Long term steroid metabolism balance studies in subjects on cholesterol-free and cholesterol-rich diets: comparison between normal and hypercholesterolemic individuals

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Abstract Regulation of cholesterol metabolism was investigated in eight hypercholesterolemic and five normal individuals by combined intravenous pulse-labeling with radioactive cholesterol and fecal steroids balance techniques. Mean serum cholesterol concentrations ranged from 168 to 717 mg/dl. Experiments were scheduled in the following sequence: a cholesterol-free diet period lasting 4–6 weeks (PI); cholesterol intake of 1350 mg/day lasting 9–10 weeks (PII); and a cholesterol-free diet for 2 weeks (PIII). It was observed that body cholesterol synthesis in PI and absorption of dietary cholesterol in PII were completely independent of the serum cholesterol levels and varied widely among the subjects. During the cholesterol intake period, seven individuals maintained a negative fecal steroid balance, whereas six others accumulated cholesterol in the body (positive balance) irrespective of cholesterol concentration. A strong positive correlation was found between dietary cholesterol absorption and cholesterol balance in PII and reflected two events: *1*) decreased synthesis as the major mechanism to prevent body storage of cholesterol, whereas the increase of fecal bile acids and endogenous neutral steroids excretion played a secondary role; *2*) increasing amounts of cholesterol accumulated in the body proportionally to the amount absorbed, whenever the latter surpassed the ability of the compensatory mechanisms. These compensatory mechanisms seem to have been equally efficient in both normal and hypercholesterolemic subjects. Changes in serum cholesterol subsequent to cholesterol feeding were also unrelated to the amount absorbed and to the steroid balance in PII.—**Maranhão, R. C., and E. C. R. Quintão.** Long-term steroid metabolism balance studies in subjects on cholesterol-free and cholesterol-rich diets: comparison between normal and hypercholesterolemic individuals. *J. Lipid Res.* 1983. **24:** 167–173.

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Hypercholesterolemia can ultimately be due to the following alterations, which are theoretically detectable by metabolic studies: *1*) increased cholesterol synthesis; *2*) increased intestinal dietary cholesterol absorption; *3*) decreased steroid excretion, either as neutral steroids or bile acids; and *4*) altered cholesterol distribution in body pools. These disturbances would reflect defective cellular or molecular events, as those described by Goldstein and Brown (1, 2) for fibroblast LDL receptors in familial hypercholesterolemia.

Studies comparing hypercholesterolemic and normal individuals by means of chemical and isotopic balance techniques have been contradictory. In hypercholesterolemic subjects, cholesterol body synthesis has been described as either enhanced, normal, or decreased (3–6). Miettinen et al. (7) and Carter et al. (4) have detected diminished cholesterol excretion as fecal bile acids, a finding not confirmed in other experiments (3, 8). On the other hand, dealing with steroid synthesis and excretion, heterogeneous results were often registered among hypercholesterolemic participants (3, 5, 9). Also, kinetic studies with radioactive cholesterol, namely compartmental and integral analysis, have often been in disagreement as to the production, removal, and distribution of cholesterol in body pools in hypercholesterolemic patients (4, 9–12).

Indeed, only two definite facts can be drawn from the overall results of these experiments: *1*) hypercholesterolemic subjects do not absorb more dietary cholesterol than normal individuals, as stated by Connor (13) and confirmed in all comparative experiments and *2*) pool A, the rapidly miscible pool of cholesterol, is expanded in hypercholesterolemics, but it also includes the serum cholesterol compartment (11, 12).

In the present series of studies, total body cholesterol synthesis as measured by the fecal steroid balance method was assessed during a cholesterol-free dietary period (PI) in 13 individuals whose serum cholesterol

Abbreviations: NS, neutral steroids; BA, bile acids.

TABLE 1. Relevant clinical data of 13 patients[a]

Patients	Sex	Age	Height	Weight	Diagnosis[b]
			cm	*kg*	
1-J.B.	F	32	170	69	N, no clinical vascular disease
2-M.R.	M	50	167	63	N, S.H. + C.H.D. + hypertrigliceridemia
3-T.N.	M	49	161	52	N, no clinical vascular disease
4-M.A.	M	60	172	70	N, no clinical vascular disease
5-F.I.	F	51	150	52	N, S.H.
6-I.P.	F	49	155	60	HCh, S.H.
7-J.M.	F	59	163	68	HCh only
8-H.F.	F	56	152	56	HCh, C.H.D.
9-A.M.	F	56	163	62	HCh, S.H.
10-R.S.	F	66	146	46	HCh, S.H.
11-A.O.	F	61	154	58	HCh, C.H.D.
12-F.B.	F	64	155	43	HCh only (probably monogenetic)
13-J.J.	F	58	157	60	HCh, probably monogenetic, S.H., C.H.D.

[a] Patients are numbered according to the increasing concentration of serum cholesterol measured during the initial cholesterol-free diet period (PI).

[b] N, normocholesterolemia; HCh, hypercholesterolemia; S.H., systemic hypertension; C.H.D., coronary heart disease.

varied over a wide range. Thereafter, their compensatory mechanisms, namely decreased synthesis and enhancement of fecal steroids reexcretion, as described in other papers (14, 15), were studied after a daily load of dietary cholesterol (1350 mg/day, PII). This intake of cholesterol was chosen to provide an absorption rate as close as possible to that of expected synthesis in a cholesterol-free diet situation (14, 15). It was hoped that the absorption values attained would be capable of triggering the compensatory mechanisms to operate fully but without leading to indiscriminate cholesterol accumulation in the body. A cholesterol-free period then followed (PIII) to evaluate the long-lasting consequences of steroid synthesis and excretion induced by the previous cholesterol feeding phase.

In this report we demonstrate that serum cholesterol concentration is independent of the parameters of cholesterol body metabolism herein measured and also that hypercholesterolemic and normal subjects have similar efficiencies in compensating for the absorption of exogenous cholesterol.

METHODS

Cholesterol balance studies were carried out in 13 nonobese subjects. Experiments were scheduled for three consecutive periods. Period I (PI) lasted 4–6 weeks while the subjects were on a diet consisting of vegetable foodstuffs with additional cottonseed oil providing the major source of fat. Egg white daily and 50 g/week of fish were also allowed as extra sources of protein. This diet is rich in polyunsaturated fatty acids and essentially cholesterol-free (less than 50 mg/day). All patients had three meals/day with the largest calorie intake around noon, as is usual in Brazil. Period II (PII) lasted 9–10 weeks, during which the diet was supplemented with 1350 mg of crystalline cholesterol/day, supplied in 270-mg gelatinous capsules administered during the lunch meal (except for Patient 13 who was given 1450 mg of cholesterol/day). Period III (PIII) lasted 2 weeks and the diet was identical to PI. Informed consent was obtained from the subjects. The research protocol was reviewed and approved by the Department of Internal Medicine.

The clinical data are displayed in **Table 1.** Basal serum cholesterol concentrations (measured during the cholesterol-free period, PI) ranged from 168 to 717 mg/dl. Five subjects (cases 1–5) were considered as normals and eight subjects were considered hypercholesterolemic (cases 6–13), i.e., serum cholesterol concentration below and above 260 mg/dl, respectively. All subjects except Patient 2 had normal serum triglyceride levels. They were studied on an outpatient basis with biweekly appointments required for body weight measurements, sample collecting, and careful monitoring of their daily records of food intake, examined independently by a nutritionist and the authors. Three of the original 16 patients initially admitted to the study were excluded for not complying strictly to the diet. Variation in body weight did not exceed ±1 kg throughout the study.

To correct for variation in daily fecal flow, the subjects were given 540 mg of chromic oxide per day during the entire study, supplied as 180-mg tablets evenly distributed through the three daily meals (16). All participants were requested to bring a complete 24-hr fecal collection from the preceding day at each visit to the outpatient clinic.

Isotopes

On the first day of PII, patients received an intravenous dose of 70 μCi of [4-^{14}C]cholesterol (New England Nuclear, Boston, MA) dissolved in 1 ml of ethanol and infused in 200 ml of saline. Radioactivity was measured in a Beckman LS-100 beta scintillation counter.

Plasma cholesterol

Plasma cholesterol concentration was determined biweekly throughout the experiment by combined GLC and TLC (17). In addition, specific activity was determined biweekly during PII.

Analysis of fecal steroids

At the end of PI and PII, from five to nine complete daily fecal collections were taken for further analysis. On the last day of PII, a single dose of carmine red was administered orally and its appearance in the feces was monitored. Immediately after its fecal disappearance, six or seven complete daily fecal excretions were collected during an approximately 2-week period (PIII). After homogenization with added water, aliquots were stored frozen and analyzed by the methods of Miettinen, Ahrens, and Grundy (17), and Grundy, Ahrens, and Miettinen (18) to measure, respectively, fecal neutral steroids (NS), bile acids (BA), and also chromic oxide (16). Aliquots corresponding to the NS fraction in PII were also assayed for radioactivity after TLC.

Calculations

Cholesterol metabolism parameters were calculated employing the equations of Method I (19), which are summarized as follows. *1*) Cholesterol synthesis in PI = steroid excretion (NS + BA). *2*) Cholesterol balance in PII = dietary cholesterol − total steroid excreted (negative balance = maintenance of cholesterol synthesis; positive balance = body cholesterol accumulation). *3*) Endogenous NS excretion = radioactivity in total fecal NS in PII ÷ corresponding plasma cholesterol specific activity. *4*) Absorption of exogenous cholesterol in PII = dietary cholesterol − (total fecal NS − endogenous NS). *5*) Daily cholesterol turnover in PII = endogenous NS + BA, which means cholesterol absorption plus synthesis. In those patients in whom cholesterol balance was positive, turnover could not be calculated since the metabolic steady state was not attained. In the present work, all data are presented in mg/kg of body weight per day.

RESULTS

Table 2 presents the parameters of whole-body cholesterol metabolism. During the cholesterol-free diet period (PI), steroid synthesis varied widely among all subjects and did not correlate with mean serum cholesterol values ($r = 0.02$). Absorption of dietary cholesterol in PII was also independent of the PI plasma cholesterol concentration ($r = 0.06$), and likewise varied greatly among the patients.

During the exogenous cholesterol absorption period (PII), six patients accumulated cholesterol in the body as shown by their positive fecal balances (cases 1, 3, 4, 9, 10, and 13) whereas in seven others synthesis continued (negative balance). Either event took place irrespective of the patient being normal or hypercholesterolemic. Cholesterol turnover measured throughout PII was also shown to be independent of the basal serum cholesterol values obtained during PI.

During cholesterol feeding (PII), serum cholesterol concentration rose significantly when compared to PI in seven patients (cases 2, 6, 7, 9, 11, 12, and 13) whereas the concentration was reasonably stable in the remaining six patients (cases 1, 3, 4, 5, 8, and 10). The variation of serum cholesterol from PI to PII also did not correlate either with the amount of cholesterol absorbed from the diet or with the steroid balance values. Consequently, in some individuals serum cholesterol remained almost unchanged in spite of accumulating cholesterol in PII (cases 1, 3, 4, and 10). Moreover, other patients (cases 2, 6, 7, 11, and 12) who happened to have absorbed less cholesterol, thus circumventing its retention in the body, nevertheless had major increments in plasma cholesterol concentration.

Fig. 1 depicts the independence of the basal cholesterol levels from steroid synthesis in PI, absorption of dietary cholesterol in PII, steroid balance in PII, and bile acid excretion throughout the study. Subsequent to the administration of dietary cholesterol, 3 of the 13 individuals (cases 8, 10, and 11) significantly increased their output of bile acids.

DISCUSSION

Since intestinal absorption varied widely among the subjects, a clear-cut comparison between the compensatory mechanisms of hypercholesterolemic and normal subjects was difficult. Nonetheless, the results of fecal steroid balance (Fig. 1) favor the hypothesis that both groups had similar abilities to compensate for the input of exogenous cholesterol since individuals either accumulated cholesterol, or its retention in the body was inhibited irrespective of basal serum cholesterol levels. In this regard, a positive correlation between absorption of exogenous cholesterol and steroid balance was found (**Fig. 2**). Accordingly, synthesis decreased as dietary cho-

TABLE 2. Parameters of daily whole body cholesterol metabolism in patients fed a

Patients[a]	1	2	3	4	5
			Mean mg/100 ml ± SD		
Basal cholesterolemia (PI)	168 ± 18	189 ± 52	227 ± 21	252 ± 25	257 ± 41
Cholesterolemia in PII	178 ± 17	410 ± 77	199 ± 38	235 ± 42	271 ± 30
Variation in cholesterolemia (PII-PI)	10	221	−28	−17	14
			Mean mg/kg body weight ± SD		
Steroid synthesis in PI	8.07 ± 3.52	10.61 ± 3.29	13.55 ± 3.40	5.74 ± 2.43	11.15 ± 6.35
Steroid balance (PII)[d]	2.66 ± 5.98	−7.43 ± 9.60	7.47 ± 7.08	3.85 ± 4.45	−3.37 ± 9.75
Steroid synthesis in PIII	7.33 ± 3.61	12.06 ± 5.08	9.72 ± 1.24	8.10 ± 4.21	10.31 ± 4.48
Endogenous neutral steroids in PII	4.32 ± 1.38	8.09 ± 3.20	6.72 ± 1.23	7.71 ± 1.95	9.91 ± 2.00
Dietary cholesterol absorption (PII)	7.35 ± 4.94	2.16 ± 9.51	15.57 ± 6.76	12.34 ± 2.87	7.71 ± 7.95
Cholesterol turnover (PII)		9.36 ± 2.83			11.07 ± 1.83

[a] Patients are numbered according to the increasing values of their basal (PI) serum cholesterol levels.
[b] Patient 13: cholesterol intake during PII was 1450 mg/day.
[c] Patient 6 was excluded for lack of fecal Cr_2O_3 correction.
[d] Positive values represent body cholesterol accumulation; negative values represent persistance of body synthesis.

lesterol absorption increased, until the compensatory mechanisms were surpassed, thereby leading to increased storage of cholesterol in the body. In fact, in all subjects who absorbed more than 12 mg/kg body weight/day of dietary cholesterol (cases 3, 4, 10, and 13) its retention in the body was not prevented. In two

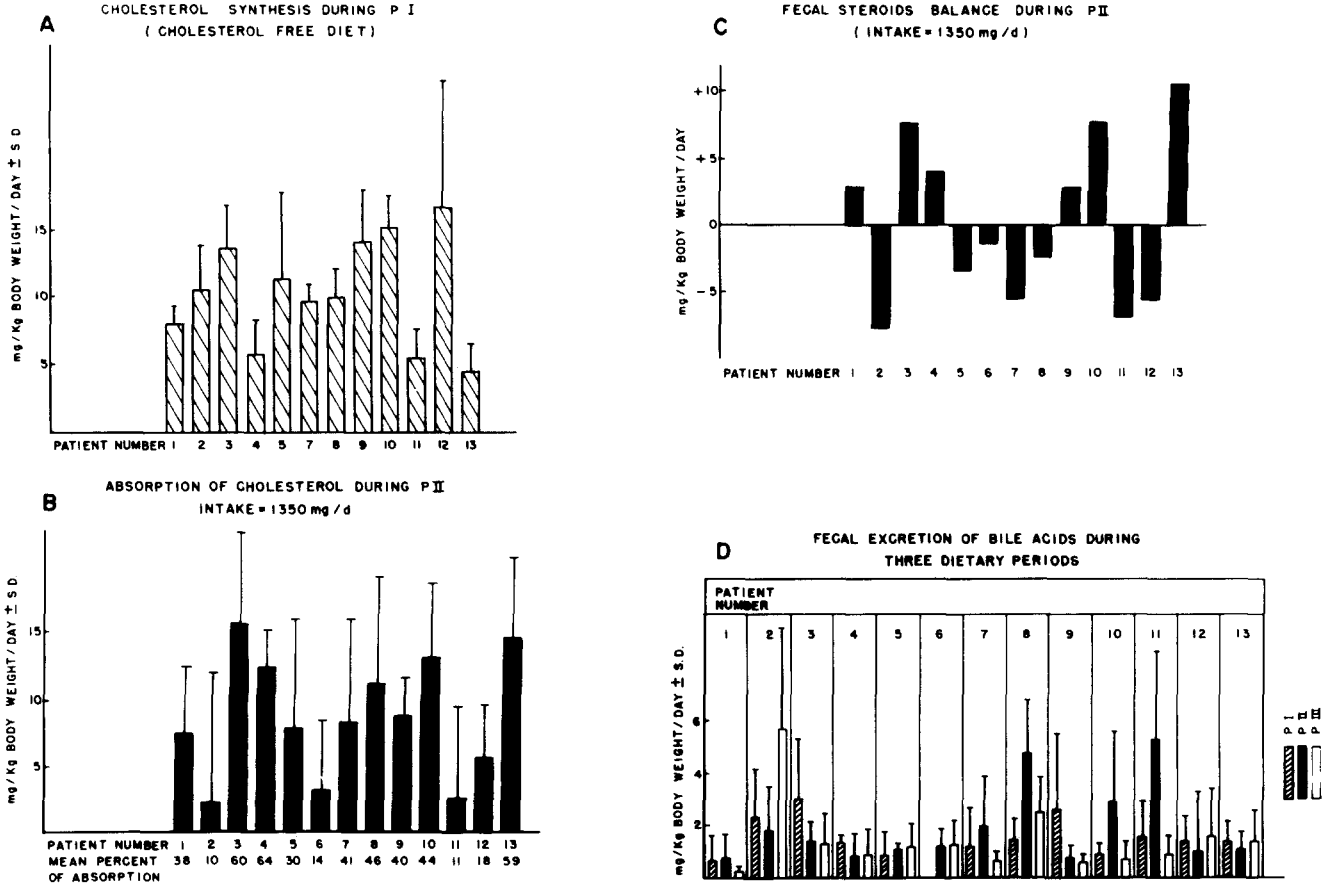

Fig. 1. Parameters of whole body cholesterol metabolism. A, Cholesterol synthesis during PI (cholesterol-free diet period); B, absorption of cholesterol during PII (intake of 1350 mg/day); C, fecal steroids balance during PII; D, fecal excretion of bile acids during the sequential three dietary periods (PI, PII, and PIII, which is also a cholesterol-free diet period). Patients are numbered according to the increasing order of their basal (PI) serum cholesterol levels. Patient 13 was fed 1450 mg of cholesterol/day during PII. The bile acid value for patient 6 (PI) was excluded for lack of a fecal chromic oxide correction.

cholesterol-poor diet (PI and PIII) as compared to a cholesterol-rich diet (PII: 1350 mg/day)

6	7	8	9	10	11	12	13[b]
268 ± 28	293 ± 56	301 ± 29	312 ± 74	318 ± 32	358 ± 44	523 ± 40	717 ± 94
342 ± 25	405 ± 59	301 ± 66	344 ± 66	300 ± 40	402 ± 48	588 ± 87	808 ± 87
74	112	0	32	−18	44	65	91
[c]	9.49 ± 2.27	9.89 ± 2.27	14.11 ± 6.35	15.20 ± 4.34	5.32 ± 2.11	16.79 ± 9.62	4.20 ± 1.70
−1.58 ± 5.43	−5.43 ± 16.13	−2.33 ± 5.82	2.73 ± 4.70	7.56 ± 6.10	−6.75 ± 7.30	−5.53 ± 4.27	10.50 ± 6.51
7.73 ± 3.56	12.08 ± 3.54	10.94 ± 3.60	4.59 ± 1.52	7.35 ± 3.23	4.72 ± 1.62	16.74 ± 13.31	4.28 ± 2.05
3.50 ± 0.50	11.63 ± 6.84	8.69 ± 1.10	5.22 ± 2.14	2.63 ± 0.81	4.11± 1.62	10.34 ± 1.07	2.67 ± 0.41
3.15 ± 5.27	8.19 ± 7.62	11.12 ± 7.47	8.69 ± 2.87	13.05 ± 5.07	2.56 ± 6.97	5.68 ± 3.95	14.30 ± 5.79
4.72 ± 1.57	13.66 ± 8.51	11.50 ± 2.10			9.31 ± 3.02	11.15 ± 1.91	

others, cholesterol accumulated despite lower absorption values (cases 1 and 9).

Ultimately, the analysis of individual responses to dietary cholesterol supports the conclusion that hypercholesterolemic individuals have actually the same capacity as compared to normals to compensate for exogenous cholesterol input. *1*) Patient 1 (normal) accumulated cholesterol while absorbing 7.35 mg/kg body weight/day, whereas two hypercholesterolemic subjects (cases 7 and 8) absorbed more and yet body accumulation did not take place. *2*) Among those who accumulated cholesterol in PII there were three normals (cases 1, 3, and 4) and three hypercholesterolemic subjects (cases 9, 10, and 13). Obviously, the larger quantities of absorbed cholesterol resulted in compensatory mechanisms operating at full capacity. Therefore, we can state that, at different absorption levels, those who absorbed more per unit of accumulated cholesterol had more efficient compensatory mechanisms. Conversely, these mechanisms were less efficient when accumulation occurred despite absorbing less dietary cholesterol. The "absorption/accumulation" index was then calculated and no significant correlation with basal serum cholesterol level was found (**Table 3**).

Previous experiments support these results. Ho, Biss, and Taylor (20) also found serum cholesterol levels to be independent of parameters of body cholesterol metabolism measured by the combined compartmental analysis and fecal steroid balance. Likewise, Goodman et al. (21) showed by compartmental analysis that no correlation exists between cholesterol production rate and serum cholesterol levels. Also Samuel and Perl (11) found no differences in cholesterol turnover when comparing hypercholesterolemic subjects to normal subjects studied by integral analysis. On the other hand, Schwartz et al. (6) found increased cholesterol body synthesis in a homozygous child aged 13 months and afterwards at 3 years of age, remarking that in the literature two other affected children also displayed the same defect. Nonetheless in older children (13–16 years), synthesis was found to be normal. In addition, in some hypercholesterolemic subjects studied by Quintão, Grundy, and Ahrens (15) and Nestel and Poyser (22), cholesterol accumulation in the body was quite efficiently blocked during cholesterol feeding in two dietary-step designed experiments, although no comparison was made to normal individuals.

Theoretically, the amount of exogenous cholesterol absorbed by the intestine is capable of eliciting dimin-

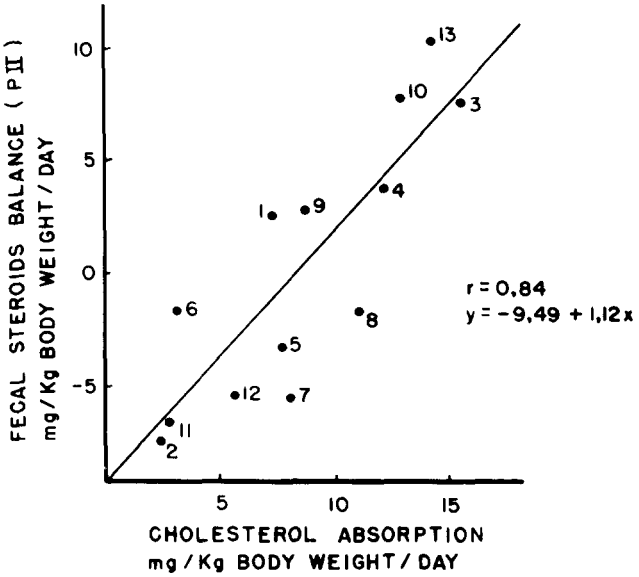

Fig. 2. Correlation between dietary cholesterol absorption and fecal steroids balance during cholesterol feeding of 1350 mg/day in PII ($P < 0.001$). Patients are numbered according to the increasing order of their basal serum cholesterol values as measured on the cholesterol-free diet (PI).

TABLE 3. Correlation between basal serum cholesterol levels (measured during PI) and the "absorption of dietary cholesterol in PII/cholesterol accumulation in PII" index (Abs./Acc.)[a]

Patients	Mean Serum Cholesterol Measured during PI	Abs./Acc.[b] Index
1	168	2.76
3	227	2.08
4	252	3.21
9	312	3.18
10	318	1.73
13	717	1.36

[a] Patients 1, 3, 4, 9, 10, and 13 are numbered according to the increasing values of their PI serum cholesterol. The other cases were excluded because body cholesterol accumulation did not occur in PII.
[b] Absorption/Accumulation index; serum cholesterol vs. index; $r = 0.65$ (not significant).

ished synthesis and enhanced fecal steroid reexcretion as endogenous cholesterol or bile acids. Patients studied by Quintão et al. (15) had compensated for exogenous cholesterol intake mainly by diminishing synthesis. In subjects studied by Nestel and Poyser (22), enhanced reexcretion of neutral steroids was an equally important compensatory system. Augmented reexcretion of cholesterol as bile acids was not demonstrated by those authors, but was recently observed by Lin and Connor (23). **Table 4** demonstrates compensatory mechanisms in those patients who maintained negative steroid balance during the cholesterol-rich diet period. It is clearly seen that a diminished synthesis was the main intervening mechanism. Increased reexcretion of neutral steroids was operative to a lesser extent. Incidentally, in two hypercholesterolemic individuals in the present study (cases 8 and 11), retention of body cholesterol was prevented by an enhanced excretion of bile acids.

Lin and Connor (23) reported accumulation of body cholesterol in a hypercholesterolemic subject but not in his normal control after eating 1000 mg/day of cholesterol, in spite of a negative fecal steroid balance in both subjects. Nevertheless, the following objections may be raised against their conclusions. *1*) The authors included the expansion of the plasma cholesterol pool elicited by the dietary load as accounting for the dietary cholesterol mass absorbed. Such an approach is rather questionable, since during cholesterol feeding plasma cholesterol originates both from the diet and from synthesis. However, the latter's share of total plasma cholesterol in PII is necessarily smaller than in PI because synthesis was markedly blocked during cholesterol feeding. Consequently, serum cholesterol of dietary origin must account for much more than simply the increment from PI to PII. *2*) In spite of their claim that endogenous neutral steroids decreased on cholesterol feeding, the opposite is found (Ref. 23, Table 5) i.e., enhancement of endogenous neutral steroids took place. *3*) When their data were recalculated according to our Table 4, the conclusion drawn was that their two studied cases did not accumulate cholesterol in the body. In similar calculations utilized by other authors (15, 22), the alterations observed in the plasma cholesterol pools were obviously already included in the variation of the whole-body cholesterol metabolism parameters elicited by cholesterol feeding.

Likewise in the present study, the near zero values obtained from the equation "compensatory mechanisms minus absorbed cholesterol" (Table 4) exclude the possibility that there was accumulation of cholesterol in the patients who maintained negative balance during PII. Similar results occurred in the cases studied by Quintão et al. (15), and Nestel and Poyser (22).

The variations in serum cholesterol due to oral cholesterol administration (Table 2) were also independent of the measured steroid metabolic parameters. Similar to what was observed in patients 2, 6, 7, 11, and 12 of the present study, it has also been reported (14, 20) that plasma cholesterol levels may rise in spite of efficient compensatory mechanisms. On the other hand, some individuals (cases 1, 3, 4, and 10) exhibited body cholesterol retention while absorbing larger amounts of cholesterol, but nevertheless their serum levels remained stable as compared to the cholesterol-poor pe-

TABLE 4. Regulation of whole body cholesterol metabolism during a high cholesterol diet (PII = 1350 mg/day)

Patients	2	5	7	8	11	12
			mg/kg body weight per day			
Variation in steroid body synthesis[a]	3.18	7.78	4.05	7.63	−1.43	11.26
Variation in endogenous neutral steroid excretion[b]	−0.15	−0.33	3.38	0.25	0.39	−4.55
Variation in bile acid excretion[c]	−0.56	0.30	0.79	3.72	3.60	−0.62
Total compensatory mechanisms (C.M.)[d]	2.47	8.04	8.22	11.60	2.56	6.09
Exogenous cholesterol absorption in PII (Abs)	2.16	7.71	8.19	11.12	2.56	5.68
C.M. − Abs.	0.31	0.04	0.03	0.48	0.00	0.41

[a] Variation in steroid body synthesis = (PI − PII).
[b] Variation in endogenous neutral steroid excretion = endogenous NS in PII − NS excretion in PI.
[c] Variation in bile acid excretion = (PII − PI).
[d] Total compensatory mechanisms = $a + b + c$.
Patients 1, 3, 4, 9, 10, and 13 were excluded because steroid balance was positive. Patient 6 was excluded for lack of fecal chromic oxide correction in PI. Patients are listed according to the increasing values of their basal serum cholesterol concentration (PI).

riod. The possibility arises that in those subjects in positive balance but with stable serum cholesterol, the liver might be the storage place for cholesterol, as demonstrated in liver biopsies by Quintão, Brumer, and Stechhahn (24). Such stored cholesterol would result in interruption of liver cholesterol production, thereby preventing the increase of serum cholesterol from taking place.

When this experimental protocol was designed, it had been expected that cholesterol stored in the body during PII would have been reexcreted in PIII, and thus the fecal steroid output in PIII would have been greater than that of PI. Contrarily, the opposite event took place; fecal steroid excretion was greater in PI than in PIII in Patients 3, 9, and 10. This suggests that cholesterol that had accumulated in PII was stored after withdrawing cholesterol from the diet while preserving local liver synthesis interrupted through PIII.

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