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Short communication

Divergent changes in serum sterols during a strict uncooked vegan diet in patients with rheumatoid arthritis

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The effects of a strict uncooked vegan diet on serum lipid and sterol concentrations were studied in patients with rheumatoid arthritis. The subjects were randomized into a vegan diet group (*n* 16), who consumed a vegan diet for 2–3 months, or into a control group (*n* 13), who continued their usual omnivorous diets. Serum total and LDL-cholesterol and -phospholipid concentrations were significantly decreased by the vegan diet. The levels of serum cholestanol and lathosterol also decreased, but serum cholestanol:total cholesterol and lathosterol:total cholesterol did not change. The effect of a vegan diet on serum plant sterols was divergent as the concentration of campesterol decreased while that of sitosterol increased. This effect resulted in a significantly greater sitosterol:campesterol value in the vegan diet group than in the control group (1·48 (SD 0·39) *v.* 0·72 (SD 0·14); $P < 0·001$). A higher concentration of campesterol compared with sitosterol is normal in omnivorous subjects and can be explained by lower absorption and esterification rates of sitosterol. Our results suggest that a strict uncooked vegan diet changes the relative absorption rates of these sterols and/or their biliary clearance.

Vegan diet: Cholesterol: Plant sterol

It has been suggested that the absorption and serum concentrations of plant sterols are correlated with the absorption efficiency of cholesterol in subjects with normal sterol intakes (Tilvis & Miettinen, 1986). Vegetarians however, have, low cholesterol and often high plant sterol intakes compared with omnivorous subjects. Dietary plant sterol intake has been shown to correlate negatively with cholesterol absorption and serum total and LDL-cholesterol (Vuoristo & Miettinen, 1994). The 'living food' diet is a strict uncooked vegan diet which contains no animal products (Dwyer, 1991; Hänninen *et al.* 1992). It is very low in cholesterol, contains plenty of seeds, nuts, cereals and legumes, and is rich in plant sterols. In the present study, we investigated the effects of the 'living food' diet on serum lipid and sterol concentrations in patients with rheumatoid arthritis. These patients were selected because they were highly motivated to follow this extreme vegan diet, as it has previously been suggested that a vegan diet may alleviate the symptoms of rheumatic disease (Haugen *et al.* 1991).

Experimental methods

The present study was part of a larger study in which the effects of an uncooked vegan diet were investigated in patients with rheumatoid arthritis (Nenonen *et al.* 1998). The study was approved by the Ethics Committee of the City of Helsinki. Sixteen subjects followed a vegan diet (all females, age 49 (SD 7) years, BMI 25·6 (SD 4·3) kg/m^2) and thirteen control subjects maintained their normal diet (one male and twelve females, age 53 (SD 11) years, BMI 22·6 (SD 2·7) kg/m^2). The subjects in the vegan diet group received their food from a specialised kitchen. The duration of the dietary period was 3 months, but eight subjects discontinued the diet after 2 months because of nausea, diarrhoea or difficulties with the taste of some food items. Nutrient intakes were calculated from 7 d food-intake records kept before and during the dietary period.

Fasting blood samples were collected before the start of the dietary period, after 1 month and at the end of the intervention period (2 or 3 months). Serum cholesterol,

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Table 1. Serum lipids and sterols at baseline and their changes during the dietary period in patients with rheumatoid arthritis following a strict uncooked vegan diet or maintaining their normal diet (control)†

	Control group (*n* 13)						Vegan diet group (*n* 16)					
	Baseline		Change in 1 month		Change in 2–3 months		Baseline		Change in 1 month		Change in 2–3 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Serum lipids (mmol/l)												
Cholesterol	4·59	0·75	0·18	0·39	0·08	0·42	5·19	1·07	−0·93***	0·51	−0·86***	0·65
LDL-cholesterol	2·98	0·68	0·22	0·40	0·04	0·42	3·52	0·91	−0·74***	0·51	−0·70***	0·18
HDL-cholesterol	1·16	0·36	−0·04	0·13	0·04	0·19	1·19	0·33	−0·14	0·15	−0·12	0·18
Triacylglycerols	0·98	0·33	0·01	0·27	0·00	0·30	1·04	0·48	−0·12	0·33	−0·11	0·40
Phospholipids	2·61	0·29	0·11	0·18	0·06	0·18	2·88	0·43	−0·45***	0·15	−0·47***	0·20
Serum sterols (mg/l)												
Cholestanol	2·13	0·73	0·17	0·35	0·16	0·42	2·56	0·67	−0·30*	0·42	−0·34*	0·74
Lathosterol	1·36	0·74	0·40	0·73	0·33	0·91	1·71	0·75	−0·18*	0·50	−0·31**	0·58
Campesterol	3·82	1·83	0·46	1·04	1·39	1·75	4·42	1·54	−1·30***	1·33	−1·30***	1·37
Sitosterol	3·21	1·15	0·25	1·05	0·47	0·85	3·82	0·78	0·24	1·00	0·47	0·79
Serum sterols (mg/100 g cholesterol)												
Cholestanol	123	44	3	16	8	30	132	43	12	28	3	47
Lathosterol	76	40	17	30	16	39	84	30	5	24	−3	29
Campesterol	221	105	16	57	73	88	226	85	−31*	78	−30**	89
Sitosterol	185	67	9	58	24	41	198	54	57	56	71*	60

Mean values were significantly different from those for the control group: *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.
† For details of experimental procedures, see p. 137.

HDL-cholesterol, -triacylglycerol and -phospholipid concentrations were determined by enzymic colorimetric methods using commercial kits (Boehringer Mannheim GmbH, Mannheim, Germany). LDL-cholesterol was calculated using the formula of Friedewald *et al.* (1972).

Sterols were analysed as steryl acetates by GC. To isolate sterols, serum (0·5 ml) was incubated with 2 M-KOH in methanol for 20 h at 30°C in a N_2 atmosphere. Epicoprostanol (5 μg) was used as an internal standard. Sterols were extracted with hexane and, after evaporation, dissolved in 1 ml of chloroform containing 3 mg dimethylaminopyridine. Acetic anhydride (0·5 ml) was added, and samples were incubated for 20 h at 40°C. The organic fraction was washed, dried and evaporated to dryness. Steryl acetates were dissolved in hexane for analysis by GC (Chrompack 438 S, Chrompack, Middelburg, The Netherlands) using a DB-FFAP capillary column (15 m, 0·32 mm i.d., 0·75 μm film thickness). The oven temperature was programmed to increase from 120 to 240°C at 6°C/min and then kept isothermal for 15 min. Baseline separation and linear calibration dependence over a range 6·25–100 ng of the injected amount was obtained. The CV for cholestanol, lathosterol, campesterol and sitosterol were 3·1, 3·2, 2·1 and 2·2 % respectively.

The Mann–Whitney U test was used for comparisons of groups. There were no differences between the results obtained after 2 or 3 months and these values have been combined.

Results

The nutrient contents of the diets used in the present study have been published previously (Rauma *et al.* 1993). The intakes of sitosterol and campesterol in the vegan diet group were calculated to be 732 (range 407–938) mg/d and 164 (range 88–207) mg/d respectively. These levels are much higher than those in a normal Finnish diet by (De Vries *et al.* 1997) (about 160 and 60 mg/d, respectively). Different seeds (sesame, sunflower), nuts (cashew, almond), legumes (beans, lentils) and cereals (buckwheat, wheat, oats) were the major plant sterol sources; vegetable oils were not used.

Serum total and LDL-cholesterol and -phospholipid concentrations decreased significantly ($P < 0·001$ in all cases) in the vegan diet group (Table 1). Serum HDL-cholesterol and -triacylglycerol concentrations also decreased slightly, but these changes were not significant. Serum cholestanol and lathosterol concentrations decreased in the vegan diet group but serum cholestanol:cholesterol and lathosterol:cholesterol did not change.

The concentration of campesterol decreased and that of sitosterol increased in proportion to cholesterol concentration in the vegan diet group. Sitosterol:campesterol was significantly greater in the vegan diet group than in the control group (1·48 (SD 0·39) *v.* 0·72 (SD 0·14); $P < 0·001$) at the end of the dietary period. There was a correlation between sitosterol and campesterol concentrations before (control group r 0·93, $P < 0·001$; vegan diet group r 0·60, $P = 0·014$) and at the end of the intervention (control group r 0·69, $P = 0·009$; vegan diet group r 0·58, $P = 0·018$), and their changes were correlated in the control group (r 0·87, $P < 0·001$), but not significantly in the vegan diet group (r 0·43, $P = 0·099$).

Discussion

Plasma cholesterol concentrations were reduced by the vegan diet, in accordance with the units of earlier studies in vegetarians and vegans (Ling *et al.* 1992; Piironen *et al.* 2000). This finding could be related to the effect of plant sterols on cholesterol absorption, to low cholesterol and high fibre intake and/or to increased unsaturation of dietary fat (Vuoristo & Miettinen, 1994). The effect of a vegetarian diet on serum phospholipids has been studied less

frequently. The unchanged cholesterol : phospholipids ratio with the vegan diet in the present study suggests that there was a decrease in lipoprotein, mostly LDL, without marked compositional changes.

Serum cholesterol precursor concentrations have been used as indicators of endogenous cholesterol synthesis (Vuoristo & Miettinen, 1994). Lathosterol : cholesterol did not change in the present study, suggesting that cholesterol synthesis was not affected. It has been shown, however, that vegetarians still have enhanced cholesterol synthesis without an increase in precursor levels (Vuoristo & Miettinen, 1994).

The absorption rate of sitosterol has been reported to be lower than that of campesterol (Heinemann *et al.* 1993). The esterification rate of sitosterol is also lower (Tavani *et al.* 1982), which in turn could increase its elimination into the bile. These differences may contribute to higher campesterol:sitosterol in serum compared with intake in omnivorous subjects (Tilvis & Miettinen, 1986). In the present study campesterol levels were also higher in the baseline samples. In addition, the concentrations of campesterol and sitosterol were correlated in both groups, and also their changes were correlated in the control group. However, in the vegan diet group the concentration of sitosterol increased and that of campesterol decreased. The 'living food' diet seems to increase dietary sitosterol : campesterol which may contribute to this finding. Adding sitosterol to a rapeseed-oil-based diet has been shown previously to have similar effect (Vanhanen & Miettinen, 1992). A vegan diet may also alter the relative absorption efficiency of campesterol and sitosterol by, for example, altering competition between sterols or the solubilisation properties of the gut. Sitosterol has been shown to be less affected by changes in the gut absorptive surface than campesterol (Pakarinen *et al.* 1998), and also seems to be absorbed with similar efficiency in all parts of the small intestine (Bhattacharyya, 1981). On the other hand, serum clearance and biliary secretion of plant sterols has been shown to be increased in vegetarians (Vuoristo & Miettinen, 1994), which may affect their serum concentrations and also their absorption in intestine.

In conclusion, a strict uncooked vegan diet decreased serum cholesterol, phospholipid, cholestanol and lathosterol concentrations without changing their ratios. The effect of a vegan diet on two major plant sterols, sitosterol and campesterol, was opposite. Whether this effect was caused by changes in absorption or clearance remains to be solved. It should be noted that the changes observed in these patients with rheumatoid arthritis may differ from those which may be seen in healthy subjects.

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