Determination of cholesterol absorption in man by intestinal perfusion

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Summary In this study a technique is described for estimating net absorption of total cholesterol (endogenous + exogenous) that enters the intestine. The method employs intubation of patients with a 3-lumen tube that contains a 10-cm mixing segment in the duodenum and a 100-cm absorption segment in the jejunum. A liquid formula diet containing varying amounts of exogenous cholesterol is infused continuously into the upper duodenum for a period of several hours; the formula diet stimulates constant contraction of the gallbladder and thus provides for continuous secretion of biliary cholesterol into the duodenum. Through constant infusion of β-sitosterol as a marker, the input of endogenous + exogenous cholesterol can be measured at the end of the 10-cm mixing segment. Net cholesterol absorption is estimated from the disappearance of cholesterol relative to β-sitosterol over the next 100-cm of jejunum. When radioactive cholesterol was also used as a marker, radioactivity usually disappeared more rapidly than the mass of cholesterol over the absorption segment; this suggests that a significant amount of isotope exchange occurs in the upper intestine. Using β-sitosterol as a marker, the extent of exchange can be determined. In six patients, cholesterol inputs ranged from 51 to 118 mg/hr, and net percentage absorption was 34–56%. When inputs of cholesterol were acutely increased by enhancing exogenous cholesterol, absolute absorption was uniformly increased, but percentage absorption either remained the same or was decreased somewhat. Changing inputs of β-sitosterol had a striking effect on cholesterol absorption, and relatively small increments of β-sitosterol almost always produced corresponding reductions in uptake of cholesterol. The intestinal perfusion method appears to provide certain advantages over previous techniques for estimating cholesterol absorption in man. It measures absorption of total cholesterol entering the upper intestine, including that derived from both endogenous and exogenous sources. Measurements can be made over short periods of time, and the method allows for determination of effects of acute changes in the intestinal milieu on cholesterol absorption. Finally, the technique defines the extent of isotope exchange between cholesterol in the intestinal mucosa and in the lumen.

Supplementary key words total cholesterol absorption · β-sitosterol

Cholesterol enters the intestinal tract from two major sources, the diet and biliary secretion. Normally, the biliary contribution is as great, or even greater, than that of the diet (1–3); thus, cholesterol

TABLE 1. Clinical profile

Patient	Age	Weight	% Ideal Weight	Plasma Lipids	
				Cholesterol	Triglycerides
	yr	*kg*		*mg/dl*	*mg/dl*
V.C.	37	125	170	173	88
J.M.	50	93	155	192	109
C.L.	41	111	148	201	178
D.L.	46	99	159	232	118
L.A.	29	131	174	186	100
L.McG.	55	113	166	242	130
A.B.	46	96	135	136	100
W.N.	56	73	112	199	294
W.V.	48	74	107	170	205
D.W.	44	80	120	144	182

that is absorbed from the intestine is derived from both endogenous and exogenous sources. Several previous attempts have been made to measure cholesterol absorption in man; however, most of the techniques developed thus far estimate absorption of exogenous cholesterol only (4–10). In the present study, a method has been developed to estimate absorption of the total quantity of cholesterol entering the intestine; this includes cholesterol from both endogenous and exogenous sources. Our technique involves intestinal intubation of patients and, by use of marker dilution techniques, uptake is measured over the upper portion of the small intestine where cholesterol is mainly absorbed. This report describes the technique and discusses its advantages, limitations, and possible applications.

Patients

Ten patients were studied on the Special Diagnostic and Treatment Unit (metabolic ward) of the Veterans Administration Hospital, San Diego, CA. Clinical data relevant to the patients are given in **Table 1.** Four of these patients (A.B., W.N., W.V., and D.W.) had mild elevations of plasma lipids as outpatients; they were not hypercholesterolemic at the time of study, but two had mild increases in triglycerides. They had been ingesting a low cholesterol, solid food diet for several weeks prior to their study (11). The remaining subjects had varying degrees of obesity and were in the hospital primarily for weight loss. These patients were studied either during weight maintenance on the same low cholesterol diet, or while they were taking a 1000 kcal diet of liquid formula (Sustacal, Mead Johnson, Evansville, IN). Thus, in all cases, the previous diet contained less than 200 mg of cholesterol per day.

Experimental technique

Intubation procedure. Patients were intubated with a 3-lumen tube on the night preceding the study, and the following morning the position of the tube was adjusted by X-ray. The tube was constructed so that the most proximal outlet (Tube 1) was adjacent to the ampulla of Vater, the second outlet (Tube 2) was 10 cm distal, just past the ligament of Trietz, and the third port (Tube 3) was 100 cm beyond the second.

The three-lumen tubes were constructed from polyvinyl chloride tubing, outside diameter 2.5 mm (Pharmaseal Laboratories, Glendale, CA) and from similar radio-opaque tubing (Ferraris Development and Engineering Company, Ltd., Edmonton, London, England). Tubes were tipped with stainless steel olives containing 16 perforations; the distal one was weighted with a mercury bag.

Preparation of infusion solutions. Liquid formulas were prepared from dextrose, fat, and protein at a concentration of 1.25 kcal per g as described by Ahrens (11). The formula contained 40% of calories as fat, in the form of lard. Protein (RI-5) was prepared from milk by Ross Laboratories (Columbus, OH). When supplemental sterols, which included cholesterol and β-sitosterol, were given, they were administered in a separate solution. When supplemental cholesterol was used, 4.4 g of cholesterol was dissolved in 40 g of triglycerol monooleate; this lipid stabilizer was made available by Mr. V. B. Babayan and Capitol City Products, Columbus, OH. The solution was added to 1 liter of water and homogenized for 3 min at high speed in a Waring blender. β-Sitosterol, which was also dissolved in triglycerol monooleate, was prepared in several concentrations; 20 g of the stabilizer were used for every g of β-sitosterol. The β-sitosterol employed in these studies was kindly supplied by Mr. Erold Diller, Eli Lilly Company, Indianapolis, IN. This preparation was derived from tall oil and contained 93% of sterols as β-sitosterol; the remaining 7% of sterols was a mixture of campesterol and stigmasterol. In most studies, radioactive cholesterol was also used as a marker; a solution of 5 g of triglycerol monooleate containing 5 mg of nonlabeled cholesterol was homogenized with 1 liter of water and to this solution approximately 2μCi of [4-^{14}C] or [1,2-^{3}H]cholesterol (New England Nuclear Corp., Boston, MA) was added in 1 ml of ethanol. Isotope purity was checked by thin-layer chromatography; greater than 95% of radioactivity had an R_f identical to that of cholesterol.

Infusion and aspiration procedure. Liquid formula, markers, and supplemental sterols were infused at a constant rate through the most proximal outlet (Tube 1). The formula was infused with a Sigmamotor pump (Model TM 20-4), Sigmamotor, Inc., Middleport, NY, and markers were delivered with a Harvard

infusion-withdrawal pump (Model 940, Harvard Apparatus Co., Inc.,Mills, MA). Formula input was adjusted to deliver 1/24 of the subject's daily caloric requirement for weight maintenance during each hour of infusion; the formula contained 1.25 kcal/g. The rate of formula infusion was approximately 100 ml/hr. Solutions containing markers and supplemental sterols were delivered at a rate of about 25 ml per hr. When β-sitosterol was used as a marker, it was infused at a rate of 7–11 mg/hr.

Constant aspiration of intestinal contents was carried out from the two more distal outlets. The rate of aspiration from each site was about 12 ml per hour, and has been shown to represent less than 5% of the flow of intestinal contents (1); aspirates were transferred to glass vials containing 12 ml of ethanol.

Analysis of intestinal sterols. On each sample of intestinal contents, the masses of cholesterol and β-sitosterol and the radioactivity of cholesterol were determined. A 10-ml aliquot of the aqueous–alcohol mixture of intestinal contents was transferred to a 50-ml centrifuge tube capped with a Teflon-lined stopper. Seven ml of ethanol and 2 ml of 10 N NaOH were added, and the mixture was heated in a water bath at 65°C for one hr. Neutral sterols were extracted with 30 ml of petroleum ether (PE); the extract was transferred to a 50-ml centrifuge tube, back-washed with 10 ml of 50% ethanol, and evaporated under nitrogen. After evaporation of the PE extract, the residue was dissolved in 10 ml of ethyl acetate containing 1 mg of 5α-cholestane. Five milliliters were counted for ratioactivity, and the remaining was analyzed by gas–liquid chromatography (GLC). Cholesterol and β-sitosterol were measured on GLC as their trimethylsilyl ethers, as described by Miettinen, Ahrens, and Grundy (12).

Calculation of cholesterol absorption. Net hourly absorption of cholesterol over the 100-cm segment of tubing was calculated as follows:

Net cholesterol absorption (mg/hr) = total cholesterol inflow at tube 2 (mg/hr) − total cholesterol outflow at tube 3 (mg/hr) Eq. 1

where cholesterol inflow (mg/hr) = cholesterol:β-sitosterol ratio at tube 2) × β-sitosterol input (mg/hr) at tube 1 Eq. 2

and cholesterol outflow (mg/hr) = (cholesterol:β-sitosterol ratio at tube 3) × β-sitosterol input (mg/hr) at tube 1 Eq. 3

Calculation of luminal–mucosal exchange (or intestinal secretion) of cholesterol. When radioactive cholesterol was used as a marker, it was noted that, in most patients, radioactivity disappeared to a greater extent than the mass of cholesterol. This difference could be explained either by isotopic exchange between mucosal and luminal cholesterol, or by secretion of cholesterol by the mucosa. As explained subsequently in the Discussion section, it is not possible to differentiate between these two processes, but in the present paper we have chosen the term "exchange". The mass of exchanged cholesterol was thus determined as follows:

Exchanged cholesterol (mg/hr) = cholesterol absorption (mg/hr) (calculated from radioactive cholesterol as marker) − cholesterol absorption (mg/hr) (calculated from β-sitosterol as marker) Eq. 4

where cholesterol absorption (mg/hr) (calculated from radioactive cholesterol) = total cholesterol inflow at tube 2 (mg/hr) − labeled cholesterol outflow at tube 3 (mg/hr) Eq. 5

where total cholesterol inflow is derived from Equation 2, and labeled cholesterol outflow = (radioactivity outflow) (dpm/hr) at tube 3 ÷ sp act (dpm/mg) of cholesterol at tube 2 Eq. 6

where radioactivity outflow = radioactivity (dpm): β-sitosterol (mg) ratio at tube 3 × β-sitosterol input (mg/hr) at tube 2 Eq. 7

and cholesterol absorption (calculated from β-sitosterol as marker) is calculated from Equation 1.

When exogenous cholesterol was perfused in our studies, the assumption was made that endogenous and exogenous cholesterol were absorbed to the same extent. This assumption seems justified because exogenous cholesterol, which is dissolved in a micellar solution of triglycerol monooleate, is mixed with micellar biliary cholesterol.

Results

Net absorption of cholesterol in the steady state. **Fig. 1** shows successive hourly values for net cholesterol absorption during 10–15 hr of steady state perfusion for six patients, and average values for each patient are given in **Table 2.**

The data show that fluctuations in absorption from hour to hour were variable. In some patients the variation was small, but in others it was considerable; because of this variability the accuracy of the method should be enhanced by carrying out the study over a period of several hours. The data suggest that a valid mean value for absorption might not be obtained if measurements were made over short periods (e.g., 1–3 hr). Despite hourly variations, there were generally no consistent trends towards higher or lower values throughout the steady state

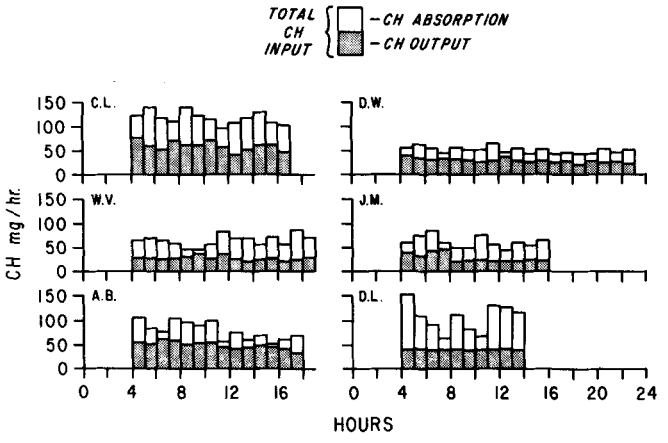

Fig. 1. Net cholesterol absorption during steady state perfusion for six patients. Each bar represents values for 1 hr. New absorption is the difference between cholesterol inflow (input) at tube 2 and outflow at tube 3. The first 4 hr were allowed for equilibration. CH = cholesterol.

period; an exception was patient A.B. in whom percentage absorption decreased in the last half of the study. It should be noted that absorption did not progressively increase in any patient, as might have been expected if the intestine had continued to "gather" upon the tube so that the absorbing surface between proximal and distal outlets was increasing throughout the period.

There could be several causes for the hourly fluctuations of cholesterol absorption noted in some of the patients. For example, the rate of cholesterol input was variable due to differences in hourly secretion rates of cholesterol by the liver. Also, fluctuations in absorption could be the result of differences in intestinal motility which could cause alterations in intestinal transit time.

Effects of low and high input of cholesterol. In five patients, absorption measurements were made at two levels of cholesterol input (**Fig. 2** and **Table 3**). In the first period, infusion rates of cholesterol were small, and most of the input was due to endogenous biliary cholesterol. In the second period, exogenous cholesterol was increased so that the input increased by 94–224%. In three patients (W.N., J.M., and V.C.), net absorption during high cholesterol inputs became reasonably constant despite some hour-to-hour fluctuations. A different pattern was seen in the other two patients (L.A. and L. McG.); in these patients, absorption declined towards the latter part of the study. Apparently, the mucosa became partially saturated in the latter part of the study by excessive cholesterol intake, the absorption decreased towards control values.

Table 3 shows that the pattern of response to high cholesterol inputs varied considerably from patient to patient. In patient V.C., increasing input from 122 to 228 mg/hr caused a marked increase in absolute net absorption without a change in percentage absorption; this patient had a remarkably constant rate of absorption throughout the study. Patient J.M. also had a distinct increment in cholesterol absorption when the input was enhanced; this increment was retained throughout the period of infusion. With increased input of cholesterol, patients L.A. and L. McG. clearly had an initial increment in absorption, but in the latter part of the study, absolute absorption was not greater than control values despite a much greater cholesterol input.

Influence of β-sitosterol on cholesterol absorption. In six studies in five patients, β-sitosterol was infused at two levels of intake (**Figure 3, Table 4**). In most patients, cholesterol inputs were approximately the same in both periods; however, for unknown reasons, one patient (V.C.) had cholesterol inputs that were unusually high during the first portion of the second period. Absolute and percentage absorption were decreased in all patients during the higher rates of infusion of β-sitosterol. In two patients (L.A. and J.M.), higher inputs of β-sitosterol caused almost total obliteration of cholesterol absorption. On the other hand, a high infusion of β-sitosterol had little effect on absolute absorption rates in patient V.C.; nevertheless, absorption rates in this patient were relatively low even during low infusion rates of β-sitosterol. It is of interest that absorption was significantly reduced even when inputs of β-sitosterol were less than those of cholesterol and, for a given increment of β-sitosterol, there was an increment in unabsorbed cholesterol corresponding to 37–113% (average 79%) of the increase in β-sitosterol.

Exchange of cholesterol between mucosa and lumen. The extent of mucosal–luminal exchange is shown in

TABLE 2. Cholesterol absorption in the steady state

Patients	No. of Hourly Determn.	Cholesterol Inflow[a]	Cholesterol Outflow	Cholesterol Absorption
		mg/hr ± SD	*mg/hr ± SD*	*mg/hr (%)*
J.M.[b]	12	61 ± 11	30 ± 9	31 ± 10 (51)
C.L.	13	118 ± 13	60 ± 10	58 ± 10 (49)
A.B.	14	79 ± 18	52 ± 6	27 ± 15 (34)
D.W.	17	51 ± 7	32 ± 5	19 ± 8 (37)
D.L.	10	106 ± 29	42 ± 0.4	64 ± 25 (60)
W.V.	15	66 ± 12	29 ± 4	37 ± 13 (56)

[a] Cholesterol inflow equals the sum of endogenous and exogenous cholesterol passing the outlet of Tube 2 per hr. In these studies the formula used was relatively low in cholesterol, and most of the inflow was from endogenous sources; i.e., from biliary secretion.

[b] Patient J.M. was on caloric restriction (1000 kcal/day) before the perfusion study.

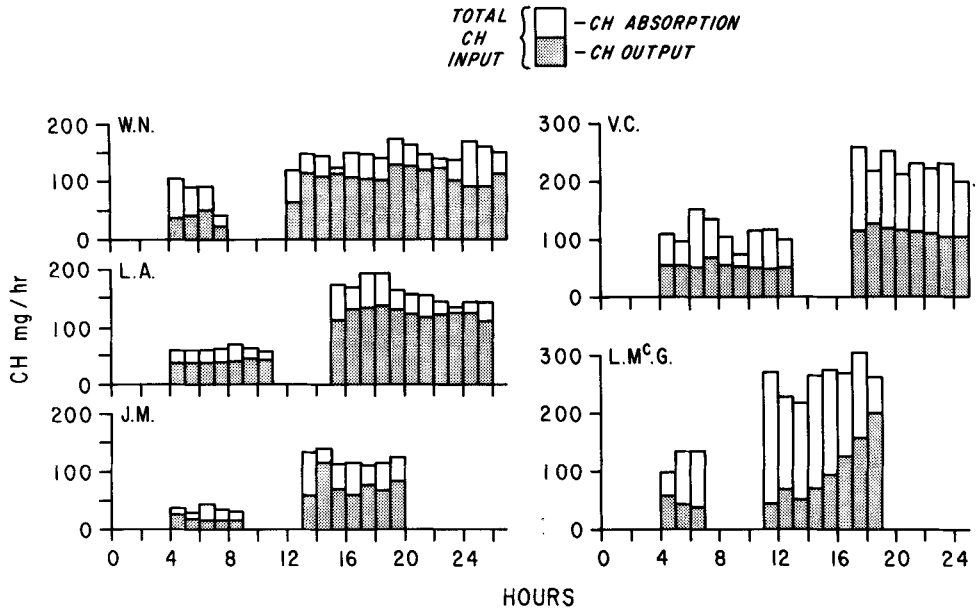

Fig. 2. Net cholesterol absorption during normal and high inputs of cholesterol. In the first period, exogenous cholesterol included only that which was inherent in the formula. In the second period, additional exogenous cholesterol was infused. A period of 4 hr equilibration was allowed, both before the first period and between the periods, after starting the high cholesterol intake.

Table 5 for those patients who were perfused with radioactive cholesterol as one marker. Most of the data are taken from those studies presented in Tables 2–4 but, in a few cases, absorption data has not been given in the foregoing results. For any given patient, the inflow of cholesterol was variable for either of two reasons: *a*) the amount of exogenous cholesterol in the perfusion was purposely altered, or *b*) the endogenous cholesterol varied because of the particular nutritional state of the patient at the time of the study, i.e., weight maintenance or reduction. The results are expressed as mg/hr of exchanged cholesterol, and they are compared to net absorption. The extent of exchange was highly variable; it ranged 0–65% of the net absorption. If the percentage absorption had been estimated from the use of radioactive cholesterol alone, absorption would have been overestimated by the extent of exchanged cholesterol.

TABLE 3. Net cholesterol absorption during normal and high cholesterol inputs

Patient	Period[a]	No. of Hourly Determn.	Cholesterol Inflow	Cholesterol Outflow	Cholesterol Absorption
			mg/hr ± SD	*mg/hr ± SD*	*mg/hr (%)*
V.C.	I	9	112 ± 23	55 ± 6	57 ± 22 (51)
	II	8	228 ± 20	113 ± 8	115 ± 19 (50)
J.M.[b]	I	5	38 ± 7	19 ± 3	19 ± 9 (50)
	II	7	123 ± 11	78 ± 20	45 ± 9 (37)
L.A.[b]	I	7	62 ± 6	40 ± 4	22 ± 6 (35)
	II	11	160 ± 20	124 ± 9	36 ± 18 (23)
L.McG.	I	3	122 ± 19	47 ± 10	75 ± 28 (61)
	II	8	247 ± 52	98 ± 18	149 ± 55 (60)
W.N.	I	4	82 ± 27	39 ± 11	43 ± 12 (52)
	II	8	148 ± 15	103 ± 31	45 ± 9 (30)

[a] In Period I, the cholesterol inflow was derived from endogenous sources; no additional cholesterol was added to the formula. In Period II, additional cholesterol was administered separately so that the inflow was significantly enhanced (incremental range 66–125 mg/hr).

[b] Patients J.M. and L.A. were on caloric restriction (1000 kcal/day) before the study.

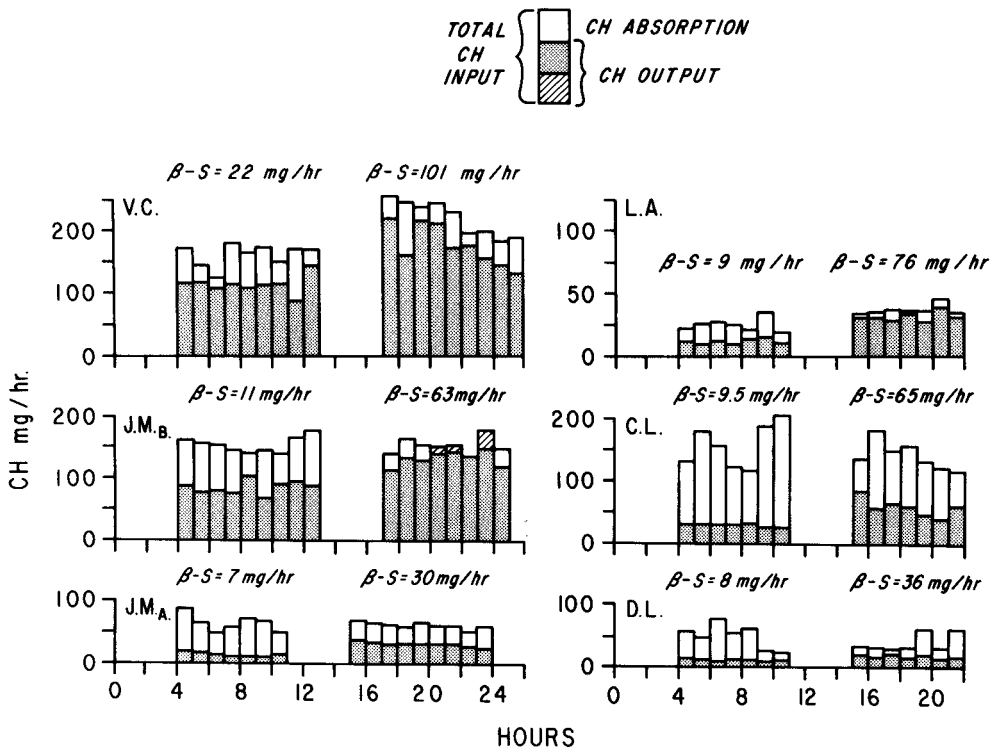

Fig. 3. Effects of β-sitosterol (β-S) on net cholesterol absorption. The intake of β-sitosterol in each of the two periods is shown on the graph. Hourly cholesterol absorption is the difference between inflow and outflow. In one patient (J.M., Study B) the outflow of cholesterol exceeded input during certain hours of the high β-sitosterol input, as shown by the striped bars.

The overestimation would have been large in some cases, but not in others.

Discussion

The purpose of this study was to develop a technique for measurement of absorption of the total quantity of cholesterol that enters the upper intestinal tract. The principle of this method was derived in large part from two previous techniques,—from a method for measuring hepatic secretion of biliary cholesterol, as described by Grundy and Metzger (1), and from a technique for estimating

TABLE 4. Effects of variable loads of β-sitosterol on cholesterol absorption

Patient	Period	No. of Hourly Determn.	β-Sitosterol Input	Cholesterol Inflow	Cholesterol Outflow	Cholesterol Absorption	Incremental Unabsorbed Cholesterol
			mg/hr	*mg/hr ± SD*	*mg/hr ± SD*	*mg/hr (%)*	*mg/hr*
V.C.	I	9	22	161 ± 18	114 ± 15	47 ± 18 (29)	
	II	9	101	219 ± 26	117 ± 33	42 ± 23 (19)	63 ± 12
J.M.[a]	IA	7	7	65 ± 13	15 ± 3	50 ± 12 (80)	
	IIA	9	30	62 ± 4	32 ± 4	30 ± 3 (48)	17 ± 5
	IB	9	11	157 ± 13	86 ± 12	71 ± 17 (45)	
	IIB	8	63	148 ± 8	145 ± 24	3 ± 9 (2)	59 ± 6
C.L.	I	7	10	158 ± 36	31 ± 4	127 ± 14 (80)	
	II	7	65	143 ± 22	60 ± 14	83 ± 10 (58)	29 ± 7
D.L.[a]	I	7	8	49 ± 20	13 ± 2	36 ± 8 (69)	
	II	7	36	39 ± 10	20 ± 3	19 ± 4 (49)	17 ± 3
L.A.[a]	I	7	9	26 ± 5	12 ± 1	15 ± 2 (54)	
	II	7	76	33 ± 4	37 ± 4	−4 ± 2 (0)	25 ± 1

[a] Patients J.M., D.L., and L.A. were on caloric restriction prior to the study.

cholesterol absorption over a segment of intestine, as presented by Simmons, Hofmann, and Theodor (13). This approach, which utilizes intestinal perfusion, was employed to overcome some of the limitations of previous methods for measuring cholesterol absorption in man. Although previous techniques have provided much useful information about absorption of dietary cholesterol, they have failed to give a complete picture of the absorptive process; that is, they have not measured absorption of the combined total of endogenous and exogenous cholesterol that enters the intestine. In the following discussion we have attempted to make a critical comparison of previous methods with the current technique.

One approach for estimating cholesterol absorption has been to determine the percentage absorption of a single dose of radioactive cholesterol. This technique was introduced by Borgström (6), who estimated absorption as the difference between oral ingestion and fecal excretion of a single dose of radioactive cholesterol. With this approach the accuracy of the measurement was enhanced by the simultaneous oral administration of nonabsorbable β-sitosterol that was labeled with another isotope. Thus, absorption could be calculated from the differences in ratios of the two sterols in the oral dose and in the feces. Since its introduction, this approach has been developed and refined by Quintao, Grundy, and Ahrens (7), Kudchodkar, Sodhi, and Holick (9), and Connor and Lin (10). Also, Zilversmit (8) has presented a modification of this method whereby one isotope of cholesterol is given orally and another is administered intravenously; absorption can thus be estimated as the isotopic ratio in plasma of the former as compared to the latter.

Although these methods have the advantage of simplicity, their accuracy and utility appear to be limited by several factors. First, since the techniques are isotopic, they basically provide a percentage value for absorption. While the mass of cholesterol in the test dose can be calculated, and hence the percentage absorption can be transformed to a value for mass absorption, this approach cannot indicate the total daily absorption of cholesterol, of either exogenous or endogenous origin. A second drawback is that a portion of the absorption of exogenous isotopic cholesterol may occur as a result of exchange between radioactive cholesterol in the lumen and unlabeled cholesterol in the intestinal mucosa; hence, it may not necessarily reflect net percentage absorption of cholesterol mass. Admittedly, resecretion (or reversible exchange) of radioactive cholesterol that is taken up but not actually absorbed by the mucosa may minimize this effect, but the extent or rate at which this process occurs cannot be determined. In this regard, the method of Zilversmit (8) should give an accurate measurement of absorption of radioactive cholesterol from the lumen to lymph (or plasma), but if the technique is employed to estimate mass absorption, it may also give a value that is greater than net absorption because of isotope exchange between cholesterol of mucosa and intestinal lipoproteins. Finally, percentage absorption of a single dose may not give a true indication of the average percentage absorption throughout the day; a fluctuation of percentage could occur because of variations in amount of cholesterol that enters the intestine from time to time throughout the day.

Another approach has been to use the cholesterol balance technique to determine the mass absorption of exogenous cholesterol; this technique estimates absorption as the difference between dietary cholesterol and fecal excretion of exogenous cholesterol (4, 5, 7). The latter term is estimated as the difference between fecal total neutral steroids and endogenous neutral steroids; differentiation of endogenous and exogenous cholesterol requires administration of

TABLE 5. Net cholesterol absorption vs. isotope exchange

Study No.	Patient	Cholesterol Inflow	Net Cholesterol[a] Absorption	Isotope Exchange[b]
		mg/hr	*mg/hr*	*mg/hr (%)*
1	V.C.	112 ± 23	57 ± 22	7.2 ± 0.8 (12)
2	V.C.	228 ± 20	115 ± 19	26 ± 1.8 (23)
3[c]	V.C.	161 ± 18	47 ± 18	18 ± 3.0 (38)
4[c]	V.C.	219 ± 26	42 ± 23	0 (0)
5	J.M.	61 ± 11	31 ± 10	3 ± 0.4 (10)
6	J.M.	38 ± 7	19 ± 9	7 ± 6.0 (37)
7	J.M.	123 ± 11	45 ± 9	0 (0)
8	J.M.	65 ± 13	50 ± 12	4 ± 3.0 (8)
9[c]	J.M.	62 ± 4	30 ± 3	8.5 ± 1.7 (23)
10	J.M.	157 ± 13	71 ± 17	46 ± 6.6 (65)
11	C.L.	118 ± 13	58 ± 10	28 ± 6.0 (48)
12	A.B.	79 ± 18	27 ± 15	11 ± 1.6 (14)
13	D.W.	51 ± 7	19 ± 8	9 ± 9.0 (37)
14	D.L.	106 ± 29	64 ± 25	12 ± 10.0 (19)
15	D.L.	49 ± 20	36 ± 8	7.1 ± 1.2 (19)
16[c]	D.L.	39 ± 10	19 ± 4	5.7 ± 3.3 (26)
17	W.V.	66 ± 12	37 ± 13	6.8 ± 1.0 (24)
18	W.N.	82 ± 27	43 ± 12	16 ± 3.0 (37)
19	W.N.	148 ± 15	45 ± 9	27 ± 4.0 (60)
20	L.A.	26 ± 4	15 ± 2	2 ± 1.6 (14)
Mean		99	43	13 (23%)

[a] Net cholesterol absorption represents the mean value for absorption calculated using β-sitosterol as a marker, as described in the Methods section.

[b] Isotope exchange represents the difference between net cholesterol absorption and absorption calculated with isotopic cholesterol as the marker (see Methods section).

[c] In all studies, except those designated by the superscript *c*, β-sitosterol was infused as a marker in the dose range of 7–11 mg/hr; in the others, rates of infusion of β-sitosterol were increased to the range of 22–101 mg/hr (see Table 4).

radioactive cholesterol to the patient. According to this approach, radioactive cholesterol is given either as a single dose intravenously (Method I, ref. 5) or by the daily oral intake of labeled cholesterol (Method II, ref. 5); by the intravenous dose, the endogenous pool of cholesterol is labeled whereas exogenous cholesterol is labeled by oral administration of radioactivity. Both methods yield similar results (5). These techniques have the advantage of measuring the average daily absorption of an absolute mass of cholesterol. On the other hand, there are at least three drawbacks: *a*) the absorption of only the exogenous fraction is measured and this may be influenced by the quantity of endogenous cholesterol that is secreted into the intestine, *b*) a prolonged period of study (e.g., several weeks) is required for an accurate estimate of absorption, and *c*) the process of isotope exchange may give an overestimate of absorption. Again, however, this latter effect may be minimized in long-term studies by reversible exchange of radioactivity between the mucosa and the lumen.

The intestinal perfusion method described in this paper appears to offer certain distinct advantages over previous ones: *a*) it measures absorption of total cholesterol entering the upper intestine (i.e., endogenous + exogenous); *b*) it can be carried out over a period of a few hours; *c*) it defines the extent of isotope exchange; and *d*) it affords the opportunity of studying effects of acute changes in the intestinal milieu on cholesterol absorption. The major drawbacks are that the procedure requires intubation of patients and must be carried out over a period of many hours in order to achieve steady state conditions. Measurements during several hours of the steady state are also required because of fluctuations in absorption from hour to hour; in other words, multiple hourly measurements are needed to give a valid mean estimate of absorption. Nevertheless, most of our patients tolerated the procedure well, and these drawbacks should not prevent the use of the method for specific studies on cholesterol absorption even though the technique may not be applicable to large numbers of patients.

While the loss of labeled cholesterol and reappearance of nonlabeled cholesterol between proximal and distal outlets has been called exchange, it might be argued that this process represents true absorption on the one hand and intestinal secretion on the other (13). Unfortunately, the present technique cannot distinguish between these two processes. If independent absorption and secretion had occurred, our technique would not detect the absorbed cholesterol that was balanced by secretion into the 100-cm segment. The fact that "exchange" was variable from one patient to another suggests that more than a physical cross-transfer process was involved. However, because of the lack of a way to differentiate exchange and absorption + secretion, and for the sake of simplicity, we have chosen to employ the term exchange.

For the purposes of illustration, total cholesterol absorption was measured before and after acute alterations in flow rates of cholesterol and β-sitosterol. When the total amount of cholesterol entering the intestine was increased acutely by the addition of more exogenous cholesterol, rates of absorption usually increased considerably; this observation indicates that absorption of total luminal cholesterol is not maximal with the usual load of endogenous cholesterol. However, in two patients (L.A. and L.McG), the increment did not persist and total hourly absorption progressively decreased after several hours of infusion at the higher levels; thus, in these patients, absorptive mechanisms became increasingly saturated towards the end of the study.

Studies with β-sitosterol at different levels of input revealed that this sterol is extremely effective in blocking absorption of cholesterol. While it is well known that plant sterols interfere with absorption of cholesterol, their high degree of potency for this action is generally not appreciated. However, the present results are in accord with other recent studies from our laboratory, which indicate that extremely high doses of β-sitosterol (e.g., 10–20 g/day) are not required for maximal inhibition of cholesterol absorption, as had been thought previously (14–16); when β-sitosterol was fed in doses of 3 g/day, average absorption was reduced by about 50%, and absorption was not reduced further by increasing dosage to 9–12 g/day (17). Furthermore, degrees of reduction in cholesterol absorption under conditions of the present study show that β-sitosterol has an even greater potential for inhibiting absorption than was obtained during dietary studies; the greater effectiveness of β-sitosterol in perfusion studies could be due to the fact that all β-sitosterol was infused in micellar solution. In dietary studies, β-sitosterol is usually given in a suspension, and less may ultimately become solubilized in mixed micelles, a condition that is probably necessary for its activity. Also, β-sitosterol is continuously present in the lumen during perfusion while it is only intermittently available to block cholesterol absorption when it is given by mouth.

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