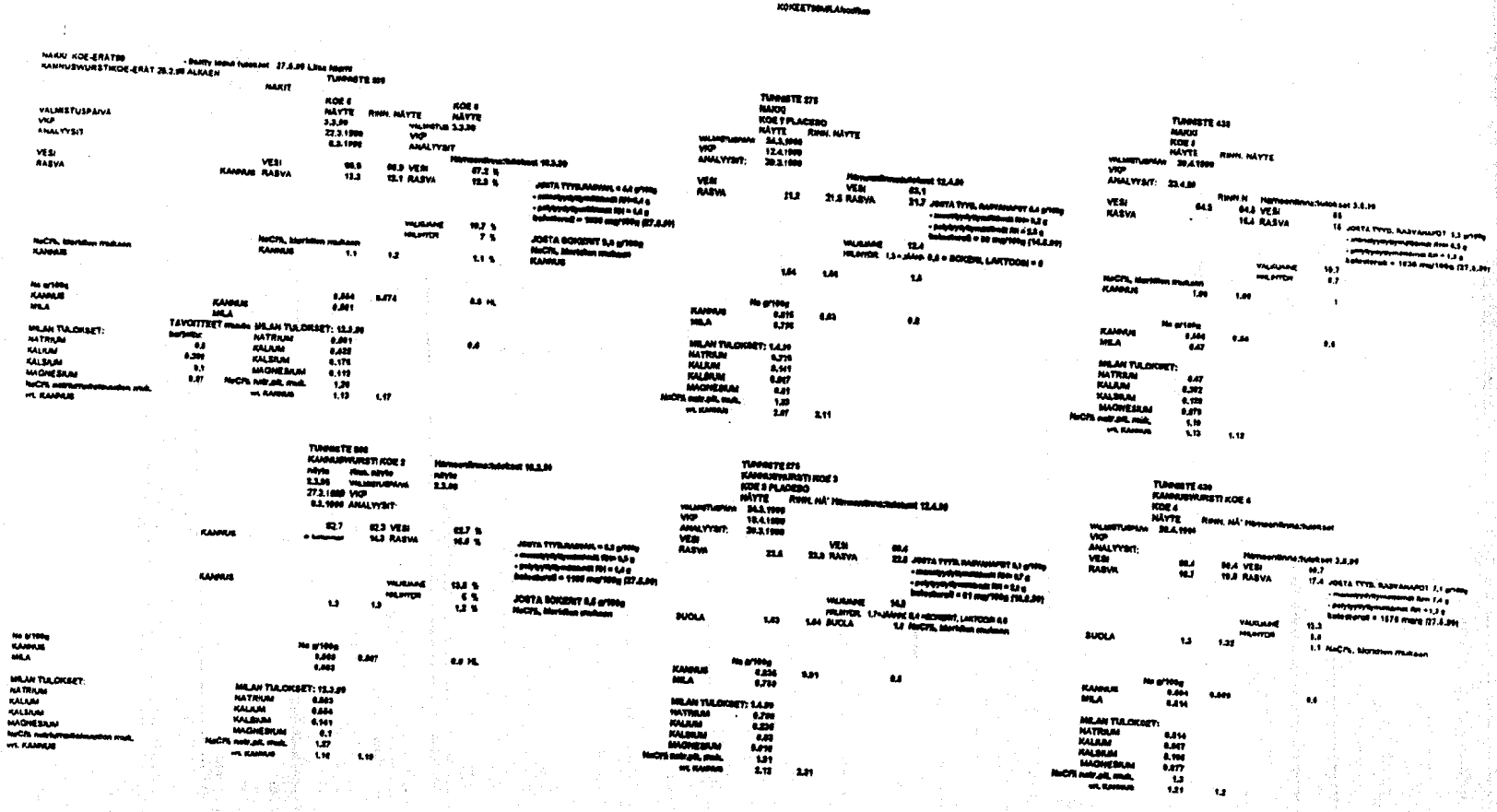
2 Kannuswurstiviipaleet: valm.pv. 20.4.99/pakk.pv. 21.4.99

Taulukko 1. Poutun lihajalosteet, steroilt hydrolyysin jälkeen, %/lp.

Näyte	%/lp	K.ste.	K.sta.	S.ste.	S.sta.
1. Kuorettomat nakit					
A	3,1	0,3	0,04	2,4	0,4
B	2,9	0,2	0,04	2,3	0,4
C	3,0	0,2	0,04	2,4	0,4
D	3,0	0,2	0,03	2,4	0,4
ka	3,0	0,2	0,04	2,4	0,4
SD	0,1				
RSD%	3				
2. Kannuswurstiviipaleet					
A	2,6	0,2	0,03	2,0	0,3
B	2,8	0,2	0,03	2,2	0,4
C	2,6	0,2	0,03	2,1	0,3
D	2,7	0,2	0,03	2,1	0,3
ka	2,7	0,2	0,03	2,1	0,3
SD	0,1				
RSD%	3				

X:\SIT0002T\990372\[TULOSX.XLS]%lp

Appendix 4c



Appendix 4 d

FOODFILES01kolesterolit

RAVINTOARVOTIEDOT: 100 g TUOTETTA SISÄLTÄÄ

8.6.99 POUTTU OY, KANNUS, LIISA NIEMI

	ENERGIA kJ	PROTEIINIT g	ilm. RASVAA g	JOSTA TYYDYTT. RASVAHAPPOJA g	HIILIHYDRAATTEJA g	JOSTA LAKTOOSIA g	PANSUOLATUT NaCl %	NATRIUM g
HERKKUNAKKI	1050	12	22	8.2	0.5	0	1.2	0.5
			kokonaiskolesteroli 54 mg/100 g					
KEVYT NAKKI	670	10	10	4	10	0	1.2	0.6
			kokonaiskolesteroli 39 mg/100 g					
KANNUSWURSTI	1080	13	22	8.6	1.6	1.1	1.4	0.7
			kokonaiskolesteroli 55 mg/100 g					
KEVYT WURSTI	695	13	10	4	6	0	1.2	0.7
			kokonaiskolesteroli 43 mg/100 g					

- kolesterolimittaustulokset olen saanut faxilla tänään, enkä tiedä, miten paljon kyseisten näytteiden rasvapitoisuudet ovat poikenneet ilmoitetusta

alemmat kokonaiskolesterolimääritystulokset (27.5.99) lisäksi tässä

		kokonaiskolesteroli mg/100 g RASVAA g	JOSTA TYYDYTT. RASVAHAPPOJA g
NAKKITYYPPINEN LIPO 275	PLACEBO	kokonaiskolesteroli 59 mg/100 g 21.7	8.4
WURSTITYYPPINEN LIPO 275	PLACEBO	kokonaiskolesteroli 61 mg/100 g 22.5	8.1
NAKKITYYPPINEN LIPO 439		kokonaiskolesteroli 1630 mg/100 g 15	5.5
WURSTTYYPPINEN LIPO 439		kokonaiskolesteroli 1570 mg/100 g 17.4	7.1
NAKKITYYPPINEN LIPO 898		kokonaiskolesteroli 1030 mg/100 g 12.3	4.4
WURSTITYYPPINEN LIPO 898		kokonaiskolesteroli 1100 mg/100 g 15.5	6.2

Appendix 5

TUTKIMUSMAKKAROIDEN NAUTTIMISOHJE

TUTKIMUSNAKKI- JA/TAI LEIKKELEMAKKARAA SYÖDÄÄN TUTKIMUSJAKSOJEN AIKANA

YHTEENSÄ 75 G PÄIVÄSSÄ

- Yksi nakkimakkara painaa 37,5 g ja leikkelemakkarasiivu painaa 12,5 g.
- Päivän aikana voit siis syödä **2 kpl nakkimakkaraa tai**
  6 siivua leikkelemakkaraa tai
  1 nakkimakkaran ja 3 siivua leikkelemakkaraa
- Syö päivittäinen tutkimusmakkara-annos **2-3:lla aterialla**. Älä siis syö koko annosta kerralla.
- Syö samanaikaisesti tutkimusmakkaroiden kanssa myös muuta ruokaa.
- On tärkeää, että syöt juuri ohjeessa mainitun määrän tutkimusmakkaraa, ei enempää eikä vähempää.
- **Säilytä** tutkimusmakkarat **kylmässä** (+ 4 °C). **Avattu** tutkimusmakkara**pakkaus säilyy 4-5 vuorokautta.**
- Korvaa tutkimusmakkaroilla aiemmin ruokavaliossasi olleita makkaroita tai muita lihavalmisteita.
  Puolet tutkimusmakkara-annoksesta (37,5 g) vastaa:
  40 g lenkkimakkaraa (1/5 lenkkiä)
  40 g nakkimakkaraa (1 nakki)
  40 g balkan, kinkkumakkara, lauantaimakkara, jahtimakkara (4 siivua)
  20 g meetvurstia (2 siivua)
  40 g jauhelihapyöryköitä, -pihvejä, kebakoita (3 kpl lihapyöryköitä, 1 pihvi, 1 kebakko)
  45 g sika-nautajauhelihaa (30 g kypsänä)
  50 g broileria nahkoineen (35 g kypsänä)
  75 g makkarakastiketta (vajaa 1 dl)
  65 g stroganoffia (vajaa 1 dl)
  45 g karjalanpaistia (½ dl)
  30 g leivitettyä porsaankyljstä
- Säilytä ruokailutottumukset muilta osin tavanomaisena koko tutkimuksen ajan.

Appendix 6

TUTKIMUSVALMISTEIDEN KÄYTTÖ JA MUU SEURANTA

- Merkitse päivittäin ja aterioittain nauttimiesi lihavalmisteiden määrä alla olevaan taulukkoon. Merkitse myös aterian nimi jolla lihavalmisteet söit. Voit käyttää seuraavia lyhenteitä: A = aamiainen, L = lounas, P = päivällinen, V = välipala, I = iltapala. **Esimerkki:** Jos syöt aamiaisella 1 siivun makkaraleikkelettä, lounaalla 1 nakkimakkaran ja päivällisellä 2 siivua makkaraleikkelettä, merkitse se oheiseen taulukkoon ensimmäisen rivin esimerkin tavoin.

- Merkitse taulukkoon myös, jos tutkimusvalmisteiden nauttimisessasi, ruokavaliossasi, liikunnassasi, terveydentilassasi tms. on ollut jotain tavanomaisuudesta poikkeavaa.

Viikot 0-3 Jakso I_I_I

Pvm/viikon-päivä		Määrä / Ateria	Määrä / Ateria	Määrä / Ateria	Erityistä tutkimusvalmisteiden nauttimisessa, ruokavaliossa, liikunnassa tms.
22.3. MA	Nakki	1 kpl/L			
	Leikkele	1 kpl/A	2 kpl/P		
	Nakki				
	Leikkele				
	Nakki				
	Leikkele				
	Nakki				
	Leikkele				
	Nakki				
	Leikkele				
	Nakki				
	Leikkele				
	Nakki				
	Leikkele				
	Nakki				
	Leikkele				
	Nakki				
	Leikkele				

TIIVISTELMÄ

Multibene®:llä rikastettujen, vähärasvaisten ja vähäsuolaisten lihavalmisteiden vaikutus kolesteroliaineenvaihduntaan ja verenpaineeseen kohtalaisessa sydän- ja verisuonitautiriskissä olevilla potilailla

Kirjoittajat: Essi Sarkkinen FT ja Niina Tapola ttyo

Tavoite

Tämän tutkimuksen tarkoituksena oli selvittää vähärasvaisten ja vähäsuolaisten sekä Multibene®:ä (mineraaleja + fysikaalisesti käsiteltyjä mäntyöljyperäisiä kasvisteroleita) sisältävien lihavalmisteiden (leikkele- ja ruokamakkaran) vaikutuksia seerumin kokonais- ja lipoproteiinien lipideihin ja verenpaineeseen tavanomaisiin lihavalmisteisiin verrattuna.

Koehenkilöt

Tutkimukseen rekrytoitiin yhteensä 33 iältään 30-65-vuotiasta vapaaehtoista henkilöä, joilla oli lievästi tai kohtalaisesti koholla oleva seerumin kolesterolipitoisuus (kokonaiskolesteroli 5,0-8,5 mmol/l ja triglyseridit alle 3,0 mmol/l), mutta ei lipidilääkitystä. Tutkimukseen soveltui 29 henkilöä, joista 21 henkilöä (15 miestä ja 6 naista) suoritti tutkimuksen loppuun asti. Koehenkilöiden keski-ikä oli 50 ± 10 vuotta ja heidän seerumin kokonaiskolesterolipitoisuus oli esivaiheen alussa 6,7 ± 0,9 mmol/l.

Tutkimusasetelma

Tutkimusasetelmana käytettiin satunnaistettua, kontrolloitua, yksöissokkoa toistettujen mittausten asetelmaa. 1-2 viikon esivaiheen jälkeen koehenkilöt nauttivat 3 kolmen viikon tuotejaksoa satunnaistetussa järjestyksessä. Tuotejaksojen järjestys oli seuraava: sitosteroli 1 jakso (kasvisteroleita 1,2 g /pv), kontrollijakso (ei lisättyjä kasvisteroleita) ja sitosteroli 2 jakso (kasvisteroleita 2,1 g/pv).

Menetelmät

Ruokavalio ja tutkimustuotteet

Koehenkilöt noudattivat tutkimuselintarvikkeita lukuunottamatta heidän tavanomaista ruokavaliotaan koko tutkimuksen ajan. He nauttivat kullakin tuotejaksolla yhteensä 75 g nakkia ja/tai leikkelemakkaraa päivittäin. Sitosteroli 1 ja 2 jaksojen makkarat olivat vähärasvaisia ja vähäsuolaisia ja niihin oli lisätty kasvisteroleita, kaliumia, kalsiumia ja magnesiumia. Kontrollijakson makkaroiden rasva- ja suolapitoisuudet olivat tavanomaiset.

Muut tutkimusmenetelmät

Tutkimuksessa tehdyt mittaukset ilmenevät loppuraportissa olevasta liitteestä 2.

Tulokset

Seerumin kokonaiskolesterolipitoisuus oli sitosteroli 2 jakson lopulla merkitsevästi pienempi kuin kontrollijakson lopulla. Myös seerumin LDL-kolesterolipitoisuus oli pienempi sitosteroli 2 jakson lopulla kuin kontrollijakson lopulla, mutta ero ei ollut tilastolliseti merkitsevä. Seerumin HDL-kolesteroli- ja triglyseridipitoisuudessa ei ollut jaksojen välillä eroja. Koehenkilöiden verenpaine ei muuttunut tutkimuksen aikana.

Koehenkilöiden paino ja elintavat eivät muuttuneet tutkimuksen aikana. Ruokavaliossa oli eroja tutkimusjaksojen välillä, mutta ne johtuivat tutkimustuotteiden valitusta erilaisesta ravintosisällöstä kontrollituotteeseen verrattuna.

Johtopäätökset

Tässä tutkimuksessa tavanomaisen ruokavalion osana nautitut, mäntyöljyperäisillä kasvisteroleilla, kaliumilla, kalsiumilla ja magnesiumilla rikastetut nakit ja leikkelemakkarat laskivat seerumin kokonaiskolesterolipitoisuutta kohtalaisessa sydän- ja verisuonitautiriskissä olevilla henkilöillä, kun päivittäinen steroliannos oli 2,1 g, mutta ei pienemmällä annoksella verrattuna koostumukseltaan tavanomaisiin lihavalmisteisiin. Kummallakaan kasvisteroliannoksella ei ollut vaikutusta verenpaineeseen.