Cholesterol absorption in man: effect of administration of clofibrate and/or cholestyramine

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Abstract Cholesterol absorption measurements were carried out in a free-living out-patient population by a plasma isotope-ratio method previously validated for in-patients (Samuel, P., J. R. Crouse and E. H. Ahrens, Jr., 1978. *J. Lipid Res.* **19:** 82–93). To test the reproducibility of the method in out-patients, 18 patients were tested twice: the mean intra-assay variability was ±6.0%. The method was then applied in 150 hyperlipidemic male out-patients, ingesting a standardized diet containing 250mg cholesterol per day, who had been randomized into four different drug-treatment groups: *1*) no medication, *2*) clofibrate, (2g/day), *3*) cholestyramine (16g/day), or *4*) both clofibrate and cholestyramine. Cholesterol absorption (as percent of the oral dose) was increased in patients receiving cholestyramine ($P < 0.02$) and decreased in those receiving clofibrate ($P < 0.02$); the group on the combined medication had the same percent absorption as the control group. In twelve patients receiving cholestyramine, a second test of cholesterol absorption was performed 30 min after each patient had received 8g of cholestyramine. The pre-test administration of cholestyramine caused a 38% decrease in cholesterol absorption ($P < 0.001$), compared to results obtained when medication was withheld prior to testing. These results demonstrate that the isotope-ratio method of measuring cholesterol absorption is a reproducible procedure applicable to a free-living out-patient population, and that the hypolipidemic drugs, clofibrate and cholestyramine, significantly affect cholesterol absorption in man. The data also show that the results of measurements of cholesterol absorption can be profoundly altered by the type and timing of medication in relationship to the test meal of labeled cholesterol.—**McNamara, D. J., N. O. Davidson, P. Samuel, and E. H. Ahrens, Jr.** Cholesterol absorption in man: effect of administration of clofibrate and/or cholestyramine. *J. Lipid Res.* 1980. **21:** 1058–1064.

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A recent report from this laboratory by Samuel, Crouse, and Ahrens (1) validated the use of an isotope-ratio method for measurement of percent cholesterol absorption in man. In contrast to previously described methods (2, 3), the isotope-ratio method does not require hospitalization of patients on a metabolic ward, eucaloric intake of a constant dietary mixture, or collection of feces for analysis of fecal neutral steroid radioactivity. Thus, the isotope-ratio method would appear to be useful for measurements of cholesterol absorption in out-patients by virtue of its simplicity and the low level of radiation imposed on the patients.

The present report describes studies conducted in a free-living out-patient population that were designed to determine the effect of two commonly used hypolipidemic drugs on cholesterol absorption. The results demonstrate that clofibrate reduced and cholestyramine increased percent cholesterol absorption; when both drugs were administered, there was no change in cholesterol absorption as compared to control patients. We also found that the timing of cholestyramine administration in relationship to the cholesterol-containing test meal significantly affected percent cholesterol absorption; these findings suggest that caution be exercised in the interpretation of absorption tests carried out by the isotope-ratio method.

MATERIALS AND METHODS

Patients

One hundred fifty patients from the Center for the Prevention of Premature Arteriosclerosis (CPPA) at The Rockefeller University Hospital were recruited for the study. Informed consent was obtained from each patient after appropriate review and approval of the study protocol by The Rockefeller University Institutional Review Board.

The CPPA population consisted of non-symptomatic hyperlipidemic males aged 27 to 61 who were maintained on a low-cholesterol diet (<300mg/day), of which 35% of total calories consisted of fat with a P/S ratio of 2. Patients were screened by oral cholecystography to exclude significant gallbladder disease. All patients in this population who had failed to normalize their plasma lipid levels after 6 months of dietary treatment were subsequently

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randomized into one of four double-blind treatment groups; placebo, clofibrate (2g/day), cholestyramine (16g/day), and patients treated with both cholestyramine and clofibrate.

After the termination of that double-blind study, 150 of these CPPA patients were reenlisted and assigned, unblinded, to their original medication group. Patients who had discontinued their medication following cessation of the double-blind study described above were required to reestablish their previous medication schedules for a minimum of 6 months prior to the present experiment.

Each patient received additional dietary counseling: he was intensively instructed in portion-size assessment and dietary record-keeping. Patients maintained 9 consecutive days' food records in order to evaluate adherence to the dietary regimen and to quantitate daily cholesterol intake (4). Food table analysis of these dietary records indicated a mean daily cholesterol intake of 247 ± 8.0 mg/day (mean ± SE); these intakes were found to be independent of hyperlipidemic phenotype and medication grouping.

Clinical procedures

The night before the absorption test, drug administration was discontinued following the evening dose, and the patients reported to the clinic in the fasting state. They were then given an intravenous infusion of [^{3}H]cholesterol as described below, immediately followed by an oral dose of [^{14}C]cholesterol. Following the test, patients resumed their normal meal and drug schedules. (In studies to be presented elsewhere, we had found that percent cholesterol absorption was unchanged by the cholesterol content of the meal following the test and was independent of the time of day the plasma sample was drawn for measurement of the isotope ratio).[2] Three to seven days after administration of the two radiolabeled cholesterols, a single blood sample was obtained and assayed in sextuplicate for the plasma cholesterol specific activity ratio.

Administration of radioisotopic compounds

For intravenous administration, 1 μCi [1,2-^{3}H]cholesterol dissolved in 1 ml of absolute ethanol was suspended in 150 ml of saline and immediately infused. For oral administration 1 μCi [4-^{14}C]cholesterol in 1 ml of absolute ethanol was mixed with 100 ml of a fat-containing liquid formula (31% of calories as cottonseed oil); after drinking the mixture, the patient rinsed the glass with, and subsequently drank, an additional 50 ml of the same formula. The total caloric intake was approximately 300 calories and was given in lieu of breakfast. Residual radioactivity in the infusion set (~12%) and glass (~5%) were determined after an ethanol rinse and hexane extraction so as to determine the actual amount of the two radiolabeled cholesterols administered. Radioactivity was measured in a Packard Tri-Carb scintillation counter (model 3380-3390, Packard Instrument Co., Downer's Grove, IL) with quench corrections performed automatically by an absolute activity analyzer (AAA model 544, Packard Instrument Co.)

Analytical methods

Concentrations of plasma cholesterol and triglycerides were determined by the method of Block, Jarrett, and Levine (6) for cholesterol, and of Kessler and Lederer (7) for triglycerides, using the Auto Analyzer II (Technicon Instruments, Corp., Tarrytown, NY).

Plasma cholesterol specific activities were measured on aliquots of the same extract used for the determination of concentration, as previously described (8).

Radioactive cholesterol

[1,2-^{3}H]Cholesterol (40Ci/mmol) and [4-^{14}C]cholesterol (40mCi/mmol) were purchased from New England Nuclear Corp., Boston, MA. They were purified by thin-layer chromatography on Florisil (Floridin Co., Tallahassee, FL) with ethyl ether–heptane 45:55 (v:v). Only material that chromatographed with the same R_f value as pure cholesterol standard was administered to patients.

The radiochemical reliability of the [1,2-^{3}H]cholesterol was determined by calculation of the dose-normalized plasma cholesterol ^{3}H/^{14}C ratio from 24 hr to 7 days after simultaneous intravenous infusion of [4-^{14}C]cholesterol and [1,2-^{3}H]cholesterol (5). The [1,2-^{3}H]cholesterol used in the present study was found to give a dose-corrected ^{3}H/^{14}C ratio of 0.79 ± 0.043 in eight tests; this correction value was used for all dosage calculations.

Calculations

Calculations of percent absorption were based on the two cholesterol specific activity values expressed in terms of percent dose per gram of plasma cholesterol, as previously described (1).

$$\% \text{ absorption} = \frac{\% \text{ dose/g of cholesterol (oral cholesterol)}}{\% \text{ dose/g of cholesterol (i.v. cholesterol)}} \times 100$$

[2] Samuel, P., D. J. McNamara, J. R. Crouse, and E. H. Ahrens, Jr. Unpublished observations.

TABLE 1. Reproducibility of the isotope-ratio method for measurement of cholesterol absorption in 18 out-patients

Patient	Cholesterol Absorption (%)		Percent Difference (II vs I)
	Test I	Test II	
1	61.1	70.6	+13.4
2	59.1	65.5	+9.8
3	49.9	52.3	+4.7
4	45.3	40.4	−12.1
5	60.4	64.1	+5.8
6	42.3	48.4	+12.7
7	50.8	49.7	−2.2
8	47.9	46.9	−2.2
9	44.2	46.8	+5.4
10	53.4	56.7	+5.8
11	44.2	46.8	+5.4
12	50.2	50.0	−0.8
13	60.3	58.5	−2.9
14	49.1	52.2	+5.9
15	54.4	57.6	+5.6
16	53.6	57.1	+6.2
17	50.6	53.6	+5.6
18	63.5	62.1	−2.2
Mean	52.4	54.4	+6.0
Standard error	1.5	1.8	0.9

Statistical analysis was carried out using a Hewlett Packard 97 calculator, with the student *t*-test program for two unpaired means and two paired means supplied in the Hewlett Packard Stat Pac I.

RESULTS

Reproducibility

Prior to the application of the isotope-ratio method for measurement of percent cholesterol absorption in a large group of out-patients on ad libitum diets, preliminary studies were conducted to determine the analytical reproducibility. In 18 patients tested twice within a 2-month period the percentage difference between the two tests was 6.0 ± 0.9 (mean ± SE), giving us 95% confidence that the inter-test variability was less than 3.6% (**Table 1**).

Out-patient characteristics

Table 2 presents the relevant clinical data for the different medication groups and for each subset of hyperlipidemic phenotype within each medication group. Relative body weights within each group did not differ significantly from each other. However, plasma cholesterol levels were significantly decreased by all three drug treatments ($P < 0.02$); plasma triglycerides were reduced by clofibrate treatment, whether cholestyramine was given ($P < 0.05$) or not ($P < 0.01$) (Table 2).

Absorption data

Table 3 presents the results of cholesterol absorption measurements performed in this out-patient population by the isotope-ratio method. It is seen that the administration of clofibrate significantly decreased percent cholesterol absorption ($P < 0.02$), while cholestyramine treatment significantly increased the percent absorption of exogenous cholesterol ($P < 0.02$). Combined therapy with clofibrate plus cholestyramine resulted in a value for cholesterol absorption identical to the control group. Further analysis of the data failed to demonstrate any significant differences in percent cholesterol absorption within treatment groups as a function of hyperlipidemic phenotypes.

Timing of cholestyramine dosage

The administration of cholestyramine (8g) 30 min prior to oral administration of the test dose of labeled cholesterol caused a profound decrease in mean percent cholesterol absorption, from 60.4 ± 4.1% to 37.0 ± 2.7% (mean ± SE, n = 12) a decrease of 38% ($P < 0.001$, paired *t*-test). Looking at the individual data, cholesterol absorption was significantly decreased in ten out of twelve patients, while in two hypertriglyceridemic patients with initially low absorption levels, pre-dosage with cholestyramine caused no significant change in absorption (**Fig. 1**).

DISCUSSION

The absorption of endogenous and exogenous cholesterol is one of several key regulatory factors responsible for the maintenance of plasma cholesterol concentrations. Accordingly, one approach to the treatment of hypercholesterolemia is to interfere with cholesterol absorption. In order to search for agents that significantly decrease cholesterol absorption, the clinical investigator must have the means to determine the absorption of exogenous cholesterol accurately and simply; ideally such measurements must be carried out in fairly large numbers of patients and under various conditions of diet, exercise, etc. Numerous methods have been developed for the measurement of exogenous cholesterol absorption in patients under metabolic ward conditions and have been extensively tested (reviewed in 1–3). However, these methods are not readily adapted to studies in a free-living out-patient population.

The isotope-ratio method for measurement of cholesterol absorption first described by Zilversmit (9) has been validated for use in the rat (10), in

TABLE 2. Clinical data on 150 hyperlipidemic male out-patients

Medication Group	Hyperlipidemic[a] Type (n)	Age[b] (years)	Relative[b,c] Body Weight	Plasma Lipids	
				Cholesterol	Triglycerides
				mg/dl[b]	
Control	HC (13)	46.8 ± 2.8	101.2 ± 2.7	295 ± 16	116 ± 9
	HTG (10)	42.5 ± 1.4	111.6 ± 3.5	217 ± 6	231 ± 27
	MHL (17)	42.4 ± 2.5	108.5 ± 2.4	282 ± 13	380 ± 64
Total	(40)	44.5 ± 1.4	106.8 ± 1.7	272 ± 9	257 ± 33
Clofibrate	HC (11)	46.6 ± 2.6	112.7 ± 2.6	257 ± 9	100 ± 5
	HTG (10)	42.3 ± 2.5	120.2 ± 4.2	218 ± 11	164 ± 20
	MHL (20)	45.3 ± 1.8	112.0 ± 2.2	252 ± 6	161 ± 48
Total	(41)	44.9 ± 1.3	114.2 ± 1.7	245 ± 5	148 ± 9
Cholestyramine	HC (12)	46.0 ± 2.9	108.3 ± 3.5	231 ± 6	134 ± 21
	HTG (10)	45.6 ± 2.1	107.0 ± 3.1	195 ± 11	248 ± 52
	MHL (15)	42.3 ± 1.9	117.2 ± 3.4	262 ± 12	353 ± 43
Total	(37)	44.4 ± 1.3	111.5 ± 2.1	233 ± 7	253 ± 27
Clofibrate plus cholestyramine	HC (9)	44.7 ± 3.5	104.6 ± 4.9	274 ± 15	106 ± 16
	HTG (9)	50.0 ± 1.9	114.9 ± 3.4	206 ± 12	215 ± 29
	MHL (14)	48.6 ± 1.3	113.6 ± 2.6	219 ± 10	177 ± 44
Total	(32)	47.9 ± 1.3	111.5 ± 2.1	231 ± 9	168 ± 22

[a] Abbreviations: HC, hypercholesterolemic; HTC, hypertriglyceridemic; MHL, mixed hyperlipidemic.
[b] Means ± S.E.
[c] Relative body weight = $\frac{\text{body weight (kg)}}{\text{height (cm)} - 100} \times 100$.

primates (11), and in man (1): it appeared to be ideally suited for use in out-patient studies.

Analytical reproducibility

Experiments to determine the reproducibility of the test were conducted in 18 out-patients; the data demonstrated that the inter-test variability was less than 3.6% (95% confidence limit). It is of interest that in comparing the isotope-ratio method with the fecal radioactivity method in in-patients, Samuel et al., (1) found that the isotope-ratio method and the fecal radioactivity method for measuring cholesterol absorption differed by less than 5% at the 95% confidence limit.

Drug effects on cholesterol absorption

It has been established that sitosterol (12, 13), sucrose polyester (14), and neomycin (15) cause a significant reduction in cholesterol absorption.

Earlier studies by Grundy et al. (16) on the effect of clofibrate on cholesterol absorption, using Methods I and II, suggested that clofibrate caused a small but significant decrease in cholesterol absorption; the present study confirms and extends that finding in a larger number of patients and with newer methodology.

We know of no previous studies of cholesterol absorption in patients given cholestyramine. However, in the present study, patients receiving cholestyramine (16g/day) showed a small but significant increase in percent cholesterol absorption when compared to either the control group ($P < 0.02$) or the clofibrate group ($P < 0.001$).

The unexpected finding of an elevated percent cholesterol absorption in the cholestyramine group prompted us to retest 12 of these patients under two conditions of the timing of drug dosage: in one case they took their morning dose of cholestyramine (8g) approximately 30 min prior to the oral administration of the cholesterol test dose, while in the comparison case the drug was taken following the cholesterol test dose. Administration of the drug 30 min prior to testing caused a significant decrease in cholesterol absorption in ten of the patients studied. (In two exceptional patients, the cholesterol absorption measurements were low initially and were not affected by cholestyramine pre-dosage. In these two cases, the cause of the initially low absorption and

TABLE 3. Cholesterol absorption in 150 out-patients on three lipid-lowering regimens compared to controls

Medication	N	Cholesterol Absorption (%) ($\bar{x} \pm SE$)	P
Placebo	40	57.2 ± 1.8	<0.02
Clofibrate	41	51.9 ± 1.7	<0.02
Cholestyramine	37	64.6 ± 2.3	NS
Clofibrate plus cholestyramine	32	58.1 ± 2.8	<0.001

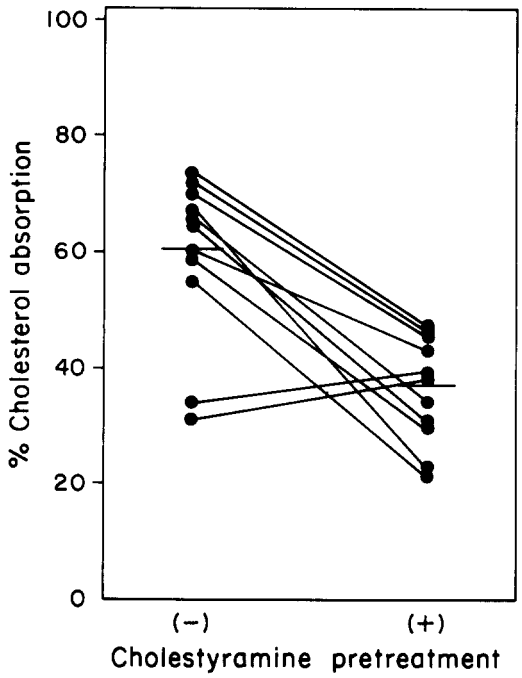

Fig. 1. Cholesterol absorption was measured by the isotope-ratio method in 12 cholestyramine-treated patients under two conditions: (−) indicates a 12-hr fast with no drug dosage prior to the oral test dose of labeled cholesterol, and (+) indicates a 12-hr fast and 8g of cholestyramine administered 30 min prior to the test meal. Data presented as individual values; the mean in each treatment schedule is shown as a horizontal bar.

lack of response to cholestyramine pretreatment are not understood).

Explanation for the drug effects

Angelin, Einarsson, and Leijal (17) studied the biliary lipid composition of patients receiving clofibrate, cholestyramine, or clofibrate plus cholestyramine: they found that clofibrate treatment resulted in an increased biliary cholesterol saturation, and that cholestyramine caused reduced biliary cholesterol saturation. The combination of clofibrate and cholestyramine resulted in a bile cholesterol saturation index equal to the pre-treatment values. If we assume that our test-population responded similarly, the decreased percent cholesterol absorption found in the clofibrate-treated group may have been caused by the secretion into the duodenum of bile supersaturated with cholesterol, especially in the fasting state, a condition not favoring the solubilization of exogenous cholesterol in the test meal. On the other hand, the elevated percent cholesterol absorption observed in our cholestyramine-treated group may have been due to drug-induced undersaturation of bile, which in turn would lead to an increased solubilizing capacity for exogenous cholesterol. A balancing of the two effects would then be expected in patients on clofibrate plus cholestyramine, as indeed we observed.

In addition to alterations in the saturation index of bile caused by administration of these two drugs, both clofibrate and cholestyramine alter the biliary flow rate and the bile acid pool size. Clofibrate treatment results in a marked, if transitory, increase in biliary cholesterol secretion (16) and a decrease in the cholic acid pool (18). Bile acid binding agents also increase biliary lipid secretion rates but, in contrast to clofibrate, cause a significant increase in the bile acid pool size (19). Combined drug treatment results in a slight increase in biliary lipid flow rates above that observed during control, and a small, but significant, decrease in the bile acid pool size (19). Since all treatments cause an increased biliary lipid flow, it is difficult to explain the observed differences on the basis of absolute biliary flow rates. Drug-induced changes in the bile acid pool size may play a role in the observed differences; yet, if flow rates of biliary bile acids were increased, one would expect that both drugs, either alone or in combination, would have similar effects.

Administration of cholestyramine prior to the test dose of labeled cholesterol, by sequestering biliary bile acids and decreasing the micellarization of exogenous cholesterol, would be expected to reduce cholesterol absorption, as we have shown. Similar findings have been reported for the rat, where simultaneous administration of cholestyramine with the cholesterol-containing test meal caused a significant decrease in cholesterol absorption (20).

These findings may have therapeutic implications: the most effective use of cholestyramine in the treatment of hyperlipidemia may depend not only on total dosage but also on the timing of the dose in relationship to meals and to gallbladder contraction.

The validity of cholesterol absorption measurements

At the present time there are eight different methods for measuring cholesterol absorption in man (1–3, 13). Certain assumptions underlie some or all of these methods: *1*) endogenous and exogenous cholesterol equilibrate completely and are absorbed equally; *2*) there is minimal isotope exchange between mucosal and lumenal cholesterol; and *3*) tests based on administration of a single bolus of labeled cholesterol (10, 21) are representative of the mean rate of absorption of cholesterol throughout the day, and, indeed from day to day.

The first assumption (equilibrium of endogenous and exogenous cholesterol within the gut lumen) has not yet been rigorously examined in a sufficient variety of patients treated in sufficiently various ways.

Studies by Simmons, Hofmann, and Theodor (22) and Grundy and Mok (13) have addressed the question of isotopic exchange. Their findings suggest that either exchange or cholesterol secretion from the mucosal cells does occur. If their findings are indeed due to simple isotopic exchange, significant errors in determination of cholesterol absorption by *any* of the currently available methods will be encountered. The present study does not address either of these two basic assumptions.

However, we have indirectly addressed the third assumption: our findings demonstrate that percent absorption of cholesterol is significantly different when the test is performed prior to or after administration of cholestyramine. Our findings suggest that any "single bolus" test is not necessarily representative of the mean daily absorption of cholesterol in patients receiving cholestyramine. It should be noted, however, that sterol balance studies performed during control and cholestyramine treatment have failed to demonstrate increased fecal neutral steroid output during the cholestyramine test period (19, 23–25), suggesting that cholestyramine treatment does not significantly alter the mean daily cholesterol absorption.

Comparisons of measurements of cholesterol absorption based on the combined use of sterol balance and isotopic balance methods (Methods I and II, ref. 26) to the single oral dose method introduced by Borgström (21) demonstrated excellent agreement when tested in non-medicated patients (2). Moreover, the Borgström method (21) agrees well with the isotope-ratio method of Zilversmit (9, 10), as Samuel et al. have shown (1). These findings suggest that under controlled conditions in which the various constituents of the gut contents are not abruptly modified, the single bolus and continuous absorption measurement methods provide comparable results. However, under conditions in which the intestinal mixture of neutral sterols, bile acids, and phospholipids is irregularly altered during any 24-hour period, as it is when cholestyramine is taken 2–4 times daily, the single bolus method provides data only on the absorption of that test dose; it may not be representative of the mean daily absorption of cholesterol.

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