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Endogenous Cholesterol Excretion Is Negatively Associated with Carotid Intima-Media Thickness in Humans

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Abstract

Objective—Epidemiological studies strongly suggest that lipid factors independent of LDL cholesterol contribute significantly to cardiovascular disease (CVD) risk. Because circulating lipoproteins comprise only a small fraction of total body cholesterol, the mobilization and excretion of cholesterol from plasma and tissue pools may be an important determinant of CVD risk. Our hypothesis is that fecal excretion of endogenous cholesterol is protective against atherosclerosis.

Approach and Results—Cholesterol metabolism and carotid intima-media thickness (CIMT) were quantitated in 86 non-diabetic adults. Plasma cholesterol was labeled by intravenous infusion of cholesterol-d_7 solubilized in a lipid emulsion and dietary cholesterol by cholesterol-d_5 and the nonabsorbable stool marker sitostanol-d_4. Plasma and stool samples were collected while subjects consumed a cholesterol- and phytosterol-controlled metabolic kitchen diet and were analyzed by mass spectrometry. CIMT was negatively correlated with fecal excretion of endogenous cholesterol (r=−0.426, P<0.0001), total cholesterol (r=−0.472, P=<0.0001), and daily percent excretion of cholesterol from the rapidly-mixing cholesterol pool (r=−0.343, P=0.0012), and was positively correlated with percent cholesterol absorption (r=+0.279, P=0.0092). In a linear regression model controlling for age, sex, systolic blood pressure, hemoglobin A1c, LDL, HDL cholesterol, and statin drug use, fecal excretion of endogenous cholesterol remained significant (P=0.0008).

Conclusions—Excretion of endogenous cholesterol is strongly, independently, and negatively associated with CIMT. The reverse cholesterol transport pathway comprising the intestine and the

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Disclosure

The authors have nothing to disclose.

rapidly-mixing plasma and tissue cholesterol pool could be an unrecognized determinant of CVD risk not reflected in circulating lipoproteins. Further work is needed to relate measures of reverse cholesterol transport to atherosclerotic disease.

Keywords

cholesterol absorption; excretion; controlled diet; stable isotopes; clinical trial; mass spectrometry

Subject Codes

Metabolism; lipids and cholesterol

Introduction

Guidelines for preventing and treating atherosclerotic disease are heavily focused on LDL cholesterol because of strong clinical trial evidence and readily-available plasma measurements.[1] Atherosclerosis originates from cholesterol deposition in the artery from β-lipoproteins, including LDL. However, circulating LDL is a small contributor to total body cholesterol, comprising about 3 g in a person with circulating LDL cholesterol concentration of 100 mg/dL compared to about 72 g of total body cholesterol.[2–6] Moreover, LDL cholesterol is a static measurement that contains no kinetic information about rates of cholesterol production or excretion.

The reverse transport of cholesterol is a multi-step pathway beginning with cholesterol extraction from peripheral tissues and macrophages, followed by transfer into a rapidly-mixing body cholesterol pool, secretion into the intestine, and excretion in the stool.[7] Reverse cholesterol transport (RCT) may mitigate the effect of LDL cholesterol by removing cholesterol deposited in the arterial wall. This work focuses on the distal portion of RCT, emphasizing the rapidly-mixing body cholesterol pool and the intestine. Using multiple stable isotopic tracers to label both dietary and endogenous cholesterol and employing a metabolic kitchen diet to provide a constant baseline for measurement, it is possible to quantitate total fecal cholesterol excretion and to characterize it as originating from the endogenous pool, the diet, or unlabeled hepatobiliary components.[8] The final common pathway for RCT is the fecal excretion of endogenous cholesterol (FEEC). That measurement, along with related cholesterol fluxes, forms the basis for this report. We define endogenous cholesterol as body cholesterol derived principally from biosynthesis and, to a lesser extent, from absorbed dietary cholesterol. The endogenous cholesterol was labeled by the intravenous administration of cholesterol-d_7 tracer.

Although little evidence exists on the relationship between FEEC and CVD risk, epidemiological studies have examined plasma biomarkers for the related processes of cholesterol absorption and synthesis, but the results have been inconsistent.[9, 10] Case-control studies also have been controversial, in part because the proposed biomarkers are not fully understood and have not been validated.[11–16] Actual measurements of whole body cholesterol metabolism, rather than its biomarkers, are needed to establish a possible relationship between whole body cholesterol metabolism and CVD.

Increased CIMT has been associated with increased risk of cardiovascular events such as myocardial infarction and stroke.[17–20] The Atherosclerosis Risk in Communities study showed a strong relationship between CIMT and cardiovascular events.[20] More recently, a study of the Framingham Offspring Study cohort showed that both the maximum internal CIMT and mean common CIMT predict cardiovascular outcomes.[21] Thus, CIMT is a well-accepted imaging biomarker of atherosclerosis and CVD risk. In the present observational study, we explored whether FEEC was related to CIMT in adults.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Subjects

Among the one hundred subjects screened, 94 were eligible and entered the study, and 86 completed the protocol. Subjects included 52 females and 34 males with mean age of 53.7 ± 13.0 years. Table 1 shows the subject characteristics upon entry into the study. They comprised a wide range of non-diabetic individuals. Subjects over age 30 were selected to increase the strength of the relation of CVD risk factors to CIMT. Statin drug use was allowed because this is a very common medication and does not appear to influence whole body cholesterol metabolism significantly.[22]

CIMT and traditional CVD risk factors

Pearson correlations between CIMT and several traditional CVD risk factors are shown in Table 2. Age, systolic blood pressure, HbA1c and fasting plasma glucose were associated with increased CIMT, as expected. However, there was no significant relation between circulating lipids or lipoproteins and CIMT in this sample.

Cholesterol metabolism

Cholesterol metabolism results are shown in Table 3. Total daily fecal cholesterol excretion measured was 0.58 g/day. Of this amount, 68% was accounted for by recovery of the plasma tracer cholesterol-d_7 and therefore had an endogenous origin, whereas 16% was due to unabsorbed dietary cholesterol measured with cholesterol-d_5. An additional 16% of fecal cholesterol was not labeled and may represent newly-synthesized or other non-equilibrating hepatobiliary cholesterol. Percent intestinal cholesterol absorption was 57.4%. The rapidly-mixing cholesterol pool, which includes both circulating lipoproteins and a portion of tissue cholesterol, averaged 25.3 g. Since the calculated plasma pool of cholesterol was only about 5.4 g, the rapidly-mixing cholesterol pool was comprised of 79% tissue cholesterol that equilibrated rapidly with plasma. Percent cholesterol excretion, the percent of the rapidly-mixing plasma cholesterol pool excreted daily into the stool as cholesterol and bacterial cholesterol metabolites, was 1.64%. These measurements emphasize that endogenous tissue cholesterol dominates whole body cholesterol metabolism.

CIMT and whole-body cholesterol metabolism

Table 3 shows that CIMT was strongly and negatively correlated with several measures of fecal cholesterol elimination, including total cholesterol excretion ($P < 0.0001$), endogenous cholesterol excretion ($P < 0.0001$), unabsorbed dietary cholesterol excretion ($P = 0.019$) and percent cholesterol excretion ($P = 0.0012$). Figure 1 shows a scatterplot of the relation between CIMT and FEEC. There was no relation of CIMT to the small amount of unlabeled stool cholesterol, suggesting that unrecognized cholesterol pools do not contribute significantly to CIMT. Percent cholesterol absorption, an important determinant of fecal cholesterol excretion, was positively correlated with CIMT ($P = 0.0092$). Secretion of endogenous cholesterol into the intestine, another determinant of fecal cholesterol excretion, was negatively correlated with CIMT ($P = 0.011$). Although turnover of plasma cholesterol was not measured in the study, it was estimated by the decline in plasma cholesterol enrichment over 13 days after infusion of intravenous tracer. This measurement was positively correlated with CIMT ($P = 0.0083$).

Potential plasma biomarkers for FEEC

Non-cholesterol sterols were measured in plasma in order to assess their potential use as biomarkers for FEEC in larger studies. FEEC was positively correlated with the ratio of plasma lathosterol/cholesterol (r = 0.283, $P = 0.0083$) and marginally negatively correlated with the ratio of plasma 5α-cholestanol/cholesterol (r = −0.211, $P = 0.051$). The ratio of 5α-cholestanol/lathosterol, reflecting the balance of cholesterol absorption to cholesterol biosynthesis, was significantly correlated with FEEC (r = −0.373, $P = 0.0004$).

Regression Model

FEEC explained 18.1% of variation in CIMT ($P < 0.0001$) in simple linear regression, and 7.6% in a multiple regression model that controlled for age, sex, systolic blood pressure, HbA1c, LDL cholesterol, HDL cholesterol, and statin drug use (model $R^2 = 0.52$, $P = 0.0008$ for FEEC).

Discussion

This work shows that FEEC, the principal pathway of cholesterol elimination in humans, is negatively correlated with CIMT. The data provide a link between whole body cholesterol metabolism and CIMT, a measure of early atherosclerosis. The association is quite strong and similar to that of traditional risk factors such as age, systolic blood pressure, and hemoglobin A1c in a study group consisting of 86 relatively healthy non-diabetic subjects between the ages of 30 and 80. Moreover, FEEC appears to be a stronger risk factor than HDL and LDL cholesterol, which were not related to CIMT in this small study.

The cholesterol production rate (1.0 g/day) in the present study was similar to previous reports of 1.1 g/day[23] and 1.14 g/day.[24] The rapidly mixing cholesterol pool of 25.3 g in the current study also was similar to the reported pool sizes of 23.4[23] and 25.9 g.[24] The cholesterol flux from the rapidly mixing cholesterol pool out of the body (production rate over the size of the rapidly mixing pool) (4.0%) in the present study was comparable to previous reported values of 4.7%[23] and 4.4%.[24] Others have reported a higher production

rate (2.1 g/day)[25] and a lower rapid pool size (11.7 g),[26] probably due to differences in tracer and/or methods used.

The primary objective in this work was to quantitate fecal cholesterol excretion and precisely determine its origins. The analytical methods also allowed us to correlate several secondary endpoints with CIMT. The complete results form a coherent model for how body cholesterol metabolism might influence CIMT and CVD risk. Figure 2 depicts a working model of cholesterol compartments (circles) and cholesterol fluxes (arrows). The rapidly-mixing body cholesterol pool includes all of the cholesterol in liver and blood as well as part of the cholesterol in other tissues. The rapid cholesterol pool is particularly important because it is the pool from which cholesterol is excreted from the body and into which cholesterol is absorbed. The size of the rapidly-mixing cholesterol pool was not directly related to CIMT, but cholesterol fluxes into and out of the pool were significantly correlated with CIMT, either negatively (solid arrows) or positively (dashed arrows). Cholesterol transport processes tending to increase the rapidly-mixing pool, such as inflow from unlabeled endogenous cholesterol and reabsorption of intestinal cholesterol, were positively correlated with CIMT, whereas processes tending to reduce the pool size, such as secretion of endogenous cholesterol into the intestine and elimination of cholesterol in the stool, were negatively correlated. Fecal bile acids were not correlated to CIMT and were not included in the model. The results suggest that cholesterol homeostasis is maintained by competing cholesterol transport processes and that individuals who have greater cholesterol excretion have more favorable CIMT values.

LDL and HDL cholesterol have great utility in assessing CVD risk, but the information obtained is limited to a static measurement, obtained at a single point in time. HDL cholesterol concentration does not provide information about the quantity of cholesterol flowing into and out of the HDL particles. Previous investigators also found that whole body cholesterol turnover studied with an intravenous cholesterol tracer was not related to circulating HDL or LDL cholesterol,[27] which may be due to the fact that RCT is the combined actions of various lipoproteins such as HDL and LDL. More attention has been given to functional assays for HDL. For example, cholesterol efflux capacity of HDL, a functional measure of cholesterol efflux from macrophages, has been shown to relate to atherosclerosis better than HDL cholesterol alone.[28] What distinguishes many cholesterol metabolic parameters in the current study is that they are kinetic and represent cholesterol flows over time. It is possible that kinetic measurements may supplement lipoprotein concentration measurements to provide a stronger prediction of CVD risk.

Reverse cholesterol transport is an important emerging pathway in which cholesterol efflux from macrophages and peripheral tissues is traced into HDL, through the plasma, and into the intestine, where it is excreted as neutral sterols and bile acids.[7, 29] Although attention has been focused on the arterial macrophage as a first step, the current work shows that cholesterol flowing from all tissues into and out of the rapidly-mixing cholesterol pool and intestine may contribute importantly to RCT. In particular, the intestine may have a central role in regulating body cholesterol metabolism and possibly in protecting against the development of atherosclerotic disease.[30] The data presented in Figure 1 show a large inter-individual variability in FEEC and support previous work demonstrating similar variability

in percent cholesterol absorption.[31] This is likely due to inter-individual differences in metabolic regulation rather than analytical error because our previous work shows that repeated FEEC measurements in the same individual have a coefficient of variation of about 10%.[8]

Because of the difficulty of performing metabolic tracer studies such as this one, very few data exist that address the relation of whole body cholesterol metabolism to CVD. It would be highly desirable to have a plasma biomarker for cholesterol excretion. Since cholesterol absorption from the intestine is not complete (averaging 57% in this study), cholesterol homeostasis must be maintained by increased cholesterol synthesis, increased cholesterol absorption, or a combination of these processes to avoid cholesterol deficiency. We used a composite plasma biomarker, the ratio of 5α-cholestanol/lathosterol, to reflect the balance between cholesterol absorption, represented by 5α-cholestanol, and cholesterol biosynthesis, represented by lathosterol, within each individual. The ratio was negatively correlated with FEEC ($r = -0.373$, $P = 0.0004$) and positively correlated with CIMT ($r = 0.415$, $P < 0.0001$). Previous investigators have shown that similar plasma absorption/synthesis ratios are associated with CVD.[16] It is possible that links to CVD in the absorption/synthesis biomarker literature might be explained, in part, by reduced FEEC as the final common pathway most closely linked to increased CIMT. Conversely, lower intestinal cholesterol absorption would result in cholesterol deficiency and increased compensatory biosynthesis, resulting in a lower absorption/synthesis ratio, higher fecal cholesterol excretion, and lower CVD risk. More work is needed to connect biomarkers with measurements of cholesterol metabolism, transport, CIMT, and/or CVD outcome.

Although CIMT is not a direct measure of clinical outcomes or events, it does reflect current information on the development of early atherosclerosis and there is abundant evidence that it is strongly related to well-validated CVD risk factors. The present study demonstrates that FEEC is negatively related to CIMT independently of established CVD risk factors, pointing to FEEC and whole-body cholesterol metabolism as a novel potential target for modulating CVD risk.

It has been demonstrated that bile acid sequestrants effectively reduce coronary artery disease outcomes, probably by reducing LDL cholesterol and increasing sterol excretion or RCT.[32, 33] Failure of CETP inhibitors to reduce cardiovascular events has indicated that it is not sufficient to simply raise HDL cholesterol.[34] The functional capacity of HDL in RCT might be more important mechanistically. Increased fecal cholesterol excretion is a prominent mechanism of action of phytosterols in foods and supplements,[35] but there have been no interventional studies with CVD outcomes to firmly establish the treatment effectiveness. A recent clinical trial of the drug ezetimibe, an inhibitor of cholesterol absorption, resulted in fewer CVD events even in the face of optimum statin drug treatment.[36] Although the result has been attributed to LDL cholesterol lowering, the actual mechanism is unknown and recent work shows that FEEC is increased about 70% by ezetimibe.[8] Further work is needed to establish the utility of treatments directed toward whole body cholesterol metabolism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

RCT	Reverse cholesterol transport
CIMT	Carotid intima-media thickness
TICE	Trans-intestinal cholesterol excretion
FEEC	Fecal excretion of endogenous cholesterol
HOMA-IR	Homeostasis model of insulin resistance

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Highlights

- The quantity and efficiency of cholesterol excretion were strongly and negatively correlated with carotid intima-media thickness (CIMT) in non-diabetic adults aged 30–80 years old.
- The association of fecal cholesterol excretion with CIMT was independent of age, sex, systolic blood pressure, hemoglobin A1c, LDL cholesterol, and HDL cholesterol.

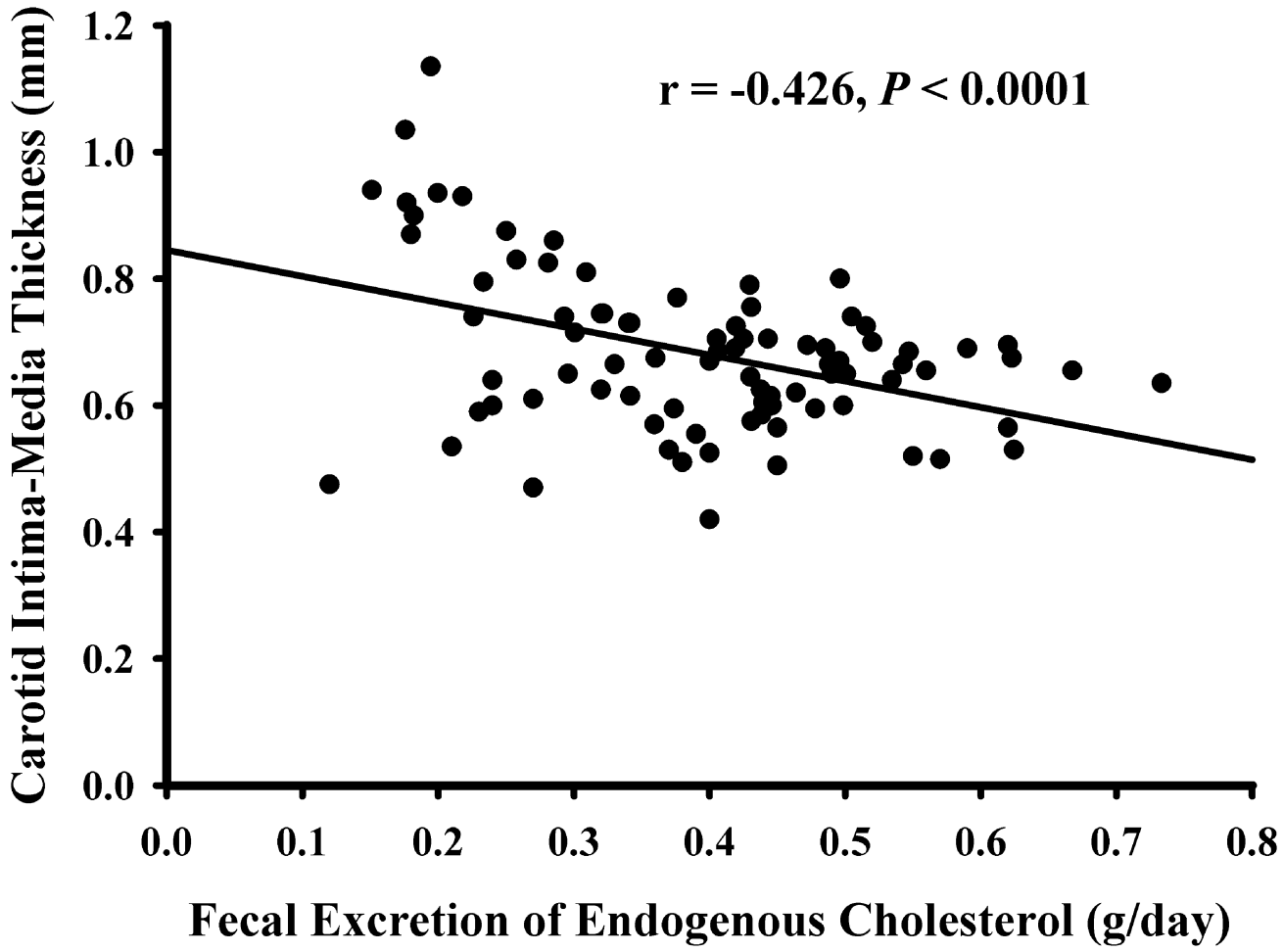

Figure 1. Relation between CIMT and FEEC

Simple regression of carotid intima-media thickness (CIMT) (mm) with fecal excretion of endogenous cholesterol (FEEC) (g/day) is presented for 86 subjects.

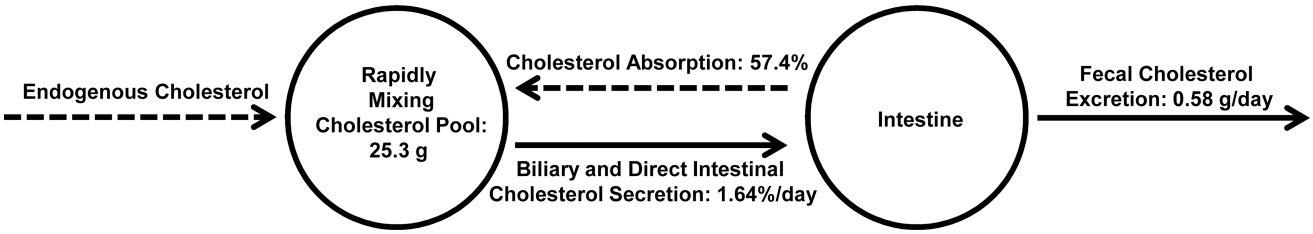

Figure 2. Partial model of body and intestinal endogenous cholesterol metabolism

Solid arrows depict cholesterol pathways with statistically significant negative correlations to carotid intima-media thickness (CIMT) while dotted lines show pathways with significant positive correlations to CIMT. Dietary cholesterol was controlled at a low level during measurements and is not included in the model. Cholesterol pathways that tend to reduce the rapidly-mixing body cholesterol pool are associated with reduced CIMT. Primary data are found in Table 3. Endogenous cholesterol input into the rapidly-mixing pool is estimated as the percent fall in plasma enrichment between days 2 and 15.

Table 1

Subject Characteristics at Baseline

	Mean ± SD
Sex: Women/Men (n)	52/34
Race: White/Black/Other (n)	62/16/8
Age (y)	53.7 ± 13.0
BMI (Kg/m^2)	28.1 ± 4.7
CIMT (mm)	0.68 ± 0.13
Lipids (mg/dL)	
Total cholesterol	181.6 ± 33.8
LDL cholesterol	101.2 ± 27.6
HDL cholesterol	59.6 ± 19.3
Triglycerides	101.1 ± 49.5
HbA1C (%)	5.51 ± 0.36
Fasting glucose (mg/dL)	95.2 ± 11.5
Fasting insulin (μU/mL)	12.4 ± 11.6
HOMA-IR	3.08 ± 3.36
Blood pressure (mmHg)	
Systolic	124 ± 17
Diastolic	74 ± 11
Non-cholesterol sterols	
5α-cholestanol (μg/mL)	2.52 ± 0.64
Lathosterol (μg/mL)	1.87 ± 0.92
5α-cholestanol/Lathosterol	1.76 ± 1.32
Medications (n):	
Statins	11
Anti-hypertensive	
Calcium channel blockers	6
Angiotensin II receptor antagonists	5
Angiotensin converting enzyme	8
Diuretic	1

Abbreviations: BMI, body mass index; HbA1C, hemoglobin A1c; HOMA-IR, homeostasis model of insulin resistance.

Table 2

Pearson Correlation between CIMT and Traditional Risk Factors

	CIMT	
	Correlation coefficient	***P* value**
Age	0.513	< 0.0001
BMI	0.200	NS
Systolic blood pressure	0.425	< 0.0001
Glucose homeostasis		
HbA1c	0.420	< 0.0001
Fasting glucose	0.272	0.0114
Fasting insulin	0.152	NS
HOMA-IR	0.143	NS
Plasma lipids and lipoproteins		
Plasma total cholesterol	−0.042	NS
Plasma LDL cholesterol	−0.061	NS
Plasma HDL cholesterol	−0.053	NS
Plasma triglycerides	0.104	NS

Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model of insulin resistance.

Table 3

Pearson Correlation between CIMT and Cholesterol Metabolic Parameters

	Mean ± SD	CIMT	
		Correlation coefficient	*P* value
Fecal excretion of total cholesterol (g/day)	0.58 ± 0.16	−0.472	< 0.0001
Endogenous origin (g/day)	0.39 ± 0.13	−0.426	< 0.0001
Dietary origin (g/day)	0.09 ± 0.02	−0.252	0.0193
Unlabeled (g/day)	0.09 ± 0.09	−0.141	0.1956
Percent cholesterol excretion (%/day)	1.64 ± 0.67	−0.343	0.0012
Fecal total fecal bile acid excretion (g/day)	0.52 ± 0.14	−0.088	0.4220
Cholesterol rapidly-mixing pool size (g)	25.3 ± 5.6	−0.075	0.4908
Endogenous cholesterol production rate (g/day)	0.89 ± 0.20	−0.425	< 0.0001
Endogenous cholesterol secretion into intestine (g/day)	0.95 ± 0.35	−0.274	0.011
Percent cholesterol absorption (%)	57.4 ± 8.4	0.279	0.0092
Plasma cholesterol-d_7 enrichment change (%)	71.1 ± 5.8	0.285	0.0083

Pearson correlations between factors related to fecal cholesterol excretion and carotid intima-media thickness (CIMT) in 86 subjects. Fecal excretion of total cholesterol includes bacterial degradation products and is also known as fecal neutral sterols. Percent cholesterol excretion is the percent of the rapidly-mixing cholesterol pool excreted in the stool per day. Endogenous cholesterol production rate is the sum of fecal cholesterol and bile acid excretion minus unabsorbed dietary cholesterol. Plasma cholesterol-d_7 enrichment change is the decline in plasma cholesterol enrichment from day 2 to day 15 expressed as a positive percent of the day 2 value; it is related primarily to the rate of dilution of plasma cholesterol by unlabeled endogenous cholesterol.