SERUM SQUALENE AND NON-CHOLESTEROL STEROLS RELATED TO CHOLESTEROL SYNTHESIS AND ABSORPTION IN TYPE 2 DIABETES

Piia Simonen[1], Helena Gylling[2], Tatu A Miettinen[1]

Department of Medicine[1], Division of Internal Medicine, University of Helsinki, Helsinki, Finland, and Department of Clinical Nutrition[2], University of Kuopio, and Kuopio University Hospital, Kuopio, Finland

Summary

Serum non-cholesterol sterols, cholestanol and plant sterols, campesterol and sitosterol, are known to positively reflect cholesterol absorption and negatively cholesterol synthesis. Opposite associations are obtained for cholesterol precursors, including squalene, cholestenol and lathosterol. We compared non-cholesterols and squalene in serum with those in lipoproteins, and related the values to absolute cholesterol synthesis and absorption percent of patients (n=33) with type II diabetes, range of body mass index (BMI) being 21-40.- Lipids in serum and ultracentrifuge fractions were measured with routine laboratory methods. Gas liquid chromatography was used to measure cholesterol, squalene and non-cholesterol sterols in serum and lipoproteins. Sterol balance technique was used to determine absolute cholesterol synthesis and a double label system for measurement of absorption percentage of dietary cholesterol -Two-thirds of the non-cholesterol sterols were carried in LDL and one-fifth in HDL, whereas squalene was mainly in VLDL and LDL. The synthesis and absorption markers were interrelated in serum and all lipoproteins suggesting intact regulation of cholesterol metabolism. The absorption and synthesis marker ratios to cholesterol were mostly similar in serum and lipoproteins, even though absorption sterols accumulated to HDL and IDL and synthesis markers in VLDL and IDL. The ratios to cholesterol of absorption markers were negatively, those of synthesis markers positively related to BMI in serum and most lipoprotein fractions, and also to respective absolute synthesis and absorption percentage of cholesterol. Also, the proportions of synthesis marker sterols to those of absorption markers (eg., lathosterol/sitosterol) were positively related to BMI and absolute synthesis and negatively to absorption percentage of cholesterol.- The findings indicate that even in patients with type II diabetes, including markedly increased body weight and altered cholesterol metabolism, measurement of serum non-cholesterol sterols and squalene reveals information of cholesterol synthesis and absorption without complicated clinical and laboratory methods.

Key words: Squalene and non-cholesterol sterols, lipoproteins, cholesterol synthesis, cholesterol absorption.

Introduction

In addition to cholesterol, serum contains small amounts of squalene and non-cholesterol sterols. From among the later ones, cholestenol, desmosterol and lathosterol reflect cholesterol synthesis**,** and plant sterols campesterol, sitosterol and avenasterol, and cholestanol**,** a metabolite of cholesterol, are markers of cholesterol absorption efficiency, especially as their ratios to serum cholesterol (1-4). Two thirds of the non-cholesterol sterols are transported by LDL, while squalene is carried effectively also by VLDL (5,6). The ratios to cholesterol vary less, even though the synthesis markers, especially squalene, tend to accumulate in VLDL, while those of absorption markers are slightly enriched in HDL as compared with the respective ratios in serum or LDL. The serum ratios of non-cholesterol sterols to cholesterol has been related to body weight (BMI), absorption percentage of dietary cholesterol and absolute synthesis of cholesterol measured by sterol balance technique in patients with type 2 diabetes and in non-diabetes subjects (2-4,7). However, since the ratios to cholesterol or the proportions of synthesis/absorption sterols in different lipoproteins have not been analyzed in details, we performed the studies on the two absolute measures of cholesterol metabolism in patients with type 2 diabetes exhibiting variable degree of obesity and altered cholesterol metabolism. In addition, associations of the proportions of absorption markers to those of synthesis markers (eg., lathostero/sitosterol) in serum and lipoproteins seemed worth of determining with BMI, and absolute absorption and synthesis of cholesterol.

Study population

The study population included 33 patients with type 2 diabetes, 23 males 10 females. Body mass index (BMI) ranged from 21 to 40, age 59 years, blood glucose 8.5 mmol/l and HbA1c 7.5 %. The patients were collected from the Outpatient Clinic, Department of Medicine, University of Helsinki. Hypoglycemic medication included diet in 16 of them, sulphonylureas in 6 biguanides in two and combinatioin in 9. Eight patients used beta blockers, 8 calcium channel blockers,8 ACE inhibitorts and 4 diereticsInclusion required fasting blood glucose >7 mmol/l, duration <5 years, lack of abnormal thyroid, renal or liver function, diabetic nephropathy or gastrointestinal complications of diabetes, unstable angina pectoris, myocardial infarction or invasive coronary treatment within one year, insulin therapy, or the use of lipid lowering drugs, including functional foods enriched with plant stanol or sterol esters. All women were postmenopausal without hormone replacement therapy.

Methods

Fasting serum cholesterol and triglycerides were measured with commercial kits (CHOD-PAP and GPO-PAP, Boehringer Diagnostica, Mannheim, Germany) and presented means of two measurements.. Blood glucose and HbA_1c were assayed by our routine hospital laboratory methods. VLDL, intermediate density lipoproteins (IDL), LDL and HDL were separated with ultracentrifugation into density classes from one overnight fasting serum sample [8]. Cholesterol, non-cholesterol sterols and squalene were determined from serum and from different lipoproteins by gas-liquid chromatography (GLC) from non-saponifiable serum and lipoprotein material on a 50 m long SE-30 capillary column [18](Ultra 1, Agilent Technologies, Wilmington, DE). This procedure measures squalene, total cholesterol, cholestanol, cholestenol, desmosterol, lathosterol, campesterol and sitosterol in the same run. Cholestenol, desmosterol and lathosterol were called the synthesis marker sterols and cholestanol, campesterol and sitosterol the absorption markers. The non-cholesterol sterol and squalene values were given as µg/dl, or as to eliminate the effect of the different cholesterol levels, they were standardized and expressed in terms of 10^2 x mmol/mol of cholesterol, i.e., ratios to serum and respective lipoprotein cholesterol values, and called ratios in the following. Using methods presented earlier (9), absolute cholesterol synthesis (mg/day) was measured with sterol balance technique and absorption percentage of dietary cholesterol. Proportions of synthesis/absorption markers were also calculated to show their possible relation to the two absolute measures of cholesterol metabolism or to their proportions. The study protocol was accepted by the Ethics Committee of the Department of Medicine, University of Helsinki.

Statistics: The statistical analyses of data were performed by using Microsoft Excel version 6 and with Biomedical Data Program (BMDP) (Berkeley, CA). The values are given as mean±SEM. The hypothesis testing between the groups was performed with one-way ANOVA, ANCOVA and consequently with Student’s two-sided t-test. The differences between the lipoprotein sterols within each group were tested with paired t-test. Pearson's and Spearman’s correlation coefficients were calculated. Most of the variables were normally distributed, and logarithmic transformations were used if skewed distributions were observed. P- value < 0.05 value was considered statistically significant.

Results

Serum total (5.23 mmol/l), HDL (1.20mmol/l), and VLDL (0.58 mmol/l) cholesterol were within normal upper levels, while those of total (1.94 mmol/l) and VLDL (1.36 mmol/l) triglycerides were slightly elevated.

About 35 % of squalene was transported by VLDL and LDL, respectively, the values, especially of squalene, being higher in obese than lean subjects. Transport of non-cholesterol sterols followed roughly that of cholesterol.

The serum ratios of squalene and non-cholesterol sterols of the patients were within the control (10) levels, but differed slightly from those in serum and lipoproteins. Thus, the lowest ratios of absorption markers were in VLDL and LDL and the highest ones in IDL and HDL, while the ratios of squalene and synthesis markers were highest in VLDL and IDL and higher than in serum. Accordingly, the proportions of synthesis markers/absorption markers were highest in VLDL (eg.,lathosterol/cholestanol =4.7) decreasing gradually with increasing density of lipoprotein (2.2 in HDL).

The ratios of synthesis and absorption markers were negatively related to each other in serum and in most lipoproteins. For instance, the correlation coefficient of cholestanol ratio to those of cholestenol desmosterol and lathosterol ranged in serum from -0.572 to -0.647, in VLDL from -0.197to -0.544, in IDL from -0.288 to -0.509 in LDL from -0.563 to -0.635 and in HDL from -0.408 to -0.665. Squalene ratio was not related to absorption sterols but was related to other synthesis markers in VLDL, IDL and LDL, absorption percent. Absorption percentage of cholesterol was positively related to ratios of absorption sterols in serum and in all lipoproteins (r-range 0.365to 0.622) but only with cholestenol in serum, VLDL and HDL. Absolute cholesterol synthesis, as shnown in Table1, was positively related to the synthesis markers in serum (including squalene) (r-range 0.477 to 0.613) and lipoproteins (r-range 0.414 to 0.620) and negatively to absorption sterols (r-range in serum and lipoproteins -0.372 to -0.457). BMI was not related to squalene in any lipoprotein, weakly to relative (lathosterol in all lipoproteins) or absolute cholesterol synthesis and negatively with absorption sterols (r=0.428 for VLDL cholestanol). The proportions of synthesis/absorption sterols were positively related to absolute synthesis (mg/day) and negatively to absorption percentage (%) of dietary cholesterol both in serum and lipoproteins. For example, the respective correlation coefficients were 0.576 and -0.542 for lathosterol/sitosterol in serum and 0.590 and -0.572 in LDL. Accordingly, the latter proportions were positively correlated with absolute synthesis/absorption % in serum (r=0.676) and LDL(r= 0.731). Further calculations showed eg., that the respective ratios of lathosterol/cholesterol and sitosterol/cholesterol correlated positively (r=0.565 and r=0.624) and negatively (r=-0.480 and r=-0.487) to the proportion of cholesterol synthesis/absorption percent (mg/day/%) both in serum and LDL (Table 2).

Table 1. Correlation coefficients in patients with type 2 diabetes (n=33) of serum, VLDL and LDL squalene and non-cholesterol sterol ratios to cholesterol (mmol x 10^2/mol of cholesterol) with synthesis (mg/day), absorption percent (%), synthesis/absorption % and BMI (kg/m^2). r-values <-0.340 or >0.340 are statistically significant, p<0.05.

	Compound	Synthesis	Absorption	Synth./Abs.%	BMI
Serum	Squalene	0.596	-0.294	0.529	0.078
	Cholestenol	0.700	-0.466	0.714	0.377
	Desmostero	0.476	-0.382	0.461	0.348
	Lathosterol	0.595	-0.381	0.565	0.324
	Cholestanol	-0.659	0.571	-0.683	-0.319
	Campestero	-0.232	0.572	-0.404	-0.340
	Sitosterol	-0.364	0.590	-0.480	-0.463
	Avenasterol	0.084	0.331	-0.124	-0.276
VLDL	Squalene	0.425	-0.159	0.271	-0.089
	Cholestenol	0.417	-0.403	0.423	0.017
	Desmostero	0.369	-0.244	0.309	0.289
	Lathosterol	0.504	-0.373	0.446	0.257
	Cholestanol	-0.638	0.458	-0.599	-0.428
	Campestero	-0.276	0.612	-0.431	-0.351
	Sitosterol	-0.455	0.479	-0.483	-0.602
	Avenasterol	0.263	0.043	0.078	-0.216
IDL	Squalene	0.145	-0.127	0.156	-0.088
	Cholestenol	0.502	-0.471	0.502	0.323
	Desmostero	0.403	-0.362	0.395	0.217
	Lathosterol	0.472	-0.306	0.401	0.252
	Cholestanol	-0.584	0.609	-0.649	-0.176
	Campestero	-0.120	0.484	-0.285	-0.261
	Sitosterol	0.001	0.255	-0.133	-0.190
	Avenasterol	0.173	0.030	0.090	-0.194
LDL	Squalene	0.441	-0.446	0.515	-0.048
	Cholestenol	0.698	-0.510	0.779	0.358
	Desmostero	0.471	-0.416	0.486	0.335
	Lathosterol	0.618	-0.423	0.624	0.316
	Cholestanol	0.656	0.553	-0.676	-0.275
	Campestero	-0.237	0.571	-0.418	-0.344
	Sitosterol	-0.356	0.589	-0.487	-0.456
	Avenasterol	0.007	0.348	-0.187	-0.317
HDL	Squalene	0.216	-0.332	0.408	-0.010
	Cholestenol	0.638	-0.312	0.523	0.403
	Desmostero	0.556	-0.416	0.514	0.365
	Lathosterol	0.561	-0.304	0.475	0.309
	Cholestanol	-0.599	0.599	-0.664	-0.272
	Campestero	-0.201	0.556	-0.357	-0.306
	Sitosterol	-0.317	0.557	-0.407	-0.404
	Avenasterol	-0.002	0.345	-0.171	-0.338

Table 2. Correlation coefficients between different synthesis and absorption variables of cholesterol metabolism in serum, VLDL and LDL of patients with type 2 diabetes (n=33). r-values <-0.340 or>0.340 are significant; p<0.05.

VARIABLE		Synthesis mg/day	Absorption %	Synth./Abs.%	BMI kg/m^2
Absorption %		-0.450			
Synth./Abs.%		0.860	-0.721		
BMI		0.489	-0.138	0.361	
Lathosterol/ Sitosterol	Serum	0.576	-0.542	0.676	0.436
	VLDL	0.546	-0.462	0.566	0.425
	LDL	0.590	-0.572	0.731	0.438
Squalene/ Sitosterol	Serum	0.639	-0.573	0.764	0.269
	VLDL	0.673	-0.364	0.572	0.184
	LDL	0.516	-0.695	0.753	0.234
Lathosterol/ Cholesterol	Serum	0.595	-0.381	0.565	0.324
	VLDL	0.504	-0.373	0.446	0.257
	LDL	0.618	-0.423	0.624	0.316
Squalene/ Cholesterol	Serum	0.596	-0.294	0.529	0.078
	VLDL	0.425	-0.159	0.271	-0.089
	LDL	0.441	-0.446	0.515	-0.048
Sitosterol/ Cholesterol	Serum	-0.364	0.590	-0.480	-0.463
	VLDL	-0.455	0.479	-0.483	-0.602
	LDL	-0.356	0.589	-0.487	-0.456

Discussion

The major findings of the present study are that, even though the ratios to cholesterol of the non-cholesterol sterols and especially of squalene, surrogate markers of cholesterol metabolism, are not exactly similarly distributed between the lipoproteins, the ratios in serum seem to reflect both absolute cholesterol synthesis and absorption percentage of dietary cholesterol.

Squalene, the last non-sterol precursor of cholesterol synthesis, known to be less good surrogate marker in non-diabetic population, was related even in many lipoproteins to other surrogate markers of normal weight and obese patients with type 2 diabetes (6). In the present population with a large BMI variation in type 2 diabetes, the squalene ratio was associated with the synthesis markers (especially cholestenol and lathosterol also in lipoproteins and with absolute cholesterol synthesis in serum, but less consistently with absorption markers or absorption percent of cholesterol.

Since cholesterol synthesis is markedly high in obese subjects, the study population may be heterogeneous, the lean ones mimicking normal subjects, resulting in variation of squalene and surrogate markers with variables of cholesterol metabolism. For instance, the squalene ratios, like other synthesis surrogates, in serum and most lipoproteins were significantly correlated with absolute cholesterol synthesis, but, even though the latter was positively related to BMI, the latter one was less significantly related to the synthesis marker ratios. However, when cholesterol metabolism was studied, in addition to the ratios of squalene and non-cholesterol sterols to cholesterol, by the proportions of synthesis/absorption markers or by absolute synthesis/absorption percent the associations with different variables were improved.

Reliable measurement of absolute cholesterol synthesis is quite complicated in man and this is the reason that the number of studies including these data is limited. The sterol balance data, the 'golden standard' -measurements of cholesterol metabolism, is the difference between dietary consumption of cholesterol and fecal output of cholesterol metabolites, bile acids and neutral sterols of cholesterol origin. Inclusion of dietary cholesterol and fecal steroid measurements complicates practical performance of 'colden standard' especially because simultaneous measurement of dietary cholesterol absorption percentage could give lot of additional information on cholesterol metabolism. This can be performed most reliably with prolonged oral consumption of labelled cholesterol and plant sterols, currently with stable isotopes, followed by determination of their ratio in stools. The present study demonstrates that the 'colden standard' of cholesterol metabolism can be replaced by measurement of the synthesis marker ratios to cholesterol in serum, and the absorption percentage of cholesterol by surrogate markers of cholesterol absorption without complicated methods or isotopes. The ratios to cholesterol of squaleneand non-cholesterol sterols or the proportions of the synthesis/absorption sterols can be easily measured from the non-saponifiable material of serum with a single gas liquid chromatographic run.

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