Intestinal cholesterol absorption in the chyluria model

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Abstract Isotopic methods for the measurement of dietary cholesterol absorption were compared with the lymph cholesterol balance procedure in filarial chyluria patients. After a single intravenous injection of radioactive cholesterol, absorption was found to be 746 ± 136 mg/day by method I, which is based upon the fecal endogenous neutral steroid mass measurement, and 471 ± 135 mg/day by the simultaneously measured lymph/plasma ratio of cholesterol specific activity (dpm/mg). The corresponding value, determined as the difference between lymph cholesterol transport on a cholesterol-containing diet (1500 mg) and on a cholesterol-free diet, was 622 mg/day. When radioactive cholesterol (1487 mg/day) was fed daily to a second patient, absorption determined by isotopic fecal recovery (353 mg/day) matched that obtained by the lymph balance procedure (326 mg/day). Transudation of plasma cholesterol into the intestinal lymph, estimated by the single intravenous injection of radioactive β-sitosterol, was independent of both the luminal content of plant sterols and the absorption of dietary cholesterol. The absorption of endogenous cholesterol was calculated by: *1*) subtracting the cholesterol originating from plasma (transudation) together with the absorbed dietary cholesterol found in lymph from the total mass of cholesterol transported in lymph, and *2*) the lymph balance method, i.e., after interrupting the endogenous cholesterol mucosal uptake by β-sitosterol feeding (9 g/day) while on a cholesterol-free diet. Endogenous cholesterol was preferentially absorbed compared to dietary cholesterol, but there was no competition for absorption. The major portion of dietary cholesterol found in lymph was esterified, but esterification was not a prerequisite for absorption.—**Doi, S. Q., H. Meinertz, K. Nilausen, E. C. Faria, and E.C.R. Quintão.** Intestinal cholesterol absorption in the chyluria model. *J. Lipid Res.* 1987. **28:** 1129–1136.

Supplementary key words dietary cholesterol absorption • endogenous cholesterol absorption • cholesterol absorption methods • cholesterol transudation • β-sitosterol feeding • cholesterol esterification

Early publications on the absorption of dietary cholesterol indicated that, in humans, there was an upper limit to the absorptive capacity (1–4). However, subsequent studies demonstrated that absorption of cholesterol was proportional to the intake and much greater than previously estimated (5–7).

Several methods dealing with the measurement of dietary cholesterol absorption in humans have been published. Most of them are based upon simultaneous measurements of labeled and unlabeled cholesterol in feces and plasma following administration of radioactive cholesterol (1, 4, 5). Other procedures are based on either the determination of plasma radioactivity only, after simultaneous oral and intravenous administration of cholesterol labeled with different isotopes (8), or on duodenal infusion of either radioactive cholesterol or cholesterol together with radioactive β-sitosterol or chromic oxide (9–11). Although they are all indirect procedures, there is considerable evidence that the values obtained by the different methods are essentially in agreement (12, 13).

A drawback common to all these methods is the possibility that isotopic exchange can cause either over- or underestimation of cholesterol absorption, depending upon the route of isotope administration (8, 9, 11, 12). Previous studies suggest that isotopic exchange does not interfere with the measurement of absorption of dietary cholesterol in rats (14 and Vasconcelos, K. S., unpublished data) or in humans (11). Chyluric patients offer a unique opportunity for the study of intestinal cholesterol absorption without the use of radioactive labels, since total cholesterol mass transported via intestinal lymphatics can be estimated when the amount of fat fed is used as a marker (15).

The uptake of intestinal luminal endogenous cholesterol into the lymph may also be measured in the chyluric patients provided that other sources of lymph cholesterol are properly taken into account. For example, *1*) the transudation of cholesterol from plasma may be evaluated after intravenous injection of radioactive β-

Abbreviations: TLC, thin-layer chromatography; GLC, gas-liquid chromatography; NS, neutral steroids.

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sitosterol (15); *2*) dietary cholesterol may be either eliminated by the use of a cholesterol-free diet, or may be measured by appropriately labeled cholesterol in the diet; and *3*) cholesterol synthesized by the intestinal mucosa may be quantified by the comparison of lymph to plasma cholesterol specific activity after the intravenous administration of radioactive cholesterol (15). Also, absorbed endogenous cholesterol in lymph may be measured as the difference between lymphatic cholesterol transport on a cholesterol-free diet, without and with simultaneous feeding of β-sitosterol, which interrupts cholesterol absorption (16).

It is not known whether the intestine absorbs exogenous cholesterol differently from the endogenous fraction, but there may be competition between exogenous and endogenous cholesterol for absorption (11, 17). Furthermore, cholesterol is esterified during absorption, but it is not known how critical the esterification is for the absorption of cholesterol in humans (18–20); so far, only data from experimental animals are available (18, 21, 22).

In the present study we have compared isotopic absorption to the value of the cholesterol mass absorbed, measured by the increment of lymph cholesterol when cholesterol was added to the diet of two patients. In one subject, the lymphatic transport of the endogenous cholesterol was also calculated after estimating other sources of lymph cholesterol such as the transudation of cholesterol from plasma. The latter was measured after a single intravenous administration of radioactive β-sitosterol. Furthermore, addition of a large dose of β-sitosterol to the cholesterol-free diet caused a decrement in lymph cholesterol, which provided an estimate of endogenous cholesterol absorption.

Finally, the mode of transport of absorbed dietary cholesterol in lymph was investigated in two studies after radioactive cholesterol was administered orally.

MATERIAL AND METHODS

Patients and diets

Steroids metabolic balance studies were carried out on three filarial chyluria patients. Their clinical data and dietary composition are presented in **Table 1.** Patient 1 was investigated on an outpatient basis with three weekly appointments for sample collection at Righospitalet, Copenhagen, Denmark. Patients 2 and 3 were hospitalized on a metabolic ward at the Hospital of the University of São Paulo Medical School, Brazil.

Patient 1 was fed exclusively oral liquid formula (A) divided into five meals a day, whereas Patient 2 was fed a liquid diet (B) divided into four meals a day, together with green vegetables and fruit juices. Diets were supplemented with mineral salts and vitamins as previously described (23). Patient 3 was maintained on a cholesterol-poor solid diet supplemented with oil at each meal.

Constant chyluria in patient 1 allowed for urine collections during long periods of time, whereas, in patients 2 and 3, collections were marred by intermittent chyluria.

In all patients body weight was maintained stable throughout the investigation. There was a minor increment of the serum cholesterol level during the initial days of cholesterol feeding in patient 1 and a decrement in patient 2; thereafter the levels remained stable.

The study protocol, approved by the local ethical committees, was explained to each patient and informed consent was obtained.

Experimental design

The study sequence of each patient is summarized in **Table 2.** Patient 1 was maintained for 20 days on cholesterol-free diet A (P_I) to which had been added 9000 mg/day of β-sitosterol (Eli Lilly Co., Indianapolis, IN) with the intent of decreasing the absorption of endogenous cholesterol from the intestinal lumen. He next received diet A without addition of β-sitosterol for another 21-day period (P_{II}). In the ensuing final period III, lasting 28 days, 1500 mg/day of cholesterol was added to the diet. This patient received a single intravenous dose of [1,2-^{3}H]cholesterol (150 μCi) and [4-^{14}C]β-sitosterol (55 μCi) on the first day of P_I. A second dose of radioactive β-sitosterol was administered on the first day of P_{III}. Radioactive cholesterol was injected to determine dietary cholesterol absorption during the log-linear phase of P_{III} by the currently used method I (1). The mass of dietary cholesterol transported in lymph was measured as the dif-

TABLE 1. *Clinical data and diet composition*

Patient	Age	Sex	Height	Weight	Calories per Day	Diet (% Calories in Parentheses) Type	Protein	Fat	Carbohydrate
	yr		*cm*	*kg*					
1	37	M	169	56.6	3162	A	soy isolate (16%)	cottonseed (37%)	dextro-maltose (47%)
2	74	F	142	57.6	1064	B	skimmed milk (15%)	soybean (40%)	saccharose + lactose (45%)
3	21	M	165	57.0	ad lib	C[a]	egg white	cottonseed	grains + starchy vegetables

[a]Ad libitum intake of egg white, grains, and vegetables together with 27.6 g/day of oil divided into two meals (% calorie distribution unknown).

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TABLE 2. Experimental design: diet periods and isotope administration

Patient	Diet Periods		
	P_I	P_{II}	P_{III}
1			
Diet	A (20)[a] + β-sitosterol (9000 mg/day)	A (21)[a]	A (28)[a] + cholesterol (1500 mg/day)
Isotope administration (intravenous)	[^{3}H]cholesterol (150 μCi)		[^{14}C]β-sitosterol (55 μCi)
Isotope administration (intravenous)	[^{14}C]β-sitosterol (55 μCi)		
2			
Diet	B (16)[a]	B (13)[a] + cholesterol (1487 mg/day)	
Isotope administration (oral)		[^{14}C]cholesterol (2.8 μCi/day)	
3			
Diet	C (19)[a]		
Isotope administration (oral)	[^{3}H]cholesterol (0.98 μCi/day)		

[a]Number in parentheses indicates the duration (days) of dietary periods.

ference in cholesterol content during the cholesterol feeding and the cholesterol-free dietary periods. Radioactive β-sitosterol provided a measurement of transudation of cholesterol from plasma to lymph (15).

Patient 2 was fed cholesterol-free diet B for 16 days (P_I); she then received 1487 mg/day of cholesterol in the diet with [4-^{14}C]cholesterol (2.8 μCi) for another 13-day period (P_{II}). Absorption of dietary cholesterol measured from radioactive cholesterol recoveries in either feces or lymph was compared to the cholesterol mass increment in lymph as described for patient 1.

Patient 3, on low-cholesterol diet C for 19 days, was given cholesterol (233 mg/day) together with [1,2-^{3}H]cholesterol (0.98 μCi/day) in gelatin capsules, divided into two daily doses, with the sole purpose of measuring the proportion of free to esterified dietary cholesterol found in lymph. Similar measurements were done in the study of patient 2.

In patient 1, dietary phytosterols, added to the diet in period I and inherent to the dietary cotton seed oil, served as a marker for the recovery of fecal neutral sterols (17, 24); chromic oxide tablets (300 mg/day) were utilized to correct for fecal bile acid recovery (25). Since the diet of patient 2 contained vegetables, the intake of plant sterols was not precisely known, and the possible intraluminal sterol degradation remained uncorrected; nevertheless recovery of neutral sterols in feces was corrected for by oral chromic oxide tablets (1000 mg/day).

Analytical procedures

Radioactive sterols were purified by Florisil TLC on the solvent system ethyl ether–heptane 55:45 (26). For the intravenous infusion of [1,2-^{3}H]cholesterol (New England Nuclear Corp., Boston, MA) and of [4-^{14}C]β-sitosterol (Amersham International, Buckinghamshire, England) sterols were dissolved in 1 ml of ethanol which was added to 100 ml of saline. [4-^{14}C]Cholesterol (Amersham International) dissolved in 1 ml of ethanol was homogenized in the dietary oil during preparation of formula feeding (patient 2). [1,2-^{3}H]Cholesterol (New England Nuclear Corp.) in ethanol was mixed with crystalline cholesterol for preparation of capsules (patient 3). Radioactivity was measured in the Beckman LS-100 beta scintillation counter as previously described (26).

Urine was completely collected in 24-hr periods and kept under refrigeration at 4°C. Aliquots were lyophilized and fat was extracted with chloroform followed by chloroform–methanol 2:1. Aliquots were taken for radioactivity measurement, to correct for losses during further steps of sterol analysis and for fat measurement by a gravimetric procedure (24, 27). Free and esterified cholesterol were separated by TLC on silica gel G with the solvent system ethyl ether–heptane 55:45. Sterol mass was then determined by GLC after mild alkaline hydrolysis (26).

Feces were collected daily and 2-day pools were analyzed. Sterols and fat were measured as previously mentioned (24, 26, 27).

Calculations

Steroid balance was carried out in patients 1 and 2. Cholesterol excreted in the urine was included in the balance calculations.

1. Method I for dietary cholesterol absorption is summarized below.

1.a. Fecal endogenous neutral steroid mass (mg/day) = total fecal NS radioactivity/specific activity of serum cholesterol 1 day previously.

1.b. Unabsorbed dietary cholesterol (mg/day) = total fecal NS mass − fecal endogenous NS mass.

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1.c. Absorption of dietary cholesterol (mg/day) = cholesterol intake − unabsorbed dietary cholesterol.

2. Dietary cholesterol absorption based on continuous oral administration of radioactive cholesterol and chromic oxide was measured as:

$$\text{Intake (mg/day)} \times \left(1 - \frac{\text{fecal } ^{14}\text{C-labeled neutral steroids (dpm)/chromic oxide (mg)}}{\text{dietary } [^{14}\text{C}]\text{cholesterol (dpm)/chromic oxide intake (mg)}}\right).$$

3. Total daily lymph cholesterol transport (C) as mg/day through the intestinal lymphatics is based upon the knowledge of the shunt size of chyle fistula as described previously (15).

3.a. Shunt size $= \dfrac{\text{urine fat (g/day)}}{\text{fat intake (g/day)}}$.

3.b. $\text{C (mg/day)} = \dfrac{\text{urine cholesterol (mg/day)}}{\text{shunt size}}$

4. Dietary cholesterol absorption measured by the decrease of the lymph specific activity relative to that of plasma:

$$\text{Absorption (mg/day)} = \left(1 - \frac{\text{sp act of lymph cholesterol}}{\text{sp act of plasma cholesterol}}\right) \times \text{C}.$$

5. Estimate of dietary cholesterol absorption derived from the recovery of the lymph radioactivity after radiolabeled cholesterol feeding:

$$\text{Absorption (mg/day)} = \frac{\text{sp act of urine cholesterol (dpm/mg)}}{\text{sp act of dietary cholesterol (dpm/mg)}} \times \text{C}.$$

6. Measurement of absorption by lymph cholesterol balance (mg/day) = C during a period on a fixed cholesterol intake − C on a cholesterol-free diet.

7. Cholesterol transudation from plasma to lymph (T):

$$\text{T (mg/day)} = \frac{\text{radioactive } \beta\text{-sitosterol as dpm/mg of cholesterol in lymph (urine)}}{\text{radioactive } \beta\text{-sitosterol as dpm/mg of cholesterol in plasma}} \times \text{C}.$$

8. Endogenous cholesterol absorption:

8.a. Lymph cholesterol of endogenous origin (indirect procedure) = total daily lymph cholesterol transport − (transudation + dietary cholesterol absorption).

8.b. Total intestinal endogenous cholesterol pool = lymph cholesterol of endogenous origin (8.a) + endogenous cholesterol excreted in feces, where the latter is represented by the GLC-measured fecal NS on the cholesterol-free diet, or fecal endogenous NS on the cholesterol-containing diet (1.a).

8.c. Percent absorption of endogenous cholesterol = lymph cholesterol of endogenous origin (8.a) × 100/total intestinal endogenous cholesterol pool (8.b).

8.d. Direct measurement in lymph of the endogenous *cholesterol absorption* = C *on a cholesterol-free diet* − C on a cholesterol-free diet containing a large amount of plant sterols.

RESULTS AND DISCUSSION

Fecal steroid balance and lymph cholesterol transport data of patients 1 and 2 are presented in **Table 3.** Changes in lymph cholesterol transport elicited by each dietary modification were abrupt and the levels were maintained steady throughout each study period.

Dietary cholesterol absorption

In both patients the exact mass of cholesterol absorbed was obtained by the lymph cholesterol balance (equation 6). Measurements based on the intravenous isotopic administration (patient 1, method I, equation 1) slightly overestimated the absorption value by 124 mg/day (20%) compared to the lymph balance procedure. As expected, the absorption of unlabeled cholesterol lowered the specific activity of cholesterol in the lymph relative to that of the plasma in P_{III} **(Fig. 1)**, and thus it was possible to calculate the absorption by the lymph/plasma radioactivity ratio (equation 4); the value obtained was 151 mg/day (24%) lower than that of the lymph balance procedure. The remarkable similarity of the degrees of over- and underestimation, 20 and 24%, respectively, by the two isotopic methods, compared to the isotope-independent lymph balance procedure, could have been attributed to random chance. However, in our view it can best be explained by the exchange of isotope among the lymph, plasma, mucosa, and intestinal content.

Although method I overestimated absorption, this error does not invalidate previous conclusions that a substantial fraction of dietary cholesterol is indeed absorbed; the lymph balance (equation 6) showed it to be 41% of the intake, which agrees well with previous studies (5–7).

Cholesterol entering the body triggers the following compensatory mechanisms: decrease of body synthesis and changes in the excretion of endogenous neutral steroids and bile acids (13, 28–30). Accordingly, in patient 1 the decrement in cholesterol synthesis, measured by the balance difference between P_{III} (−200 mg/day) and P_{II} (−856 mg/day), was 656 mg/day, a value closer to the absorption by the lymph balance method (622 mg/day) than by the isotopic method I (746 mg/day).

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TABLE 3. Fecal steroid excretion, lymph cholesterol balance, dietary cholesterol absorption, transudation of cholesterol from plasma into lymph, and endogenous cholesterol absorption

Parameters	Patient 1			Patient 2	
	P_I (20)[a]	P_{II} (21)[a]	P_{III} (28)[a]	P_I (16)[a]	P_{II} (13)[a]
Serum cholesterol (mean mg/dl ± SD)	135 ± 11	139 ± 31	157 ± 19	357 ± 45	314 ± 58
Intake (mg/day)					
Cholesterol	0	0	1500	0	1487
β-Sitosterol	9000	392[b]	392[b]	[c]	[c]
Chromic oxide	300	300	300	1000	1000
Fecal recovery (mean %)					
β-Sitosterol	54.3	102.8	78.1		
Chromic oxide	88.4	96.1	116.8	73	58[d]
Fecal steroids (mean mg/day ± SD)	(16)[e]	(14)[e]	(22)[e]	(6)[e]	(7)[e]
Neutral	998 ± 105	456 ± 28	1172 ± 156		
Acidic	374 ± 246	110 ± 88	82 ± 103		
Total	1372 ± 234	566 ± 88	1254 ± 203		
Urine cholesterol (mean mg/day ± SD)	235 ± 43 (18)[e]	272 ± 69 (17)[e]	445 ± 90 (26)[e]	37 ± 30 (6)[e]	252 ± 48 (7)[e]
Urine fat (mean g/day ± SD)	22.88 ± 4.30	21.97 ± 6.14	26.61 ± 7.35	1.73 ± 1.08	8.86 ± 0.92
Fat intake (g/day)	130	130	133	36	38
Total steroid balance (mean mg/day ± SD)[f]	− 1603 ± 234 (16)[e]	− 856 ± 125 (14)[e]	− 200 ± 237 (22)[e]		
Total daily lymph cholesterol transport[g] (mean mg/day ± SD)	1334 ± 160 (18)[e]	1612 ± 214 (17)[e]	2234 ± 417 (26)[e]	756 ± 359 (6)[e]	1082 ± 167 (7)[e]
Dietary cholesterol absorption[h] (mean mg/day ± SD)					
Isotopic method I (Eq. 1.c)			746 ± 136 (49.7%)		
Isotopic fecal recovery method (Eq. 2)					353 ± 852 (23.7%)
Lymph/plasma radioactivity ratio (Eq. 4)			471 ± 135 (31.4%)		
Isotopic lymph recovery (Eq. 5)					300 ± 30 (20.2%)
Lymph cholesterol balance (mg/day) (Eq. 6)			622 (41.5%)		
Transudation of cholesterol from plasma (Eq. 7) (mean mg/day ± SD)	971 ± 194	(1028)[i]	1086 ± 246		
Endogenous cholesterol absorption (Eq. 8.a) (mg/day)	363	584	526		

[a] Duration of study period (days) is in parentheses.
[b] Intrinsic to the diet.
[c] Unknown.
[d] Utilized for the fecal recovery of oral labeled cholesterol.
[e] Number of days analyzed is in parentheses.
[f] Intake − (total fecal steroid + urine cholesterol).
[g] See calculation section (C value : Eq. 3.b).
[h] Values in parentheses represent cholesterol absorption as percentage of intake.
[i] Mean of transudation in P_I and P_{III} was utilized for calculation of endogenous cholesterol absorption in P_{II}.

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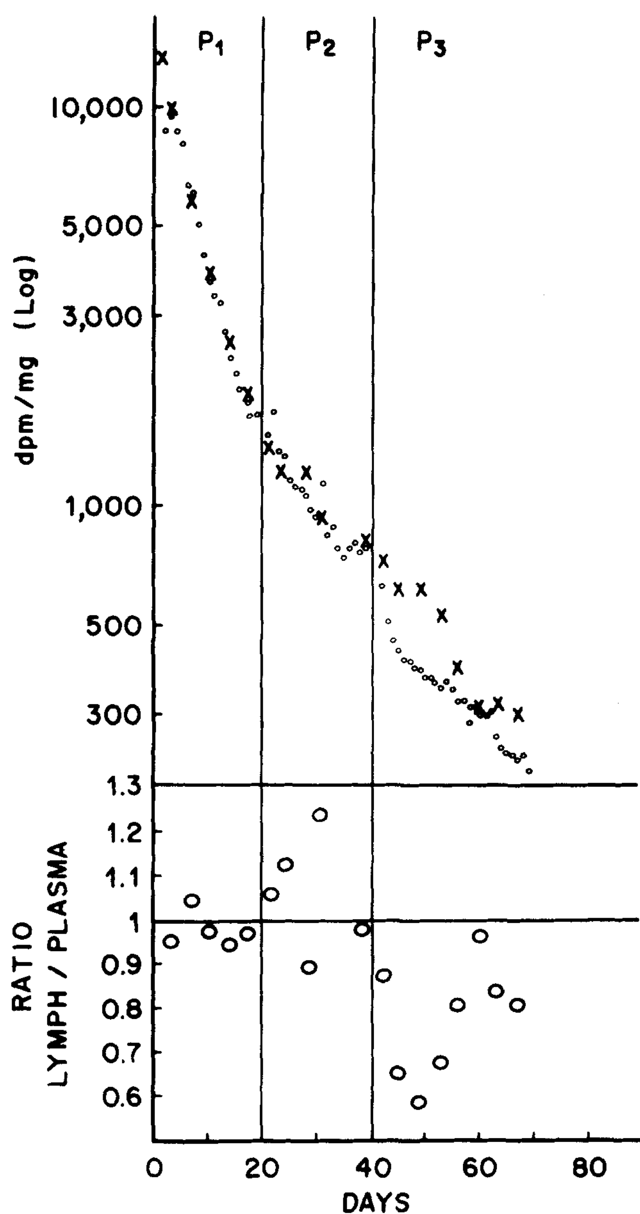

Fig. 1. Upper panel shows the specific activity of [^{3}H]cholesterol in plasma (X) and lymph (O) during the experimental periods of patient 1. The lower panel shows the lymph/plasma specific activity ratio (O).

In patient 2 (Table 3), absorption by the isotopic fecal recovery procedure (equation 2) was virtually identical to that obtained by the lymph balance method (equation 6) and therefore did not seem influenced by isotopic exchange, probably because measurements were carried out several days after starting radioactive cholesterol feeding (12). In this patient, radioactivity recovery in lymph (corrected for shunt size) together with feces (corrected for chromic oxide) accounted for 96% of the radioactivity fed daily. Reexcretion of absorbed isotope through the biliary system must have been negligible, for otherwise the measurement of absorption based upon the fecal recovery would have been smaller than that obtained by the lymph balance. Besides, finding radioactivity in bile would have meant that radioactivity in plasma cholesterol, which is the bile precursor, ought to influence the radioactivity of lymph because a substantial share of the lymph cholesterol is known to be of plasma origin.

Transudation of cholesterol from plasma

In patient 1, independently calculated transudation in PI and PIII (Table 3) did not differ significantly despite the large amounts of dietary cholesterol absorbed during PIII. This finding is in agreement with a previous study (15) suggesting that transudation is a passive process independent of the intestinal cholesterol content. We have obtained similar results in lymph duct-cannulated rats (Sipahi, A.M., K. Stechhahn, H.C.F. Oliveira, and E.C.R. Quintão, unpublished observations). The mean of PI and PIII transudation values was used in PII in calculations discussed in the next section.

Endogenous cholesterol absorption

An estimate of endogenous cholesterol absorption was available from the study of patient 1 only (Table 3). It was based on the assumption that the contribution of newly synthesized cholesterol to lymph is negligible, since the lymph/plasma cholesterol specific activity ratio was close to 1 throughout PI and PII as well as in cases described in another report (15). Accordingly, endogenous cholesterol mass in lymph is obtained by equation 8.a. During PIII the exact amount of dietary cholesterol in lymph (determined by the lymph balance procedure, equation 6) was used in this calculation.

Fecal endogenous neutral steroids were measured by GLC in PI and PII (Table 3) and isotopically in PIII (431 ± 142 mg/day). Therefore, absorbed endogenous cholesterol (equation 8.c.) represented 27%, 56%, and 55%, respectively, of the total intestinal endogenous cholesterol pool that was estimated (equation 8.b) to be 1361 mg/day (PI), 1040 mg/day (PII), and 957 mg/day (PIII). In PIII, 41.5% of dietary cholesterol was absorbed (equation 6). Thus, endogenous as compared to exogenous cholesterol was preferentially absorbed, in agreement with previous studies (11).

The amount of endogenous cholesterol absorbed during PI was the lowest value obtained in all of the three periods, owing to a partial inhibition due to a high intake of phytosterols. It is noteworthy that, compared to the control period (PII), β-sitosterol lowered the amount of endogenous cholesterol in PI lymph by 278 mg/day (equation 8.d), even though it simultaneously increased the fecal endogenous neutral steroids output by 542 mg/day. This discrepancy can only be explained through an action of β-sitosterol on the intestinal mucosa eliciting a greater excretion of cholesterol independent of its transport into the lymph. In other words, phytosterols trigger an in-

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crease in fecal output of cholesterol of mucosal origin, arising either from local synthesis or from plasma.

The inhibition of the endogenous cholesterol absorption by β-sitosterol was only partial, since endogenous cholesterol was present in P_I lymph (363 mg/day). If ordinary dietary doses rather than pharmacological doses of phytosterols had been used, the amount of endogenous cholesterol carried in P_I lymph would have been 641 mg/day (278 + 363), which is remarkably close to the estimated amount of lymph endogenous cholesterol in P_{II} (584 mg/day) when only a trace amount of β-sitosterol was fed (equation 8.a).

Role of esterification in the absorption of dietary cholesterol

In patient 2, the distribution of cholesterol between the free and the esterified fraction was 36.5% and 63.5%, respectively, when measured as radioactivity, but 45.5% and 54.5%, respectively, when measured by mass. In patient 3, free and ester radioactivity fractions were 46.5% and 53.6%, respectively, whereas by mass measurement 42.4% was free and 57.5% was esterified.

In both cases the major share of dietary cholesterol in lymph was represented by the ester component. This was most pronounced in patient 2.

However, as shown in Table 3, absorption measured by the lymph balance method and by the lymph radioactivity recovery were virtually identical in this patient. Consequently, the finding of only 63.5% of labeled cholesterol in the ester form supports the conclusion that esterification was not essential for absorption of dietary cholesterol.

Previous studies have also shown that the larger share of radioactive lymph cholesterol was esterified after its administration by mouth. But inasmuch as the cholesterol mass involved was negligible as opposed to a regular diet, these findings may not have been representative (27, 31).

The amount of esterified dietary radioactive cholesterol in lymph may depend on the relative proportions of cholesterol and fat in the diet fed; cholesterol, in percent of the fat fed, was 3.9% in patient 2 and 0.8% in patient 3. In other words, there may have been a preferential esterification of cholesterol when the mass was high relative to the fat intake, as shown by others in rats and nonhuman primates (14, 17, 32). In the present experiments, total lymph cholesterol included cholesterol from plasma, which is known to be largely esterified, thus explaining the differences in the distribution between the free and ester fractions in terms of radioactivity and in terms of mass. ■

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