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Atherosclerosis

Plant Sterols in Serum and in Atherosclerotic Plaques of Patients Undergoing Carotid Endarterectomy

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OBJECTIVES The purpose of this research was to determine whether serum plant sterol levels are associated with those in atheromatous plaque.

BACKGROUND Cholesterol of low-density lipoprotein (LDL) particles contributes to atheromatous plaque formation; LDL also contains most serum non-cholesterol sterols, including plant sterols. The role of plant sterols in atheromatous plaque formation is open.

METHODS Free, ester, and total cholesterol and the respective non-cholesterol sterols were measured by gas-liquid chromatography in serum and arterial tissue of 25 consecutive patients undergoing carotid endarterectomy. The population was ranked to triads according to tissue cholesterol concentration.

RESULTS Cholesterol concentration increased markedly in tissues but not in serum with triads. The ester percentage was lower in the third than in the first triad (47% vs. 56%; $p < 0.01$) and lower than in serum triads (70%; $p < 0.001$). Ratios to cholesterol of non-cholesterol sterols decreased in increasing tissue triads, but were unchanged in serum. A major new observation was that the higher the ratio to cholesterol of the surrogate absorption sterols (cholestanol, campesterol, sitosterol, and avenasterol) in serum, the higher was their ratio also in the carotid artery wall (e.g., $r = 0.683$ for campesterol). Despite undetectable differences in serum and tissue cholesterol concentrations off and on statins, an additional important novel finding was that statin treatment was associated with increased ratios of the absorption sterols in serum and also in the arterial plaque.

CONCLUSIONS The higher the absorption of cholesterol, the higher are the plant sterol contents in serum resulting also in their higher contents in atherosclerotic plaque. However, the role of dietary plant sterols in the development of atherosclerotic plaque is not known. (J Am Coll Cardiol 2005;45:1794–801) © 2005 by the American College of Cardiology Foundation

Atherothrombotic disease is the major source of mortality and morbidity in the Western world. Although it may affect any part of the arterial tree, its main clinical manifestations are coronary heart disease (CHD), cerebrovascular disease, and occlusive arterial disease of lower extremities. The threatened sequelae of cardiovascular diseases (CVDs) are myocardial infarction, stroke, threatening limb ischemia, and death. Increased serum total and low-density lipoprotein (LDL) cholesterol concentrations are among the major risk factors of arterial CVD. It is believed that cholesterol from blood is incorporated through arterial endothelium to subendothelial extracellular connective tissue mainly as LDL in proportion to its serum level (1). Thus, as the occurrence of clinical manifestations of CVD is higher, the higher is the serum level of LDL cholesterol. In fact, recent clinical statin trials have convincingly shown that effective LDL cholesterol lowering reduces the incidence of clinical manifestations of CVD (2). Even though LDL cholesterol has been suggested to be below 2.5 mmol/l in coronary and diabetic patients, more current targets suggested that it should be even <1.6 mmol/l for a high-risk population (3).

Low-density lipoprotein particles contain not only cholesterol, however, but also small amounts of squalene and other sterols, called non-cholesterol sterols, including cholesterol precursors cholestenol, desmosterol, and lathosterol, and sterols reflecting cholesterol absorption (i.e., cholestanol and plant sterols campesterol, sitosterol, and avenasterol). In serum, cholesterol precursors, especially their ratios to cholesterol, reflect cholesterol synthesis, whereas the respective ratios of cholestanol and plant sterols are markers of cholesterol absorption (4). Because patients with high LDL cholesterol usually have high cholesterol absorption efficiency and high ratios of plant sterols to cholesterol in serum (5), mainly in LDL, we considered that plant sterols should also be rich in arterial atheromas. Postmortem plant sterol concentrations have previously been studied in arterial walls of a few infants, children, and adults (6), but comparison of serum non-cholesterol sterols and their ratios to cholesterol with those in atheromas have not been previously studied in vivo. In addition, long-term statin treatment, especially with large doses of effective drugs, is known to increase serum plant sterols reflecting cholesterol absorption (4,7–9), suggesting that increased plant sterol ratios to cholesterol in

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Abbreviations and Acronyms

BMI = body mass index
CHD = coronary heart disease
CVD = cardiovascular disease
GLC = gas-liquid chromatography
HDL = high-density lipoprotein
LDL = low-density lipoprotein
4S = Scandinavian Simvastatin Survival Study

serum might increase the ratios also in atheromatous tissues. So, to study this question more closely, non-cholesterol sterols, including plant sterols, were studied in serum and compared with those in carotid artery plaques obtained during endarterectomy of patients with signs of disturbed cerebral circulation. The samples were obtained from consecutive patients undergoing carotid surgery.

METHODS

Study population. A carotid endarterectomy specimen was obtained from 25 consecutive patients who underwent the procedure in order to prevent stroke or stroke-related death. Sixteen patients were men and nine were women, and their mean age was 67 years, range 52 to 84 years. All patients were symptomatic having transient ischemic attacks, amaurosis fugax, or recent stroke with favorable recovery as the clinical indication for operation. Duplex ultrasound evaluation and digital subtraction angiography were used for carotid artery assessment. All carotid arteries operated on showed a maximum diameter reduction of at least 70%.

Mean body mass index (BMI) of the study population was 25.0 ± 0.7 kg/m^2. Thus, the patients had mostly a normal body weight, and only three of them were obese with BMI >30 kg/m^2. The population included 5 patients with type 2 diabetes, 8 with chronic obstructive pulmonary disease as a consequence of smoking, 14 were current or ex-smokers, 14 had intermittent claudication, 6 had signs of CHD, and 7 had had an earlier stroke. Diagnosis of hypercholesterolemia had been made in 10 patients, yet serum cholesterol levels were relatively low (Table 1) in these patients, partly due to the fact that 12 of 25 were on statins (mainly simvastatin and atorvastatin). Unfortunately, the initial serum total or LDL cholesterol levels could not be explored.

All participants volunteered for the study and gave their informed consent. The study protocol was approved by the ethics committees of the Departments of Medicine and Surgery, Helsinki University Hospital.

Table 1. Serum and Lipoprotein Lipids (mmol/l) Off and On Statin Treatment

Variable	Total (n = 25)	Statin − (n = 13)	Statin + (n = 12)
Cholesterol	3.56 ± 0.15	3.68 ± 0.17	3.43 ± 0.27
LDL cholesterol	2.23 ± 0.14	2.44 ± 0.17	1.99 ± 0.22
HDL cholesterol	0.82 ± 0.06	0.82 ± 0.08	0.82 ± 0.08
Triglycerides	1.13 ± 0.14	0.93 ± 0.10	1.35 ± 0.25

Mean ± SE. For cholesterol, to obtain mg/dl, multiply by 38.6; for triglycerides, multiply by 88.2.
HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Techniques. Arterial blood sample was obtained during the surgery at the time of removing the carotid arterial plaque. Serum was separated from the blood sample by mild centrifugation. Carotid artery plaque was washed in saline and sent to the laboratory with the serum sample. Attempts were made to separate from each arterial plaque macroscopically less atheromatotic endothelial parts in addition to more tight plaque areas by one researcher (T.A.M.) blinded to the history of the patients, clinical data, and laboratory results. Thus, one to five different-looking samples were prepared from each arterial sample. Macroscopic differentiation of the tissue specimen to clear endothelial parts without or with some atheroma plaques or with more severely atheromatotic areas was not possible. Thus, 68 vascular and 25 serum samples were collected.

Serum total and high-density lipoprotein (HDL) cholesterol and triglycerides were analyzed by the routine methods of our hospital (using kits from Boehringer Diagnostica, Mannheim, Germany, and Waco Chemicals, Neuss, Germany). Exact amounts of tissue samples, 50 to 200 mg each, were carefully weighted, 5-α cholestane and epicoprostanol added for internal markers, homogenized with chloroform/methanol, and the homogenate was extracted three times with the mixture. The extract was evaporated and applied in a small amount of ethyl ether on a thin-layer chromatography plate (on silica gel) for separation of free and ester sterols with hexane/ethyl ether 50:50. The fractions were extracted from the plate, and the ester fraction, containing also 5-alpha-cholestane and squalene, was saponified, and non-saponifiable lipids were extracted with ethyl ether. The sterol fractions were silylated, and the sterols and squalene were quantitated with gas-liquid chromatography (GLC) using epicoprostanol as the internal standard for free sterols and 5-alpha-cholestane for squalene and ester sterols (10). Serum squalene and free and ester sterols were analyzed in the same way; GLC was performed using a 50-m long ULTRA-1 SE-30 column (Hewlett-Packard, Wilmington, Delaware). The values are given as mg/100 g of tissue or mg/dl of serum for cholesterol. Squalene and non-cholesterol sterols are expressed as μg/100 g of tissue or μg/dl of serum, or as ratios to respective cholesterol value ($10^2 \times \mu$g/mg of cholesterol). The sum of free and ester fractions reveals the total concentration of each compound in tissues and serum. The study population was also ranked to triads according to 66 (the weight of two tissues were missed) total cholesterol concentrations in tissues (upper limit for 22 cases in triad 1 was 838 mg/100 g and 2,020 mg/100 g for 22 cases in triad 2). So as to have comparable values, this case-ranking was also used for grouping of respective GLC values in serum. Large concentration differences of tissue cholesterol of the same individual case resulted in that several patients could be in all triads; the number of arterial samples was 22 in each triad. On the