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Serum cholesterol and cholesterol and lipoprotein metabolism in hypercholesterolaemic NIDDM patients before and during sitostanol ester-margarine treatment

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Summary Cholesterol absorption and metabolism and LDL and HDL kinetics were investigated in 11 hypercholesterolaemic non-insulin-dependent diabetic men off and on a hypolipidaemic treatment with sitostanol ester, (3 g sitostanol daily) dissolved in rapeseed oil margarine, by a double-blind crossover study design. Serum total, VLDL and LDL cholesterol and apoprotein B fell significantly by 6 ± 2, 12 ± 6, 9 ± 3 and 6 ± 2 %, mean ± SEM, and HDL cholesterol was increased by 11 ± 4 % ($p < 0.05$) by sitostanol ester. LDL cholesterol and apoprotein B were significantly decreased in the dense (1.037–1.055 g/ml), but not light, LDL subfraction due to a significantly diminished transport rate for LDL apoprotein B, while the fractional catabolic rate was unchanged. HDL kinetics, measured with autologous apoprotein A I, was unaffected by sitostanol ester. Cholesterol absorption efficiency was markedly reduced from 25 ± 2 to 9 ± 2 % ($p < 0.001$) during sitostanol ester followed by proportionately decreased serum plant sterol proportions. Cholesterol precursor sterol proportions in serum, fecal neutral sterol excretion, and cholesterol synthesis, cholesterol transport, and biliary secretion were all significantly increased by sitostanol ester. We conclude that the sitostanol ester-induced decrease in cholesterol absorption compensatorily stimulated cholesterol synthesis, had no effect on fractional catabolic rate, but decreased transport rate for LDL apoprotein B so that serum total, VLDL and LDL cholesterol levels were decreased. Dietary rapeseed oil margarine rich in sitostanol ester was well tolerated, appears to be safe from the nutritional point of view and effective for lowering VLDL and LDL cholesterol and increasing HDL cholesterol in hypercholesterolaemic non-insulin-dependent diabetic subjects. [Diabetologia (1994) 37: 773–780]

Key words Cholesterol, cholesterol absorption, cholesterol synthesis, lipoprotein kinetics, sitostanol, plant sterols, precursor sterols, non-insulin-dependent diabetes.

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Abbreviations: Apo, apoprotein; NIDDM, non-insulin-dependent diabetes mellitus; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; FCR, fractional catabolic rate; TR, transport rate.

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NIDDM is associated with accelerated atherosclerosis, and the risk of coronary artery disease is three- to fourfold increased compared with the non-diabetic population [1–3]. Atherogenic risk factors are the same in NIDDM as in the general population [4], although the occurrence of hypercholesterolaemia is less consistent, and hypertriglyceridaemia and low HDL cholesterol levels are the frequently observed lipid abnormalities.

Plant sterols, particularly sitosterol, have been known since the 1950s to reduce serum cholesterol by inhibiting cholesterol absorption [5–13], but the large doses needed and the crystalline form has limited its wider use. Sitostanol is a 5α-saturated derivative of sitosterol. It lowers the serum cholesterol level more effectively than sitosterol [14], and is completely unab-

sorbable in humans [15–17]. In addition, sitostanol ester is fat soluble which facilitates its consumption e. g. when dissolved in rapeseed oil mayonnaise [16–17]. In normal subjects cholesterol absorption efficiency and cholesterol synthesis are inversely interrelated and regulate serum total and LDL cholesterol levels [18, 19]. In a recent study, small amounts of sitostanol ester reduced cholesterol absorption efficiency by 5 % and LDL cholesterol level by 7 %, and with larger doses of sitostanol the reduction of LDL cholesterol was more marked [17]. The question now arises whether serum total and LDL cholesterol could be diminished by sitostanol ester in hypercholesterolaemic patients with NIDDM and with similar mechanisms as in normal subjects. Very little is known about cholesterol absorption in NIDDM, and interrelations between cholesterol absorption and synthesis, LDL receptor activity and LDL cholesterol levels have not been studied in these patients either. Accordingly, the aim of the present study was to investigate the suitability and the mode of action of a margarine with a high sitostanol ester content for the hypolipidaemic treatment of hypercholesterolaemic NIDDM men by studying changes in serum lipids, cholesterol absorption, and cholesterol, LDL and HDL metabolism.

Subjects and methods

Subjects. The study group consisted of 11 NIDDM men with a mean age of 57.8 ± 1.9 (SEM) years. None of the patients were undergoing insulin or hypolipidaemic therapy. Three patients were on diet only, five were treated orally with glibenclamide and two with biguanides, and two were on a combination therapy of these two regimens. The glycaemic control was good to moderate during the metabolic studies with a mean blood glucose value of 8.9 ± 0.5 mmol/l and glycated haemoglobin of 7.6 ± 0.5 %. None of the patients had microalbuminuria, retino- or neuropathia or hepatic or gastrointestinal diseases. One patient had been treated with thyroxin for several years. His serum thyroid stimulating hormone (TSH) level was normal, 5.0 mU/l. Four patients used calcium channel blockers, three were treated with beta blocking agents, two with angiotensin converting enzyme inhibitors and two with isosorbide mononitrates. Two subjects were only being treated with glibenclamide. Neither their weight nor serum lipids, dietary nor metabolic parameters differed from those subjects taking other medications. In addition, serum cholesterol and the metabolic parameters did not differ between patients with or without beta blocking therapy. All the subjects volunteered for the study, which had been approved by the Ethical Committee of the University Hospital.

Study design. After a run-in period of 4 weeks with the subjects eating their normal diet, a control period of 6 weeks was started, during which five of the subjects consumed 30 g of rapeseed oil margarine daily followed by a 6-week treatment period, during which 30 g of margarine was dissolved with sitostanol ester (3 g sitostanol/day) into three 10-g buttons. One of the buttons was spread on a sandwich during each of the three daily meals. Six of the patients started first with the sitostanol ester margarine followed by the pure margarine period. The subjects continued unchanged with their regular diets except for a reduction in fat intake respective to the 30 g of daily rapeseed oil margarine, according to detailed instructions of our dietician. The study was completed as an outpatient study on a cross-over, double-blind basis. This was possible because the two types of margarines had similar taste and appearance, and the subjects could not distinguish between the different periods.

The rapeseed oil margarine contained plant sterols campesterol, sitosterol and avenasterol 209, 288 and 19 mg/100 g of margarine, respectively. Sitosterol was hydrated to sitostanol (Kaukas Inc., Lappeenranta, Finland) and transesterified with rapeseed oil fatty acids and dissolved in the margarine as 1 g/10 g buttons (Raisio Inc., Raisio, Finland).

At the end of the control and sitostanol ester treatment periods, metabolic and kinetic studies were performed. The subjects kept a food record for 7 days, from which the dietary constituents were calculated [20]. Also, they were given a capsule containing 4-^{14}C-cholesterol, 22,23^{3}H-beta-sitosterol, and 200 mg Cr_2O_3 three times a day with their regular meals during the 7-day period. Cholesterol absorption and fecal steroids were analysed from a 3-day stool collection. During the LDL and HDL turnover studies serum lipids, lipoproteins and apoproteins and serum non-cholesterol sterols were analysed four times from serum samples after a 12-h fast. The glycaemic control was monitored during each 6-week period by analysing fasting blood glucose and urine sediment four times and glycated haemoglobin and the presence of microalbuminuria twice.

Methods. Serum total and free cholesterol, triglycerides, phospholipids and apo A I, A II and B were analysed with commercial kits (Boehringer Diagnostica, Mannheim Germany; Wako Chemicals, Germany, and Orion Diagnostica, Espoo Finland). Serum lipoproteins were separated by ultracentrifugation into density classes as described previously [21]. HDL_2 and HDL_3 were separated by ultracentrifugation. In addition, LDL was fractionated into subfractions by density gradient ultracentrifugation [22]. The LDL subfractions of hydrated densities from 1.019 to 1.036 g/ml (light LDL) and from 1.037 to 1.055 g/ml (dense LDL) were pooled separately.

Serum cholesterol precursors squalene, Δ8-cholestenol, desmosterol and lathosterol, and serum plant sterols campesterol and sitosterol, and cholestanol, were quantitated by gas-liquid chromatography on a 35-m long SE-30 capillary column [22, 23]. The serum values are expressed in terms of 10^2 mmol/mol of cholesterol.

Cholesterol ester transfer protein activity was measured according to a method described by Groener et al. [24]. Vitamin E concentration was measured with HPLC [25], and sialic acids by a calorimetric reaction [26, 27]. Cholesterol absorption was measured by the peroral double-isotope continuous feeding method [28] and by analysing serum plant sterol levels. Chromic oxide was analysed from the 3-day fecal specimen [29] and fecal sterols with gas-liquid chromatography using the 50-m long SE-30 capillary column [23, 30, 31]. Cholesterol synthesis and its changes were measured by the sterol balance technique and the cholesterol precursor sterol proportions in serum.

For the kinetic studies, after the subjects had fasted 50 ml of EDTA plasma was drawn, and autologous HDL and autologous total and dense LDL were separated by serial preparative ultracentrifugations. Apo A I was isolated from HDL as described previously [32]. Dense LDL and HDL were iodinated with ^{125}I and total LDL with ^{131}I by a modification of the iodine-monochloride method [33, 34]. Three days before injection the subjects started to take peroral potassium iodide. Approximately 1 mg of the labelled total LDL and apo A I and 0.5–1 mg of dense LDL were mixed with 5 % human serum albumin, filtered, and injected simultaneously. The total amount of radioactivity varied from 40 to 50 μCi.

Table 1. Weight, body mass index and dietary parameters before and during sitostanol ester treatment in eleven NIDDM men

Parameters	Control	Sitostanol ester
Weight (kg)	81.2 ± 3.1	80.8 ± 3.0
Body mass index (kg/m^2)	26.5 ± 0.7	26.4 ± 0.7
Dietary cholesterol (mg/day)	324.4 ± 39.5	356.8 ± 43.2
Dietary fat (g/day)	92.1 ± 7.3	98.6 ± 7.7
Calorie intake (kcal · kg^{-1} · day^{-1})	26.8 ± 1.3	28.8 ± 2.1
Dietary campesterol[b] (mg/day)	114 ± 8	129 ± 8
Dietary sitostanol[b] (mg/day)	29 ± 4	3091 ± 172[a]
Blood glucose (mmol/l)	8.8 ± 1.3	9.0 ± 1.1
Glycated haemoglobin (%)	7.5 ± 0.7	7.6 ± 0.7
Serum vitamin E (μmol/l)	50.9 ± 4.3	45.9 ± 4.1

Data given as mean ± SEM.
[a] $p < 0.001$; [b] Measured from fecal sterols

After the injection, blood samples of 10 ml were collected and the radioactivity counted for 14 days. The die-away curves were constructed in whole plasma for ^{131}I total LDL and after ultracentrifugation for ^{125}I dense LDL and ^{125}I HDL. FCR for total and dense LDL and apo A I HDL were determined using a two-pool model [35]. TR was calculated by multiplying FCR by the pool size, which was calculated to be 4.5 % of body weight. LDL density gradient spin was performed four times from the post-injection samples. Cholesterol, apo B and sialic acid contents were measured from the fractions, and TR for dense LDL was calculated similarly to total LDL. The FCR and TR for light LDL were calculated from the total and dense kinetic data.

Calculations. Cholesterol synthesis was calculated as the difference between the fecal steroids (neutral and acidic) of cholesterol origin and dietary cholesterol. Total intestinal cholesterol flux was calculated by dividing fecal neutral sterols by (1-fractional cholesterol absorption). Cholesterol transport or turnover was the sum of cholesterol synthesis and absorbed dietary cholesterol (cholesterol absorption efficiency multiplied by dietary cholesterol). Biliary cholesterol secretion was calculated as total intestinal flux minus dietary cholesterol.

Statistical analysis

Statistical significances were tested with two-tailed Student's *t*-test, paired *t*-test and analysis of variance with repeated measurements. Correlations were calculated as Pearson's product-moment correlation coefficients. A *p*-value of less than 0.05 was considered statistically significant.

Results

All subjects completed the studies, and the sitostanol ester dissolved in rapeseed oil margarine was well tolerated. Weight, body mass index, glycaemic control and serum vitamin E levels (even without correction for changes in lipoprotein levels) and daily cholesterol, fat and calorie intakes were similar during the control and sitostanol ester treatment periods (Table 1). The compliance was good as indicated by the increase of fecal sitostanol output from 29 mg/day to 3091 mg/day.

Lipoprotein concentrations. Serum total, VLDL and LDL cholesterol were significantly lowered by 6 ± 2, 12 ± 6 and 9 ± 3 %, respectively, and HDL cholesterol was 11 ± 4 % higher during sitostanol ester treatment (Tables 2 and 3). Serum triglyceride and phospholipid levels were unchanged. In serum and VLDL and IDL the amount of esterified cholesterol was reduced while free cholesterol, triglycerides and phospholipids did not show a constant change. However, in the LDL particle both esterified and free cholesterol as well as phospholipids and apo B were significantly lowered. The cholesterol/apo B ratio was significantly reduced in LDL, but LDL size was practically unchanged. Subfractionation of LDL showed that no significant changes were occurring in the light fraction, while cholesterol and apo B were significantly reduced in the dense fraction, and a significant elevation of apo B and a reduction in the cholesterol/apo B ratio was found in the very dense (1.056–1.063 g/ml) fraction.

The sialic acid content in LDL was significantly reduced (28 ± 6 %) by sitostanol ester, but the respective sialic acid/apo B ratio only tended to decrease from 63.6 ± 6.3 to 48.0 ± 4.7 μg/mg ($p = 0.06$). The decrease was exclusively found in the dense LDL subfraction.

In HDL, free, and to a lesser extent esterified cholesterol, and apo A I were significantly elevated by 15 ± 5 and 4 ± 2 %, respectively (Table 2), while the increment of phospholipids and apo A II were less consistent. Cholesterol ester transfer protein activity was practically unchanged (1.043 ± 0.106 vs 1.070 ± 0.106, arbitrary units).

Lipoprotein kinetics. The significant reduction of LDL cholesterol and apo B during sitostanol treatment resulted from a diminished TR for LDL apo B (Table 3). The catabolism of LDL apo B, measured by FCR, was unchanged. The kinetic data of the light LDL fraction, calculated from the differences between the total and dense LDL apo B kinetics, were practically unchanged. Sitostanol ester significantly reduced the contents of cholesterol and apo B in the dense LDL fraction because of a diminished TR for LDL apo B ($p = 0.05$).

Despite the significant elevation in HDL cholesterol and apo A I, FCR and TR for apo A I were unchanged by sitostanol ester (Table 4).

Intestinal transport and synthesis of cholesterol. Cholesterol absorption efficiency was markedly reduced during sitostanol ester treatment from 25 ± 2 to 9 ± 2 % (Table 5). Accordingly, the absolute amount of cholesterol absorbed was less than one-half the baseline value. Cholesterol synthesis and transport and its excretion as neutral sterols and intestinal cholesterol flux were all significantly increased. In addition, biliary secretion of cholesterol was increased. Bile acid synthesis was unaltered by sitostanol ester.

Table 2. Serum and lipoprotein lipids before and during sitostanol ester treatment in eleven NIDDM men

Variables	Control	Sitostanol ester	Change, %
Total serum cholesterol (mmol/l)	5.98 ± 0.22	5.62 ± 0.21[a]	−5.8 ± 2.3[a]
esterified (mmol/l)	4.36 ± 0.16	4.08 ± 0.15[a]	−6.1 ± 2.4[a]
free (mmol/l)	1.62 ± 0.07	1.54 ± 0.07	−4.5 ± 2.4
Serum triglycerides (mmol/l)	2.14 ± 0.17	2.08 ± 0.21	−1.3 ± 17.8
Serum phospholipids (mmol/l)	3.15 ± 0.11	3.04 ± 0.10	−3.2 ± 2.1
Total VLDL cholesterol (mmol/l)	0.72 ± 0.07	0.63 ± 0.08[a]	−12.5 ± 5.6[a]
esterified	0.38 ± 0.04	0.32 ± 0.04[a]	−13.1 ± 7.9
free (mmol/l)	0.34 ± 0.04	0.31 ± 0.04	−6.6 ± 7.9
triglycerides (mmol/l)	1.56 ± 0.15	1.46 ± 0.19	−4.0 ± 10.8
phospholipids (mmol/l)	0.54 ± 0.05	0.48 ± 0.06	−9.4 ± 8.8
IDL cholesterol, total (mmol/l)	0.30 ± 0.03	0.28 ± 0.02	−5.2 ± 3.7
esterified (mmol/l)	0.20 ± 0.02	0.18 ± 0.01[a]	−10.7 ± 3.6[a]
free (mmol/l)	0.10 ± 0.01	0.10 ± 0.01	+5.8 ± 4.9
triglycerides (mmol/l)	0.12 ± 0.01	0.13 ± 0.01	+17.2 ± 5.9[a]
phospholipids (mmol/l)	0.14 ± 0.02	0.12 ± 0.01	−5.9 ± 6.0
LDL cholesterol, total (mmol/l)	3.83 ± 0.16	3.46 ± 0.16[a]	−9.3 ± 2.8[a]
esterified (mmol/l)	2.87 ± 0.12	2.58 ± 0.12[a]	−9.5 ± 2.9[a]
free (mmol/l)	0.96 ± 0.04	0.88 ± 0.04[a]	−8.3 ± 3.0[a]
triglycerides (mmol/l)	0.29 ± 0.02	0.30 ± 0.02	+1.1 ± 3.9
phospholipids (mmol/l)	1.27 ± 0.06	1.16 ± 0.05[a]	−8.8 ± 1.9[a]
LDL size (Å)	243.9 ± 2.2	241.9 ± 2.5	−0.3 ± 0.7
HDL cholesterol, total (mmol/l)	1.13 ± 0.03	1.24 ± 0.05[a]	+10.7 ± 4.4[a]
esterified (mmol/l)	0.91 ± 0.03	1.00 ± 0.05	+9.6 ± 4.4
free (mmol/l)	0.21 ± 0.01	0.24 ± 0.01[a]	+15.2 ± 5.1[a]
triglycerides (mmol/l)	0.17 ± 0.01	0.18 ± 0.01	+12.3 ± 5.8
phospholipids (mmol/l)	1.20 ± 0.04	1.28 ± 0.05[a]	+7.8 ± 3.8
apo A I (mg/100 ml)	123.5 ± 3.7	128.8 ± 3.7[a]	+4.5 ± 1.9[a]
apo A II (mg/100 ml)	28.8 ± 0.7	30.1 ± 0.8	+4.9 ± 2.3

Data are given as mean ± SEM.
[a] $p < 0.05$ or less

Table 3. LDL kinetics before and during sitostanol ester treatment in eleven NIDDM men

LDL	Cholesterol (mmol/l)	Apoprotein B (mg/100 ml)	Cholesterol/ apoprotein B	Sialic acids (μg/100 ml)	FCR (pools/day)	TR ($mg \cdot kg^{-1} \cdot day^{-1}$)
LDL, total (1.019–1.063 g/ml)						
Control	3.8 ± 0.2	65.2 ± 2.5	2.13 ± 0.04	41.1 ± 4.1	0.339 ± 0.019	10.1 ± 0.5
Sitostanol ester	3.3 ± 0.2[a]	60.5 ± 2.8[a]	2.06 ± 0.02[a]	28.5 ± 2.6[a]	0.314 ± 0.010	8.5 ± 0.4[a]
LDL, light (1.019–1.036 g/ml)						
Control	1.2 ± 0.1	20.2 ± 2.6	2.24 ± 0.08	12.7 ± 1.4	0.209 ± 0.039	2.0 ± 0.5
Sitostanol ester	1.0 ± 0.2	20.4 ± 3.1	1.92 ± 0.19	10.8 ± 1.3	0.159 ± 0.035	2.3 ± 0.4
LDL, dense (1.037–1.055 g/ml)						
Control	2.5 ± 0.1	42.8 ± 2.1	2.26 ± 0.04	23.7 ± 2.6	0.420 ± 0.032	8.4 ± 0.8
Sitostanol ester	2.1 ± 0.1[a]	36.3 ± 2.2[a]	2.30 ± 0.08	14.0 ± 1.5[a]	0.387 ± 0.027	6.5 ± 0.8[b]
LDL, very dense (1.056–1.063 g/ml)						
Control	0.1 ± 0.01	2.1 ± 0.3	2.81 ± 0.18	4.7 ± 0.7	–	–
Sitostanol ester	0.2 ± 0.05	3.8 ± 0.8[a]	2.27 ± 0.07[a]	3.7 ± 0.5	–	–

Mean ± SEM.
[a] $p < 0.05$ or less; [b] $p = 0.05$

Table 4. HDL apo A I kinetics before and during sitostanol ester treatment in eleven NIDDM men

Treatment	HDL cholesterol (mmol/l)	Apo A I (mg/dl)	FCR (pools/day)	TR ($mg \cdot kg^{-1} \cdot day^{-1}$)
Control	1.13 ± 0.03	123.5 ± 3.7	0.230 ± 0.013	12.9 ± 0.8
Sitostanol ester	1.24 ± 0.05[a]	128.8 ± 3.7[a]	0.226 ± 0.010	13.0 ± 0.7

Mean ± SEM.
[a] $p < 0.05$

Table 5. Cholesterol absorption and metabolism before and during sitostanol ester treatment in eleven NIDDM men

Variables	Control	Sitostanol ester
Cholesterol absorption (%)	25.3 ± 2.1	8.6 ± 1.8[a]
Dietary cholesterol absorbed ($mg \cdot kg^{-1} \cdot day^{-1}$)	0.96 ± 0.12	0.42 ± 0.12[a]
Total cholesterol absorbed ($mg \cdot kg^{-1} \cdot day^{-1}$)	4.5 ± 0.4	1.8 ± 0.4[a]
Fecal steroids of cholesterol origin ($mg \cdot kg^{-1} \cdot day^{-1}$)		
Bile acids	8.6 ± 1.1	8.4 ± 1.2
Neutral sterols	13.5 ± 1.1	18.7 ± 0.8[a]
Total sterols	22.1 ± 1.9	27.1 ± 1.7[a]
Fecal plant sterols ($mg \cdot kg^{-1} \cdot day^{-1}$)		
Campesterol	1.4 ± 0.1	1.6 ± 0.1
Sitosterol	3.9 ± 0.4	5.0 ± 0.3[a]
Sitostanol	0.4 ± 0.1	38.3 ± 2.1[a]
Fecal fat ($mg \cdot kg^{-1} \cdot day^{-1}$)	68.0 ± 10.0	70.0 ± 8.0
Cholesterol synthesis ($mg \cdot kg^{-1} \cdot day^{-1}$)	18.2 ± 1.6	22.7 ± 1.3[a]
Intestinal cholesterol flux ($mg \cdot kg^{-1} \cdot day^{-1}$)	18.0 ± 1.1	20.6 ± 1.0[a]
Biliary cholesterol secretion ($mg \cdot kg^{-1} \cdot day^{-1}$)	14.0 ± 0.8	16.2 ± 0.6[a]
Cholesterol transport ($mg \cdot kg^{-1} \cdot day^{-1}$)	19.1 ± 1.6	23.1 ± 1.4[a]

Data are given as mean ± SEM. [a] $p < 0.05$ or less

Table 6. Serum squalene and non-cholesterol sterol proportions[a] before and during sitostanol ester treatment in eleven NIDDM men

Variables	Control	Sitostanol ester
Cholesterol, mg/100 ml	222.1 ± 8.1	206.6 ± 8.0[b]
Squalene[a]	31.4 ± 3.7	32.5 ± 2.9
Δ 8-cholestenol[a]	32.4 ± 2.3	35.8 ± 3.0[b]
Desmosterol[a]	109.3 ± 6.0	121.3 ± 5.7[b]
Lathosterol[a]	185.3 ± 12.5	212.3 ± 19.1[b]
Campesterol[a]	240.7 ± 27.3	134.9 ± 15.0[b]
Sitosterol[a]	110.3 ± 12.2	62.9 ± 6.5[b]
Cholestanol[a]	94.4 ± 6.6	84.0 ± 6.0[b]

Data are given as mean ± SEM.
[a] $10^2 \times$ mmol/mol of cholesterol; [b] $p < 0.05$ or less

The unchanged fecal campesterol output suggested that the amount of dietary plant sterols were unchanged during the intervention, while the small increment in fecal sitosterol was due to its presence in the sitostanol preparation.

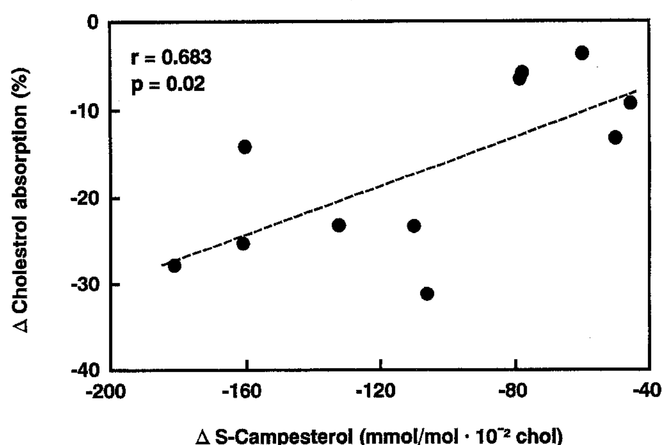

Fig. 1. The relationship between sitostanol ester margarine-induced changes in cholesterol absorption efficiency and serum campesterol proportion in eleven men. $y = -1.83 + 0.14x$

Non-cholesterol sterols. During sitostanol ester treatment, serum cholesterol precursor proportions, except squalene, were significantly elevated and serum plant sterol proportions were reduced to one-half (Table 6). In addition, serum cholestanol proportion was significantly lowered.

Correlations. During the control period, LDL cholesterol concentration was significantly negatively correlated with FCR for LDL apo B, and cholesterol and bile acid synthesis ($r = -0.732$, -0.633 and -0.681, $p < 0.05$). Cholesterol synthesis was related to the proportion of serum squalene ($r = 0.715$, $p < 0.05$), LDL cholesterol level ($r = -0.633$, $p = 0.05$) and cholesterol absorption efficiency ($r = -0.601$). In addition, the serum lathosterol proportion was significantly related to FCR for LDL apo B ($r = 0.667$, $p < 0.05$). Cholesterol absorption efficiency was significantly related to the proportions of serum campesterol and sitosterol ($r = 0.717$ and 0.661, $p < 0.05$).

During sitostanol ester treatment, LDL cholesterol was significantly related to TR for LDL apo B but not to other metabolic parameters. The reduction in cholesterol absorption efficiency was correlated with that of the proportion of serum campesterol ($r = +0.683$, $p < 0.05$, Fig. 1) and HDL cholesterol level ($r = +0.638$, $p = 0.05$).

Discussion

The present results showed that sitostanol ester-containing rapeseed oil margarine was an effective and well-tolerated hypocholesterolaemic nutrient in hypercholesterolaemic NIDDM. Cholesterol absorption was reduced to one-third, cholesterol synthesis was enhanced and TR for LDL apo B was reduced so that the serum levels of total, VLDL and LDL cholesterol and LDL apo B were reduced, and those of HDL cholester-

ol and apo A I were increased by sitostanol ester. The reduced serum levels during sitostanol ester treatment occurred mainly in the dense (1.037–1.055 g/ml), virtually not at all in light (1.019–1.036 g/ml) LDL fraction, and was caused by reduced TR for LDL apo B.

The control cholesterol absorption efficiency was lower and cholesterol synthesis, measured by the balance technique or the cholesterol precursor proportions, was markedly higher in the NIDDM men than in a group of 50-year-old men [19, 36]. Cholesterol absorption in NIDDM has been shown to be low in a hypertriglyceridaemic group but not in other diabetic subgroups [37]. The low cholesterol absorption efficiency in the present series could result from diabetes itself or be contributed to by rapeseed oil fat or a relatively large plant sterol intake. Thus, the serum plant sterol proportions, instead of being low as suggested by low cholesterol absorption efficiency, were above the level of the 50-year-old men [36]. Dietary plant sterols can be absorbed [16] and inhibit cholesterol absorption. However, low plant sterol proportions in NIDDM subjects were suggested to reflect a lowered cholesterol absorption efficiency, although unchanged lathosterol proportion did not indicate compensatorily increased cholesterol synthesis [38]. In fact, the latter and high bile acid synthesis have been observed in some NIDDM subgroups [37, 39, 40], these findings being in agreement with the present results. In vitro studies have shown that an altered LDL composition and hyperglycaemia in the presence of hyperinsulinaemia [41, 42] stimulate cholesterol synthesis in mononuclear cells. According to the present sterol balance and non-cholesterol sterol values low cholesterol absorption efficiency activates cholesterol synthesis and turnover even during good glycaemic control in hypercholesterolaemic patients with NIDDM.

In the present series the decrease in serum total and LDL cholesterol by up to 16 % is less than the respective 15–33 % reduction in children with familial hypercholesterolaemia consuming a daily sitostanol dose of 1.5 g [14]. In contrast to non-diabetic subjects [14, 15, 17], in the present diabetic population also VLDL and IDL cholesterol were significantly decreased and HDL cholesterol and apo A I increased by sitostanol. These changes decreased core cholesterol from VLDL and IDL and increased surface lipids in HDL. The percent of esterified cholesterol and cholesterol ester transfer protein activity, in general low in NIDDM [43], were unaffected by sitostanol treatment. Despite the slight but significant increase in the apo A I concentration its kinetic parameters were inconsistently changed during the sitostanol ester treatment. The accumulation of free cholesterol in HDL suggests that the reverse cholesterol transport may be accentuated during sitostanol ester treatment.

Enhanced cholesterol synthesis increases biliary cholesterol and plant sterol secretion [44]. Thus, the decrease in serum plant sterols was apparently caused by both improved biliary output and reduced absorption. The decrease of the serum plant sterol proportions was related to that of cholesterol absorption efficiency, indicating that the quantitation of serum plant sterols offers a potential, less laborious means with which to study changes in cholesterol absorption without isotopes and fecal collections.

Hypolipidaemic therapy with cholesterol malabsorption by ketoconazole [22], neomycin [45] or sitostanol ester in the present study decreases TR, less so fractional catabolism for LDL apo B. The main reduction of LDL cholesterol, apo B and TR occurred in the dense LDL fraction with no compositional change, while the very dense fraction became apo B enriched. These results are in concert with simvastatin-induced changes in primary hypercholesterolaemia [46]. It could be hypothesized that sitostanol ester decreased cholesterol absorption efficiency so that less intestinal chylomicron cholesterol entered the liver, resulting in upregulation of hepatic cholesterol synthesis and probably LDL receptor activity. The possibly upregulated LDL receptor activity could not be seen by increased FCR for any LDL subfraction, but the decreased VLDL and IDL cholesterol levels suggest that removal of these cholesterol-rich particles was increased by upregulated receptor activity lowering LDL removal and decreasing their conversion to LDL. The decrease of LDL apo B transport could, according to a previous report [47], indicate enhanced receptor activity. The finding agrees with an earlier study in which simvastatin increased the direct catabolism of VLDL particles and FCR of IDL, not of LDL, so that the synthesis of LDL was reduced [46].

Desialylated LDL has been considered to be atherogenic [48]. Thus, in patients with coronary artery disease [49, 50] and in both types of diabetes [51] the sialic acid content is decreased in LDL. Although desialylated and glycated LDL are effectively taken up by human aortic intimal cells [51], in the NIDDM subjects studied here the LDL sialic acid/protein ratio was not correlated with removal of total or dense apo B (data not shown).

The present kinetic results for total LDL apo B were similar to those found in non-diabetic subjects [22]. The previous kinetic studies for total LDL have shown varying FCR and TR values in NIDDM [52–54], while those for dense LDL particles have not been studied previously in NIDDM. In most but not all non-diabetic hyperlipidaemic subjects the dense LDL fraction was cleared slower than the light fraction [55–57], while in almost all of our diabetic patients the dense LDL fraction was cleared faster than the light fraction. In contrast to earlier findings in familial hypercholesterolaemia [55], none of the radioactivity in the dense fraction was transferred to the light fraction in our diabetic subjects. Thus, the kinetics of total LDL is normal in hypercholesterolaemic NIDDM under good glycaemic control.

In conclusion sitostanol ester margarine treatment reduced serum total, VLDL, and LDL cholesterol levels by inhibiting cholesterol absorption, increasing cholesterol synthesis and reducing TR of the dense LDL fraction. A simple and well-tolerated dietary use of the sitostanol ester margarine preparation in doses of 30 g with 3 g of sitostanol per day offers a significant cholesterol-lowering measure in NIDDM subjects with mild to moderate hypercholesterolaemia.

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