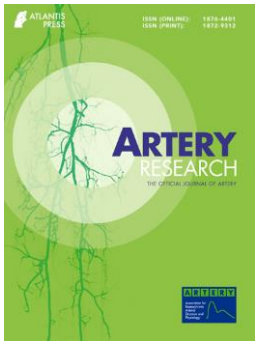

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Cholesterol metabolism, endothelial dysfunction, and carotid artery stiffness in type 1 diabetes☆

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Desmosterol;
Sitosterol;
Type 1 diabetes

Abstract *Background:* Type 1 diabetes is associated with increased risk of cardiovascular diseases and altered metabolism of cholesterol. We studied whether the markers of arterial stiffness reflecting preclinical atherosclerosis are related to markers of cholesterol metabolism in type 1 diabetes.

Methods: In eighteen type 1 diabetes subjects aged from 20 to 56 years, serum squalene and non-cholesterol sterols were measured with gas–liquid chromatography, and carotid arterial stiffness (elastic and Young's modulus, beta index, distensibility, and compliance), intima-media thickness (IMT), and brachial artery endothelial function (flow-mediated dilatation, FMD) were measured with ultrasound imaging.

Results: Variables of arterial stiffness were not related to serum lipids or HbA_{1c} except Young's modulus and compliance to triglycerides ($r = 0.541$ and $r = -0.552$, $p < 0.05$ for both, respectively). Stiffness of carotid artery was related to mean blood pressure (elastic modulus $r = 0.590$, distensibility $r = -0.486$, $p < 0.05$ for both). Stiffness of carotid artery was associated with serum desmosterol concentration, marker of cholesterol synthesis (e.g. compliance $r = -0.600$, $p < 0.01$), and with markers of cholesterol absorption (e.g. distensibility and sitosterol to cholesterol ratio $r = 0.628$, $p < 0.01$), and the associations between

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absorption markers and arterial stiffness remained significant after adjustment on age and mean blood pressure.
Conclusions: Carotid arterial stiffness was associated with markers of cholesterol metabolism, but not with serum lipid levels. Low absorption-high synthesis of cholesterol was related to increased arterial stiffness. Cholesterol metabolism seems to play a role in vascular health beyond serum lipids in type 1 diabetes.

Introduction

Atherosclerosis develops under several years, even for decades, and is frequently undetectable until the appearance of clinical signs. However, early vascular changes, such as increased arterial intima-media thickness (IMT), impaired endothelial function measured as flow-mediated dilatation (FMD), and stiffening of arterial wall can be detected years before the clinical manifestations of atherosclerosis.[1]

Previous studies have shown that the risk of vascular events, such as myocardial infarction, cerebral stroke, and peripheral arterial insufficiency, increases with IMT[2] and with decreasing endothelial function,[3] and there is a good correlation between IMT and atherosclerosis.[4] Arterial stiffening has been associated to myocardial infarction and atherosclerosis,[5] and also to hypertension.[6] IMT is a well-established method for assessment of preclinical atherosclerosis. However, FMD, despite its wide use in research, is not yet introduced in clinical practice because of the lack of a standardized method. On the contrary, arterial stiffness, as measured with pulse wave velocity, is incorporated in clinical practice as is also suggested by the ESC/ESH guidelines.[7]

Macrovascular complications, such as coronary artery disease, stroke, and peripheral obliterate arterial disease, are major causes of morbidity and mortality in type 1 diabetes.[8] Accordingly, type 1 diabetes can be considered as a risk factor for atherosclerosis, and it has been associated with increased IMT and decreased endothelial function even in childhood.[3] It has been proposed that increased serum blood glucose level affects vascular stiffness through glycosylation.[9] Although type 1 diabetes patients may frequently have normal levels of LDL cholesterol, diabetic patients have altered LDL cholesterol characteristics and enhanced foam cell formation.[3] These changes are known to be associated with thicker intima-media layer,[3] decreased endothelial function,[3] and increased arterial stiffening.[10] In addition, we have shown earlier that cholesterol metabolism is perturbed in type 1 diabetes, so that cholesterol absorption efficiency is enhanced and cholesterol synthesis is downregulated.[11] Furthermore,[12] in type 1 diabetes IMT was associated with LDL cholesterol, brachial artery diameter was inversely associated with HDL cholesterol, and endothelial function was associated with serum sitosterol to cholesterol ratio, a marker of cholesterol absorption.[13] Accordingly, the objective of this study was to evaluate in more detail the interrelation of different vascular markers of preclinical atherosclerosis, especially those of arterial stiffness, and cholesterol metabolism. As a high-risk population of atherosclerosis, we recruited type 1 diabetes subjects without earlier macrovascular atherosclerotic complications.

Research design and methods

Subjects

Twenty-two subjects with type 1 diabetes were recruited to the cross-sectional study from our earlier study populations and through announcements in the local newspaper. The inclusion criteria were normal liver, kidney and thyroid function. The exclusion criteria were the presence of cardiovascular diseases, active inflammatory gastrointestinal disease, or lipid-lowering medication. Four subjects dropped out because ultrasound images of their carotid arteries were not possible to analyze adequately. Accordingly, eighteen subjects were included in the following analyses (Table 1).

Five subjects were males and 13 were females. The age of the subjects ranged from 20 to 56 years, and $HbA1_{1c}$ from 5.9% to 12.2%. The duration of diabetes was 13.3 $\pm$ 2.1 years with a range of 2—31 years. Mean body mass index (BMI) was 25.1 kg/m^2. Two subjects had microalbuminuria. Two were smokers. Serum total and LDL cholesterol values varied from 3.6 to 6.0 and from 1.3 to 3.8 mmol/l, and HDL cholesterol from 1.3 to 2.4 mmol/l, respectively. Serum triglycerides varied from 0.5 to 2.0 mmol/l.

Of the medication of the subjects in addition to long- and short acting insulin, one subject had calcium channel blockers, and four had angiotensin converting enzyme- or angiotensin receptor blocking agents, and one subject had a diuretic for hypertension. One subject used hormone replacement therapy, and two subjects used hormonal contraceptives. All medications and doses had been unchanged at least for three previous months. All subjects gave their written informed consent. The investigation was carried out in accordance with the principles of the Declaration of Helsinki. The study protocol was accepted by the Ethics Committee of the University of Kuopio.

Methods

Blood samples were drawn after 12-h fasting. Serum total and HDL cholesterol and serum triglycerides were analyzed using routine enzymatic methods, and LDL cholesterol was calculated with the Friedewald equation.

Serum cholesterol, squalene and non-cholesterol sterols were analyzed with gas—liquid chromatography (GLC) with

Table 1 Description, clinical characteristics, and lipid values of the study population ($n = 18$).

Variables	Mean ± SEM
Age (yrs)	36.4 ± 2.3
Gender (male/female)	5/13
Microalbuminuria (yes/no)	2/16
Body mass index (kg/m^2)	25.1 ± 0.9
Systolic blood pressure (mmHg)	123.4 ± 2.0
Diastolic blood pressure (mmHg)	78.2 ± 2.1
HbA1c (%)	8.2 ± 0.3
Plasma glucose (mmol/l)	9.0 ± 0.8
Serum cholesterol (mmol/l)	4.8 ± 0.2
LDL cholesterol (mmol/l)	2.5 ± 0.2
HDL cholesterol (mmol/l)	1.8 ± 0.1
Serum triglycerides (mmol/l)	1.0 ± 0.1
Serum cholesterol, (mg/dl) (GLC analysis)	170.3 ± 5.3
Serum markers of cholesterol synthesis	
Squalene (μg/dl)	29.4 ± 3.4
Desmosterol (μg/dl)	125.2 ± 6.8
Lathosterol (μg/dl)	185.7 ± 17.1
Squalene (10^2 μmol/mmol of cholesterol)	17.0 ± 1.6
Desmosterol (10^2 μmol/mmol of cholesterol)	73.6 ± 3.4
Lathosterol (10^2 μmol/mmol of cholesterol)	108.9 ± 9.2
Serum markers of cholesterol absorption	
Campesterol (μg/dl)	591.7 ± 57.9
Sitosterol (μg/dl)	282.6 ± 24.2
Avenasterol (μg/dl)	91.6 ± 8.1
Cholestanol (μg/dl)	281.0 ± 14.2
Campesterol (10^2 μmol/mmol of cholesterol)	354.4 ± 37.2
Sitosterol (10^2 μmol/mmol of cholesterol)	168.2 ± 15.2
Avenasterol (10^2 μmol/mmol of cholesterol)	53.5 ± 4.0
Cholestanol (10^2 μmol/mmol of cholesterol)	165.9 ± 8.2
Vascular variables	
Intima-media thickness (mm)	0.54 ± 0.03
Flow-mediated dilatation %	7.7 ± 0.9
Beta index (no unit)	4.7 ± 0.4
Peterson's elastic modulus (kPa)	66.0 ± 5.7
Young's elastic modulus (kPa/mm)	125.7 ± 12.0
Compliance by area (Mm^2/kPa)	2.56 ± 0.20
Distensibility (kPa^{-1})	3.86 ± 0.35

a 50 m long SE-30 capillary column (Ultra-2, Agilent Technologies, Wilmington, DE) equipped with flame ionization detector. The procedure uses 5α-cholestane as internal standard, and it measures the concentrations of squalene, cholesterol, cholestanol, desmosterol, lathosterol, campesterol, sitosterol and avenasterol in the increasing order of refractory time.[14] The values of squalene and non-cholesterol sterols were expressed in μg/dl and also in terms of 10^2 × μmol/mmol of cholesterol (called ratio in the text) dividing the concentrations by the cholesterol value of the same GLC run in order to eliminate the different carrier lipoprotein concentrations (mainly LDL).

Of the non-cholesterol sterols, squalene, desmosterol, and lathosterol are markers of cholesterol synthesis, and those of plant sterols campesterol, sitosterol, and avenasterol, and cholestanol, a metabolite of cholesterol, are markers of cholesterol absorption.[13]

Vascular ultrasound imaging

Three vascular properties were measured using ultrasound imaging with a 14 Mhz linear array transducer and Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA). All measurements were performed by a single person.

Arteries were measured using functional and structural viewpoints. Functional changes were measured by assessment of the endothelium-dependent vasodilatation, a non-invasive ultrasound-based method as described by Celermajer et al. in 1992[15] with automated vessel wall detection.[16] Postischemic vasodilatation was induced by 4.5 min occlusion of the brachial artery. Structural changes of inner part of the vessel were measured by IMT, which was measured from left common carotid artery 10 mm from proximal to the carotid bulb by using high-resolution ultrasound. Structural changes of muscular layer of the vessel were measured by using carotid artery stiffness (elasticity), which was measured from left common carotid artery after subjects lying for 5 min. Common carotid artery was imaged for 2 min with B-mode ultrasound. Diameter of the carotid artery was measured from B-mode ultrasound image with frame rate 25 per second, by using automated edge detection and distension was calculated. The distension data was combined with ECG and blood pressure. Continuous blood pressure recording was performed on the middle finger of the right hand with a Finapres plethysmograph (Ohmeda Englewood, CO). Blood pressure level was also controlled from the brachial artery. Data were exported to personal computer to custom-made analytic software. Analysis was performed twice by the same observer. Our continuous beat-to-beat analysis method was repeatable and feasible. Standard deviation between ten measurements of diameter was 0.03 mm (CV 0.4%). Intraclass correlations were 0.988 for diameter of vessel, 0.989 for elastic modulus, and 0.989 for beta index.

Stiffness of common carotid artery was measured using Peterson's elastic modulus (elastic modulus) and beta index, which are described earlier by O'Rourke et al.,[17] and Young's modulus, distensibility and compliance, which are described earlier by Bussy et al.[18] Formulas of variables and endothelial function are presented in Table 2.

Statistical analyses

Statistical analyses were performed with SPSS for Windows 14.0 statistics program (SPSS, Chicago, IL, USA). Normality was tested with Shapiro–Wilk's *W*-test before further analysis. Data with skewed distributions were transformed

Table 2 Attributes of arterial stiffness.

Attribute	Formula	Unit	Interpretation
Peterson's elastic modulus (E_p)	$E_p = dP * D_{max}/dD;$	kPa	Lower value indicates more elastic vessels
Young (E_y)	$E_y = \frac{(SBP-DBP)*D_{min}}{(D_{max}-D_{min})*IMT}$	kPa/mm	Lower value indicates more elastic vessels
Beta index (β)	$\beta = \frac{\ln(SBP/DBP)}{dD/D_{min}}$	No unit	Lower value indicates more elastic vessels
Compliance (C)	$C = dLCSA/dP$	Mm^2/kPa	Higher value indicates more elastic vessels
Distensibility (D)	$D = dLCSA/dP * LCSA_{min}$	kPa^{-1}	Lower value indicates more elastic vessels
Flow-mediated dilatation (FMD%)	$FMD\% = \frac{D_{dilatated}-D_{normal}}{D_{normal}}$	No unit	Higher value indicates better endothelial function

dP = Change of the blood pressure during cardiac cycle (SBP − DBP) SBP = systolic blood pressure and DBP = diastolic blood pressure dLCSA = Change of the lumen cross-sectional area of carotid artery diameter during cardiac cycle.
dD = Change of the carotid artery diameter during cardiac cycle ($D_{max} - D_{min}$) D_{max} = maximal and D_{min} = minimal diameter of carotid artery. D_{normal} = brachial arterial diameter before dilatation and $D_{dilatated}$ = brachial artery diameter after dilatation; IMT = Intima-media thickness.

logarithmically. Correlations of variables with normal distribution were tested with Pearson coefficient test, and those with skewed distribution were tested with Spearman's coefficient variation test. To evaluate the effects of age and mean blood pressure on associations of vascular variables and cholesterol metabolism, a linear regression analysis was used. Grouping variables (gender, smoker) were tested with Mann–Whitney's t-test. A p-value of <0.05 was considered statistically significant. The results of continuous variables are presented as mean ± SEM.

Results

Description of the study population including plasma glucose, glycemic control, and serum lipids, squalene and non-cholesterol sterols, and vascular variables are presented in Table 1. The mean HbA1c value was 8.2% suggesting moderate glucose control. Serum total and LDL cholesterol and triglyceride concentrations were on recommended levels, and HDL cholesterol was high-normal. All continuous variables in Table 1 except serum desmosterol concentration and desmosterol ratio to cholesterol did not differ significantly between men and women (data not shown).

Vascular variables

Elastic modulus, Young's modulus, and beta index were interrelated, and inversely associated with compliance (Table 3). Elastic modulus was also related to FMD ($r = -0.479$, $p < 0.05$). None of these variables were related to IMT.

Age was positively related to IMT (Table 3). Mean blood pressure was positively related to elastic modulus, and inversely to distensibility and FMD. Fasting plasma glucose, HbA_{1c}, or serum and lipoprotein lipids were not related to any of the vascular variables, except serum triglycerides were positively related to Young's modulus and negatively to compliance. Elastic modulus ($r = 0.562$, $p = 0.015$), Young's modulus ($r = 0.509$, $p = 0.031$), beta index ($r = 0.546$, $p = 0.019$), compliance ($r = -0.483$ $p = 0.042$) and distensibility ($r = -0.589$ $p = 0.010$) were related to the intake of carbohydrates (g/day), and Young's modulus to the intake of polyunsaturated fatty acids ($r = 0.589$ $p = 0.010$). None of the vascular variables were related to gender, smoking habits, height, weight, BMI, or dietary intakes of saturated or monounsaturated fatty acids, cholesterol or fibre (data not shown).

Cholesterol metabolism

Of the markers of cholesterol synthesis, desmosterol concentration was related to elastic modulus and beta index, and inversely to compliance, distensibility and FMD (Table 3). Serum lathosterol concentration was negatively related to compliance. When adjusted on age and mean blood pressure, these associations were no more statistically significant (Table 4).

Of the absorption markers of cholesterol, sitosterol and cholestanol to cholesterol ratios were inversely related to elastic modulus (Table 3). Campesterol, sitosterol, and cholestanol ratios to cholesterol were positively related to compliance and distensibility, and that of campesterol ratio also inversely to IMT. However, when adjusted with age and mean blood pressure, sitosterol concentration and sitosterol and cholestanol ratios to cholesterol were still related to distensibility (Table 4). In addition, cholestanol and sitosterol ratios were almost significantly related to compliance ($\beta = 0.449–0.477$, $p = 0.057–0.058$). The concentrations of plant sterols and cholestanol and the ratio to cholesterol of avenasterol were not related to any of the vascular variables.

Discussion

Preclinical atherosclerosis was measured from structural (IMT) and functional viewpoints of endothelium (FMD), and from muscular structures of the vessel reflecting arterial stiffness (elastic modulus, Young's modulus, beta index, compliance, and distensibility). The markers of arterial stiffness were interrelated, though compliance and distensibility were inversely related to elastic and Young's modulus and beta index. The markers of arterial stiffness

Table 3 Correlation coefficients between clinical characteristics, variables of cholesterol metabolism, and carotid arterial stiffness and endothelial function in subjects with type 1 diabetes ($n = 18$).

Variables	Peterson's elastic modulus	Young's modulus	Beta index	Compliance	Distensibility	FMD%	IMT
Age (y)	0.178	−0.135	0.216	−0.309	−0.294	0.034	0.595**
Body mass index (kg/m^2)	−0.163	−0.287	−0.228	0.051	0.126	0.230	0.257
Mean blood pressure (mmHg)	0.590**	0.396	0.465	−0.457	−0.486*	−0.617**	0.237
HbA1c (%)	0.350	0.178	0.256	−0.413	−0.436	−0.095	0.212
Plasma glucose (mmol/l)	0.081	−0.025	0.166	0.002	−0.104	0.057	0.068
Serum cholesterol (mmol/l)	0.277	0.024	0.144	−0.408	−0.295	−0.352	0.431
LDL cholesterol (mmol/l)	0.245	−0.011	0.122	−0.290	−0.199	−0.348	0.448
HDL cholesterol (mmol/l)	−0.126	−0.205	−0.049	0.080	0.036	0.230	0.160
Serum triglycerides (mmol/l)	0.360	0.541*	0.221	−0.552*	−0.467	−0.453	−0.272
Serum markers of cholesterol synthesis							
Squalene (μg/dl)	0.303	0.298	0.308	−0.350	−0.215	−0.213	0.051
Desmosterol (μg/dl)	0.595**	0.347	0.515*	−0.600**	−0.606**	−0.534*	0.332
Lathosterol (μg/dl)	0.272	0.171	0.185	−0.469*	−0.310	−0.253	0.208
Squalene[a]	0.209	0.276	0.267	−0.163	−0.137	0.026	−0.041
Desmosterol[a]	0.448	0.293	0.445	−0.392	−0.437	−0.378	0.163
Lathosterol[a]	0.157	0.129	0.114	−0.349	−0.203	−0.133	0.087
Serum markers of cholesterol absorption							
Campesterol (μg/dl)	−0.310	−0.039	−0.244	0.396	0.452	0.227	−0.411
Sitosterol (μg/dl)	−0.435	−0.301	−0.366	0.454	0.508	0.258	−0.157
Avenasterol (μg/dl)	0.056	0.026	0.111	0.000	0.043	0.002	0.057
Cholestanol (μg/dl)	−0.258	−0.224	−0.261	0.213	0.315	0.039	−0.053
Campesterol[a]	−0.400	−0.087	−0.300	0.507*	0.544*	0.322	−0.485*
Sitosterol[a]	−0.541*	−0.345	−0.421	0.574*	0.628**	0.383	−0.237
Avenasterol[a]	−0.058	−0.051	0.051	0.160	0.159	0.123	−0.025
Cholestanol[a]	−0.538*	−0.376	−0.462	0.561*	0.632**	0.275	−0.259
Vascular variables							
Young's modulus (kPa/mm)	0.822***						
Beta index (no unit)	0.960***	0.807***					
Compliance by area (mm^2/kPa)	−0.802***	−0.744***	−0.774***				
Distensibility (kPa^{-1})	−0.887***	−0.804***	−0.876***	0.932***			
FMD%	−0.479*	−0.386	−0.351	0.363	0.385		
IMT (mm)	0.127	−0.430	0.088	−0.015	−0.020	−0.128	

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
[a] $10^2 \times$ μmol/mmol of cholesterol. IMT = intima-media thickness, FMD% = flow-mediated dilatation.

were also related to FMD, but not to IMT, and arterial stiffness and endothelial function were associated to mean blood pressure. Association between arterial stiffness and mean blood pressure may be explained by the pretension of artery.

The novel observations of the present study were that arterial stiffness (elastic modulus, beta index, compliance, and distensibility) were related to cholesterol synthesis assayed with the synthesis markers of cholesterol, especially that of serum desmosterol concentration. The associations between serum desmosterol level and elastic modulus and beta index were positive, whereas those with compliance and distensibility were negative. The disappearance of the significant regression between desmosterol and arterial stiffness after adjustments on age and mean blood pressure may result from the interrelation between desmosterol and mean blood pressure, which weakens the association between desmosterol and arterial stiffness. Furthermore, compliance and distensibility were related to markers of cholesterol absorption. Accordingly, increased arterial stiffness was associated with low cholesterol absorption and high cholesterol synthesis in type 1 diabetes subjects. This kind of profile of cholesterol metabolism has been observed earlier in insulin resistance,[19] obesity,[20] and in type 2 diabetes.[21] Even in healthy adolescents, the low absorption-high synthesis profile of cholesterol metabolism was characteristic in subjects with variables of the metabolic syndrome.[22] It might be concluded that low absorption-high synthesis of cholesterol seems to be proatherogenic.

Vascular variables were related only to serum triglyceride levels, but not to serum total or HDL cholesterol or variables of glucose metabolism. These findings are different from a previous study in type 1 diabetics, in whom carotid artery stiffness was associated with high serum cholesterol value and poor glucose control.[23] However, in

Table 4 Regression coefficients adjusted with age and mean blood pressure between variables of cholesterol metabolism and carotid arterial stiffness and endothelial function in subjects with type 1 diabetes ($n = 18$).

Variables	Peterson's elastic modulus	Young's modulus	Beta index	Compliance	Distensibility	FMD%	IMT
Serum markers of cholesterol synthesis							
Desmosterol (μg/dl)	0.357	0.172	0.365	−0.538	−0.510	−0.227	0.251
Lathosterol (μg/dl)	0.001	−0.006	−0.039	−0.322	−0.105	0.033	0.109
Desmosterol[a]	0.261	0.143	0.321	−0.271	−0.309	−0.145	0.133
Lathosterol[a]	0.044	0.037	0.029	−0.284	−0.124	0.002	0.080
Serum markers of cholesterol absorption							
Campesterol (μg/dl)	−0.213	−0.007	−0.155	0.301	0.360	0.157	−0.302
Sitosterol (μg/dl)	−0.326	−0.182	−0.295	0.409	0.457*	0.087	−0.204
Cholestanol (μg/dl)	−0.292	−0.214	−0.299	0.262	0.365	0.042	−0.134
Campesterol[a]	−0.222	0.005	−0.135	0.366	0.404	0.173	−0.343
Sitosterol[a]	−0.343	−0.188	−0.270	0.477	0.527*	0.109	−0.203
Cholestanol[a]	−0.377	−0.283	−0.336	0.449	0.520*	0.076	−0.174

*$p < 0.05$.
[a] 10^2x μmol/mmol of cholesterol. IMT = intima-media thickness, FMD% = flow-mediated dilatation.

the present study the lack of correlation between glucose control and arterial stiffness may be related to the small sample size, because there is a negative trend between HbA_{1c} and distensibility.

When using surrogate serum markers of cholesterol metabolism, the relevant question to be addressed is whether the markers are valid in the current population. In a previous study including also subjects of the present population, cholesterol precursor sterols were interrelated.[12] Similarly, the absorption markers plant sterols and cholestanol were interrelated. Moreover, cholesterol precursor sterols were inversely related to plant sterols and cholestanol suggesting, first, that cholesterol homeostasis was intact, and second, the non-cholesterol sterols reflected cholesterol metabolism in type 1 diabetes. It was interesting to note that the variables of arterial stiffness but not FMD or IMT were related to cholesterol metabolism. In our previous study in non-diabetic mildly to moderately hypercholesterolemic subjects, large brachial artery diameter was associated with high cholesterol synthesis (serum desmosterol ratio) and low absorption.[24] In this respect, brachial artery diameter behaved in a similar way as the variables of arterial stiffness in the present study confirming the association between vascular wall well-being and cholesterol metabolism.

In conclusion, in type 1 diabetes carotid artery stiffness was related to cholesterol metabolism beyond serum cholesterol level. Increased arterial stiffness was related to high cholesterol synthesis and low cholesterol absorption, and the latter association remained significant after adjustment on age and mean blood pressure. IMT was not related to serum lipids or cholesterol metabolism.

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