Effect of aging on cholesterol 7α-hydroxylation in humans[1]

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Abstract In order to investigate the alterations of bile acid synthesis in aging, we studied the rates of cholesterol 7α-hydroxylation, the rate-limiting step, in 28 patients of different ages (34–83 years old, 14 below and 14 above the age of 65) of both sexes. Hydroxylation rates were determined by tritium release assay after an intravenous bolus of [7α-^{3}H]cholesterol. Cholesterol 7α-hydroxylation was significantly decreased in the older age group, compared to middle-aged subjects, both in males and females; moreover, a significant inverse correlation between hydroxylation rates and age was found in the whole sample ($r = -0.56$; $P < 0.01$) and in females, but not in males. The percent concentration of deoxycholic acid in plasma (determined by gas-liquid chromatography) was increased in older subjects. Plasma cholesterol and triglyceride levels were not related with age even though triglyceride concentrations tended to be lower in the older age group. Triglyceride, but not cholesterol levels, were directly correlated with hydroxylation rates ($r = 0.45$, $P < 0.05$). After cholestyramine treatment (8–12 g/day for 4 weeks) a sharp increase in 7α-hydroxylation rates was observed in three elderly patients, accompanied by reduced levels of dihydroxylated bile acids. Our data are consistent with a reduced rate of conversion of cholesterol to bile acids with aging, particularly in females, and suggest a coordinate reduction of triglyceride production. Alterations of the quantitative and/or qualitative pattern of the bile acid pool recirculating to the liver may be responsible, at least in part, for the changes observed.—**Bertolotti, M., N. Abate, S. Bertolotti, P. Loria, M. Concari, R. Messora, F. Carubbi, A. Pinetti, and N. Carulli.** Effect of aging on cholesterol 7α-hydroxylation in humans. *J. Lipid Res.* 1993. **34:** 1001–1007.

Supplementary key words bile acid synthesis • cholesterol homeostasis • plasma cholesterol • plasma triglycerides • bile acid pool • cholestyramine

Increasing age is associated with important alterations of cholesterol metabolism in humans. As shown by epidemiological studies, plasma cholesterol levels tend to increase with advancing age (1, 2) and the prevalence of cholesterol gallstone disease definitely increases with aging (3, 4). The latter phenomenon is likely accounted for by increased biliary secretion of cholesterol (5), whereas the increase in plasma cholesterol levels was related to reduced turnover of low density lipoprotein (6, 7).

Alterations in bile acid metabolism could also be expected in the elderly; in fact, the rates of bile acid synthesis, evaluated by the isotope dilution technique, were found to be inversely correlated with age (5).

We recently validated a technique to evaluate the rates of cholesterol 7α-hydroxylation in humans in vivo with no need for duodenal intubation (8, 9). As 7α-hydroxylation is the first and rate-limiting step in the pathway leading from cholesterol to primary bile acids (10, 11), its evaluation leads to an estimate of bile acid synthesis rates. The ultimate mechanisms of regulation of bile acid synthesis and of its rate-limiting step are not completely understood; in this control, factors related to the recirculation of bile acids seem to play a role (12), most likely at a transcriptional level (13, 14).

In the present work we investigated the influence of age on the rates of cholesterol 7α-hydroxylation in male and female patients and how these parameters relate to plasma lipid concentrations and recirculating bile acid pattern.

MATERIALS AND METHODS

Subjects

Twenty-eight patients (10 females, 18 males), admitted to the 1st Division of General Medicine (Istituto di Patologia Medica) of the University of Modena or to the Division of Geriatrics of the Giovanni XXIII Hospital of Albinea were investigated. The patients ranged between 34 and 83 years of age (9 males and 5 females, below age

[1]This paper is dedicated to Professor Dr. Gustav Paumgartner in honor of his sixtieth birthday.

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65, and 9 males and 5 females over age 65). Criteria of inclusion were: normal function of liver, intestine, and thyroid, and normal nutritional state, no history of alcohol abuse, no diagnosed malignancy, and serum levels of cholesterol and triglycerides below 250 mg/dl (**Table 1**). Four subjects (patients 2, 5, 14, and 18) showed impaired glucose tolerance. None of the patients were taking insulin or other drugs known to affect lipid metabolism. The subjects gave their consent to the study protocol, which was approved by the Ethical Committee of the University of Modena.

The patients were administered an isocaloric diet containing about 400 mg of cholesterol per day. After clinical and laboratory evaluation, the rates of cholesterol 7α-hydroxylation were studied once in all subjects. Three elderly patients (one female, two males) were reinvestigated after a 4-week treatment with cholestyramine (8–12 g/day given twice or thrice daily before meals). Cholestyramine was administered as 4-g packages (Questran, Bristol Italiana, Roma, Italy).

Methods

[7α-^{3}H]cholesterol (sp act 3–9 mCi/mmol) was synthesized as described (8, 15). The rates of cholesterol 7α-hydroxylation were assayed by tritium release assay; the technique has been previously validated under in vitro conditions (16, 17) and subsequently in vivo (8, 9). Trace amounts of [7α-^{3}H]cholesterol (200–400 μCi), dissolved in ethanol and then in 50 ml of human albumin, were injected intravenously after an overnight fast; blood or urine samples were taken at fixed intervals thereafter for 5–6 days. The water distilled from erythrocyte or urine samples was assayed for radioactivity; the specific activity of plasma cholesterol was determined after extraction, as the radioactivity/mass ratio.

As 7α-hydroxylation of cholesterol is a highly stereospecific reaction, the amount of tritium removed from the 7α position of the sterolic nucleus and joining the body water pool as tritiated water reflects the extent of hydroxylation. Cholesterol 7α-hydroxylation could be

TABLE 1. Clinical data and 7α-hydroxylation rates of subjects

Patient	Sex	Age	Body Weight	Body Mass Index	Cholesterol	Triglyceride	Cholesterol 7α-Hydroxylation
		yr	*kg*	*kg/m²*	*mg/dl*		*mg/day*
1	M	34	74	25.6	247	244	507
2	M	35	69	23.9	171	62	323
3	F	36	54	26.8	182	97	475
4	F	37	70	28.8	81	74	457
5	M	37	56	23.3	162	105	295
6	M	43	60	21.8	166	160	477
7	F	45	73	29.6	249	151	706
8	M	51	62	20.2	197	224	304
9	F	53	93	29.9	179	122	391
10	F	55	61	25.7	194	117	333
11	M	57	81	28.4	226	213	377
12	M	57	73	25.9	224	234	601
13	M	59	68	24.1	248	148	296
14	M	60	87	28.4	214	238	785
Mean ± SD			70 ± 11	25.9 ± 3.0	196 ± 45	156 ± 64	452 ± 155
15	M	68	82	26.8	204	89	389
16	F	70	70	27.3	220	97	191
17	M	71	71	26.4	246	133	138
18	M	73	77	25.7	145	72	364
19	M	73	68	24.1	121	109	133
20	M	74	67	24.3	151	71	460
21	M	75	63	25.2	194	78	221
22	M	76	64	24.4	202	88	351
23	F	78	65	25.4	209	109	99
24	M	81	68	22.7	231	98	61
25	F	81	59	20.9	235	120	92
26	M	82	64	24.1	160	51	408
27	F	82	62	25.8	219	92	164
28	F	83	73	28.5	185	92	89
Mean ± SD			68 ± 6	25.1 ± 1.9	194 ± 37	92 ± 21[a]	226 ± 139[a]

Patients were divided into two age groups, below and above age 65; n = 14 in each group.
[a] $P < 0.01$ compared to younger subjects; Student's t test for paired data.

calculated as the ratio between the increment of body water radioactivity in a fixed time interval (usually 60–72 h after tracer infusion) and the mean specific activity of plasma cholesterol in the same interval. Hydroxylation rates were expressed as the amount of cholesterol undergoing 7α-hydroxylation per day, after correcting for the degree of stereospecificity of the label on the 7α position (which averaged 75% in this study) (8). Total body water volume was assumed to equal 60% of ideal body weight, expressed in kilograms and estimated as height (cm) – 100.

In a preliminary study, conducted in four subjects with normal liver function (not included in the present paper) and two patients with advanced liver cirrhosis showing markedly reduced 7α-hydroxylation rates, a good correspondence was found between bile acid synthesis rates, calculated with the isotope dilution technique (18), and 7α-hydroxylation as estimated by our tritium release method (M. Bertolotti, M. Con :ari, and N. Carulli, unpublished observations, 1992).

Routine laboratory evaluation was performed by automated analysis. Plasma cholesterol and triglyceride concentrations were determined by enzymatic techniques; lipoprotein cholesterol was determined in the different density fractions, separated by sequential ultracentrifugation of plasma. Plasma bile acid composition was determined on fasting samples by gas–liquid chromatography as described (19), on a Fractovap 4200 equipment (Carlo Erba, Milano, Italy).

Statistical evaluation

When appropriate, data were expressed as the mean ± SD and statistical differences between groups were evaluated according to Student's t test for unpaired data.

Simple and multiple regression analysis among various parameters was performed. Multiple regression was conducted only with the data from both sexes; parameters investigated included age, cholesterol 7α-hydroxylation, body mass index, plasma cholesterol, and triglyceride concentrations. Hydroxylation rates, cholesterol levels, and triglyceride levels were used as dependent variables on separate evaluations, with the others as independent variables. Analysis was made according to the SPSS/PC statistical package utilizing an IBM PS2 workstation.

RESULTS

Table 1 shows plasma lipid concentrations and cholesterol 7α-hydroxylation rates for all patients. Hydroxylation rates were reduced by 50% in elderly patients; a significant difference was observed both among males (below 65 years: 440 ± 169 and above 65: 281 ± 144 mg/day, $P < 0.05$) and females (below 65 years: 472 ± 142 and above 65: 127 ± 47 mg/day, $P < 0.01$). Cholesterol concentrations were not different in the two age groups, whereas plasma triglyceride levels were 41% lower in the elderly.

Fig. 1 illustrates the relationship between age and cholesterol 7α-hydroxylation rates in all patients, showing a significant inverse correlation ($r = -0.56$; $P < 0.01$). The equation of the regression line was $y = 719.3 - 6.17\ x$. A highly significant correlation was observed when data from only female patients were analyzed ($r = -0.89$; $P < 0.01$) whereas plotting the values of males alone showed a nonsignificant negative trend with increasing age ($r = -0.32$; $P > 0.1$).

When multiple regression analysis was performed considering cholesterol 7α-hydroxylation as the dependent variable, age was the only parameter showing a significant correlation; adding either body mass index, plasma cholesterol, or plasma triglyceride levels did not contribute significantly to the regression.

No significant correlation was detected between age and body mass index ($r = -0.12$, $P > 0.1$). A correlation of borderline significance was present between age and plasma triglycerides ($r = -0.36$, $P < 0.07$), whereas no correlation was present with cholesterol levels ($r = 0.14$, $P > 0.1$) as illustrated in **Fig. 2.**

Fig. 3 illustrates the relationship between cholesterol 7α-hydroxylation rates and plasma cholesterol (upper panel) and triglycerides (lower panel). Cholesterol levels showed no correlation, whereas a significant direct correlation was present between 7α-hydroxylation and triglycerides ($r = 0.45$, $P < 0.05$).

As could be expected, plasma cholesterol and triglyceride levels were significantly correlated ($r = 0.49$, $P < 0.01$). In multiple regression analysis with triglycerides as the dependent variable, adding 7α-hydroxylation rates to the

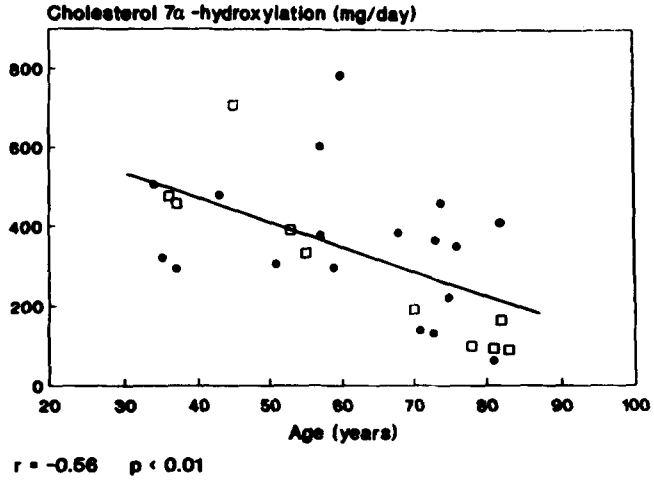

Fig. 1. Correlation between age and the rates of cholesterol 7α-hydroxylation, expressed as the amount of cholesterol undergoing 7α-hydroxylation per day, in the patients studied (closed circles, males; open squares, females). Equation of the regression line: $y = 719.3 - 6.17\ x$; $r = -0.56$, $P < 0.01$.

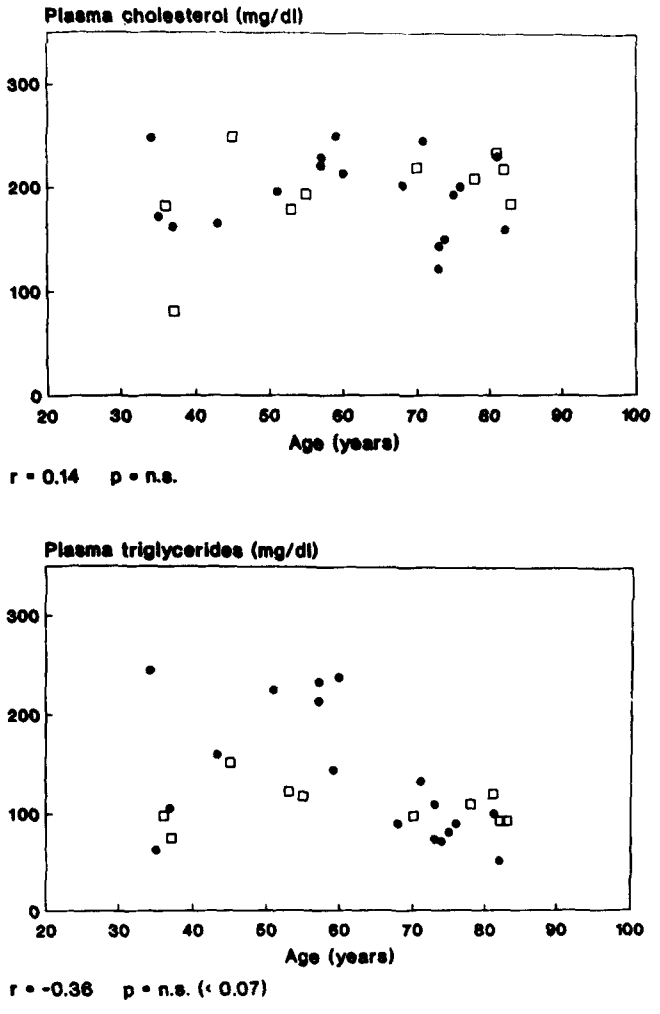

Fig. 2. Correlation between age and plasma concentration of total cholesterol (upper panel) and between age and plasma triglycerides (lower panel). Closed circles, males; open squares, females; n.s., not significant ($P > 0.1$ unless indicated).

regression equation already containing cholesterol as independent variable contributed significantly to the regression, still explaining more than 20% of plasma triglyceride variance. On the other hand, with cholesterol as the dependent variable, by adding age to the equation containing triglyceride levels as the independent variable, a significant contribution to the regression could be obtained, accounting for nearly 11% of plasma cholesterol variance.

No correlation was detected between hydroxylation rates and cholesterol concentration in low density lipoproteins, which paralleled total plasma cholesterol, and in the other fractions as well (data not shown).

A trend toward a modification of the plasma bile acid pool composition between the two age groups was observed: percent deoxycholic acid content was increased from 19 ± 4% in the younger subjects to 23 ± 5% in the older subjects ($P < 0.05$); cholic acid tended to decrease from 40 ± 6% to 36 ± 9% while chenodeoxycholic acid tended to increase from 36 ± 6% to 38 ± 9% in the older age group.

Table 2 shows the effect of cholestyramine treatment on the three subjects studied. Two of the patients could not tolerate more than 8 g (two packages) of cholestyramine per day, because of the onset of constipation. Even when statistical analysis was not performed due to the small number of patients and to the different doses used, a sharp increase of cholesterol 7α-hydroxylation was evident after treatment; this was accompanied by a shift of the recirculating bile acid pattern toward increased concentrations of cholic acid and decreased levels of chenodeoxycholic and deoxycholic acids.

Plasma total cholesterol decreased by 12-20% after treatment, whereas low density lipoprotein cholesterol

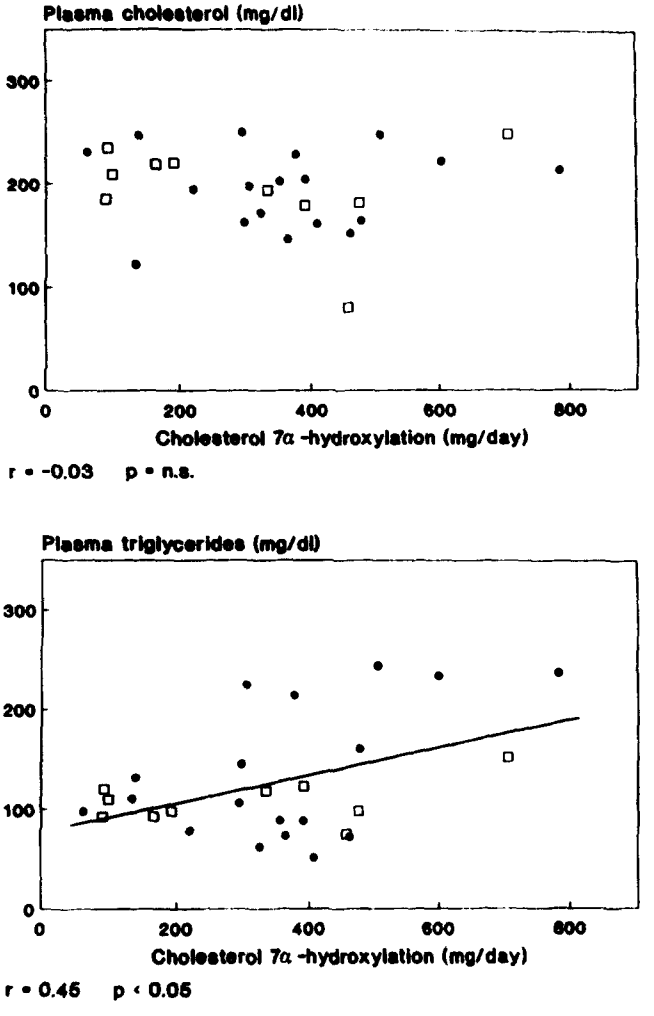

Fig. 3. Correlation between cholesterol 7α-hydroxylation and plasma cholesterol (upper panel) and plasma triglycerides (lower panel). Closed circles, males; open squares, females. Equation of the regression line between cholesterol 7α-hydroxylation rates and plasma triglycerides: $y = 77.7 + 0.14\ x$; $r = 0.45$, $P < 0.05$; n.s., not significant ($P > 0.1$).

TABLE 2. Effect of cholestyramine treatment on cholesterol 7α-hydroxylation and plasma bile acid pool composition in three elderly patients

Patient	Sex	Age	Treatment	C7αOH	Bile Acid Concentration			
					CA	CDCA	DCA	Others
		yr	*g/day*	*mg/day*		%		
17	M	71	–	138	32	48	17	3
			8	472	44	41	11	4
21	M	75	–	221	26	39	30	5
			8	554	37	35	24	4
28	F	83	–	89	31	44	24	1
			12	925	50	27	18	5

Patients were studied before and after treatment for 4 weeks with cholestyramine, at the dosage indicated; C7αOH, cholesterol 7α-hydroxylation; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid.

decreased by 15–26%. No changes were detected on the other lipoprotein fractions. Plasma triglycerides increased by 13 and 16% in two patients and remained unchanged in the third.

DISCUSSION

Cholesterol 7α-hydroxylation is the rate-limiting step of bile acid biosynthesis, a key event in the maintenance of cholesterol balance in the liver and in the whole organism (20). The present technique can quantitate the rates of 7α-hydroxylation in vivo, overcoming the pitfalls of in vitro assays of this metabolic step, and with the advantage over other techniques that measure bile acid synthesis, such as by isotope dilution (18), of providing a more direct estimate of the amount of cholesterol diverted to the bile acid synthetic pathway.

The technique has been validated and has proved to give results comparable to others in physiological and pathological situations. Possible sources of error have already been discussed (8, 9). Furthermore, this method relies on the determination of body water tritium and the effect of changes in body water turnover deserves consideration, particularly in the elderly where the volume of body water was found to be smaller (21) and its turnover might also be slower. Our older patients did not show any clinical evidence of dehydration or features suggesting alterations of water metabolism; moreover, had some changes occurred, they would have tended to overestimate body water radioactivity and 7α-hydroxylation rates, i.e., to minimize the difference with the younger group. We therefore conclude that this should bear no relevance to the interpretation of our data.

The observed reduction in the rates of cholesterol 7α-hydroxylation in the elderly supports previous findings by Einarsson et al. (5) where bile acid synthesis was evaluated by isotope dilution. An older report by Valdivieso et al. (22) instead showed no reduction in cholic acid synthesis in older women, even though the small number of subjects investigated in that study makes their conclusions uncertain. According to the present findings, it can be estimated by linear regression analysis (see Fig. 1) that the amount of cholesterol undergoing 7α-hydroxylation per day decreases by about 60 mg (nearly 150 μmol) every 10 years; this is in close agreement with the data from Einarsson and coworkers, that showed a decrement of bile acid synthesis of about 80 mg (200 μmol)/day per 10 years (5).

Such reduction in the rate of conversion of cholesterol to bile acid could induce important modifications of intracellular free cholesterol content and consequently lead to changes in the rates of cholesterol synthesis, lipoprotein uptake, and biliary cholesterol output as well. The observed changes in cholesterol 7α-hydroxylation are consistent with results previously obtained in the experimental animal and in humans. In rats and hamsters, aging was shown to associate with reduced hepatic cholesterol synthesis (23, 24) and data in humans showed a reduced turnover of low density lipoprotein in the elderly, which could be reversed by treatment with cholestyramine (7). The previously observed increase in biliary cholesterol secretion was also related to reduced utilization of cholesterol for bile acid synthesis (5).

In the present study, no simple correlation between aging and plasma cholesterol levels was observed. Only with multiple regression analysis, adding age to the equation containing cholesterol as the dependent variable and triglycerides as the independent variable significantly contributed to the regression. Moreover, no correlation was present between 7α-hydroxylation rates and plasma cholesterol. These findings could be explained by the relatively small number of subjects investigated and by the fact that patients with overt hypercholesterolemia and hypertriglyceridemia were excluded from the study. We believe that the observed decrease in cholesterol degradation to bile acid could induce a metabolic situation that favors the occurrence of hypercholesterolemia only with the coexistence of other factors, possibly environmental (dietary) and/or genetic. In other words, at least in this Mediterranean group of patients, the reduction in 7α-hydroxylation could be a prerequisite, although not sufficient per se to down-regulate the activity of low density lipoprotein receptors.

This study, both for the number of patients and for its design, cannot allow epidemiological evaluations. Nonetheless, the finding that females showed a more evident inverse correlation between 7α-hydroxylation and age is consistent with the pattern of increase of plasma cholesterol with age which was shown to take place, in a large Italian population, from young to middle age in men and from middle to old age in women (Multicentrica Italiana

Colelitiasi (MICol) Study Group, unpublished observations, 1991). Because, in the present study, mainly patients of middle to old age were investigated, alterations occurring in males at an earlier age might have been missed.

Plasma triglycerides were lower in the older age group of patients with a trend toward an inverse correlation with age, and a significant correlation was present between 7α-hydroxylation rates and triglycerides. The lack of a correlation between age and body mass index seems to rule out the occurrence of malnutrition in older patients. The correlation between 7α-hydroxylation and plasma triglycerides supports the hypothesis of a coordinate regulation of the limiting enzymes of hepatic bile acid and triglyceride synthesis (respectively, cholesterol 7α-hydroxylase and phosphatidic acid phosphatase), previously suggested as a possible pathogenetic explanation for the changes observed in familial hypertriglyceridemia (25).

The mechanisms by which aging accompanies reduced cholesterol 7α-hydroxylation and bile acid synthesis are not clear. The finding that cholestyramine restores normal or supranormal hydroxylating capacity (Table 2) is against a specific enzymatic deficiency taking place with aging. The stimulated values of cholesterol 7α-hydroxylation appear to be somewhat lower, though, compared to the values obtained in middle-aged patients treated with comparable doses of cholestyramine, which ranged from 800 to 2000 mg/day in a previous report from our group (9). This trend toward a reduced reserve in 7α-hydroxylating capacity is consistent with the finding of reduced liver mass in the elderly, observed by Marchesini et al. (26). Alterations in the levels of growth hormone, which were found to increase bile acid synthesis (27), could play a role in these changes.

We finally looked at the composition of the recirculating bile acid pool. The qualitative and quantitative patterns of bile acid in peripheral plasma are certainly different from those in the portal blood, which are more likely to reflect the flux of bile acids through the liver (28). With these limitations, evaluation of bile acids in peripheral plasma may still be of some value, especially when used for comparative purposes. In our patients we observed a higher percentage of cholic acid compared to most previous studies (28, 29), probably due to technical factors related to the assay. On the other hand there are reports showing bile acid profiles more similar to ours (29, 30). The percent content of dihydroxy bile acids in plasma, in particular of deoxycholic acid, tended to be higher in the elderly; moreover, cholestyramine treatment (Table 2) reduced the concentration of dihydroxy bile acids and increased 7α-hydroxylation rates. Previous results also showed an increase of secondary bile acids with aging, possibly due to increased formation of deoxycholic acid in the gut (31, 32). The data are consistent with a body of evidence, including our own, showing an inhibitory effect on bile acid synthesis and on cholesterol 7α-hydroxylation by hydrophobic bile acids, such as chenodeoxycholic and deoxycholic acids, and not by hydrophilic ones, such as ursodeoxycholic acid (9, 33–35). It is possible that changes in the size and composition of the bile acid pool recirculating from the intestine to the liver may underlie a more active feed-back inhibition of cholesterol 7α-hydroxylation. Alterations in transit time and in the bacterial flora of the intestine might play a role in this respect.

Ongoing research on the molecular biology of cholesterol 7α-hydroxylation will hopefully provide a better understanding of the regulation of this metabolic step in physiology and disease and of the metabolic consequences of its alterations.

This work was supported in part by the Research Funds (60%) of the University of Modena.

Manuscript received 25 September 1992 and in revised form 31 December 1992.

REFERENCES

1. Heiss, G., I. Tamir, C. E. Davis, H. A. Tyroler, B. M. Rifkind, G. Schonfeld, D. Jacobs, and I. D. Frantz, Jr. 1980. Lipoprotein-cholesterol distributions in selected North American populations: the Lipid Research Clinics Program Prevalence Study. *Circulation.* **61:** 302–315.
2. Abbott, R. D., R. J. Garrison, P. W. F. Wilson, F. H. Epstein, W. P. Castelli, M. Feinleib, and C. LaRue. 1983. Joint distribution of lipoprotein cholesterol classes. The Framingham Study. *Arteriosclerosis.* **3:** 260–272.
3. Friedman, G. D., W. B. Kannel, and T. R. Dawber. 1966. The epidemiology of gallbladder disease: observations in the Framingham Study. *J. Chronic Dis.* **19:** 273–292.
4. Rome Group for the Epidemiology and Prevention of Cholelithiasis (GREPCO). 1984. Prevalence of gallstone disease in an Italian adult female population. *Am. J. Epidemiol.* **119:** 796–805.
5. Einarsson, K., K. Nilsell, B. Leijd, and B. Angelin. 1985. Influence of age on secretion of cholesterol and synthesis of bile acids by the liver. *N. Engl. J. Med.* **313:** 277–282.
6. Miller, N. E. 1984. Why does plasma low density lipoprotein concentration in adults increase with age? *Lancet.* **Feb. 4:** 263–267.
7. Ericsson, S., M. Eriksson, S. Vitols, K. Einarsson, L. Berglund, and B. Angelin. 1991. Influence of age on the metabolism of plasma low density lipoproteins in healthy males. *J. Clin. Invest.* **87:** 591–596.
8. Bertolotti, M., N. Carulli, D. Menozzi, F. Zironi, A. Digrisolo, A. Pinetti, and M. G. Baldini. 1986. In vivo evaluation of cholesterol 7α-hydroxylation in humans: effect of disease and drug treatment. *J. Lipid Res.* **27:** 1278–1286.
9. Bertolotti, M., N. Abate, P. Loria, M. Dilengite, F. Carubbi, A. Pinetti, A. Digrisolo, and N. Carulli. 1991. Regulation of bile acid synthesis in humans: effect of treatment with bile acids, cholestyramine or simvastatin on cholesterol 7α-hydroxylation rates in vivo. *Hepatology.* **14:** 830–837.
10. Myant, N. B., and K. A. Mitropoulos. 1977. Cholesterol 7α-hydroxylase. *J. Lipid Res.* **18:** 135–153.
11. Björkhem, I. 1985. Mechanism of bile acid biosynthesis in

mammalian liver. *In* Sterols and Bile Acids. H. Danielsson and J. Sjövall, editors. Elsevier, Amsterdam. 231–278.
12. Einarsson, K., K. Hellström, and M. Kallner. 1973. Feedback regulation of bile acid formation in man. *Metabolism.* **22:** 1477–1483.
13. Jelinek, D. F., S. Andersson, C. A. Slaughter, and D. W. Russell. 1990. Cloning and regulation of cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis. *J. Biol. Chem.* **265:** 8190–8197.
14. Pandak, W. M., Y. C. Li, J. Y. L. Chiang, E. J. Studer, E. C. Gurley, D. M. Heuman, Z. R. Vlahcevic, and P. B. Hylemon. 1991. Regulation of cholesterol 7α-hydroxylase mRNA and transcriptional activity by taurocholate and cholesterol in the chronic biliary diverted rat. *J. Biol. Chem.* **266:** 3416–3421.
15. Corey, E. J., and G. A. Gregoriou. 1959. Stereospecific synthesis of the 7-deuterio and 7-tritio cholesterols. The mechanism of enzyme-catalyzed hydroxylation at a saturated carbon atom. *J. Am. Chem. Soc.* **81:** 3127–3133.
16. Van Cantfort, J., J. Renson, and J. Gielen. 1975. Rat liver cholesterol 7α-hydroxylase. I. Development of a new assay based on the enzymic exchange of the tritium located on the 7α position of the substrate. *Eur. J. Biochem.* **55:** 23–31.
17. Carulli, N., M. Ponz de Leon, F. Zironi, A. Pinetti, A. Smerieri, R. Iori, and P. Loria. 1980. Hepatic cholesterol and bile acid metabolism in subjects with gallstones: comparative effects of short-term feeding of chenodeoxycholic and ursodeoxycholic acid. *J. Lipid Res.* **21:** 35–43.
18. Lindstedt, S. 1957. The turnover of cholic acid in man. *Acta Physiol. Scand.* **40:** 1–9.
19. Ali, S. S., and N. B. Javitt. 1970. Quantitative estimation of bile salts in serum. *Can. J. Biochem.* **48:** 1054–1057.
20. Turley, S. D., and J. M. Dietschy. 1988. The metabolism and excretion of cholesterol by the liver. *In* The Liver: Biology and Pathobiology. I. M. Arias, W. B. Jakoby, H. Popper, D. Schachter, and D. A. Shafritz, editors. Raven Press, New York. 617–641.
21. Schoeller, D. A. 1989. Changes in total body water with age. *Am. J. Clin. Nutr.* **50:** 1176–1181.
22. Valdivieso, V., R. Palma, R. Wünkhaus, C. Antezana, C. Severìn, and A. Contreras. 1978. Effect of aging on biliary composition and bile acid metabolism in normal Chilean women. *Gastroenterology.* **74:** 871–874.
23. Stange, E. F., and J. M. Dietschy. 1984. Age-related decreases in tissue sterol acquisition are mediated by changes in cholesterol synthesis and not low density lipoprotein uptake in the rat. *J. Lipid Res.* **25:** 703–713.
24. Spady, D. K., and J. M. Dietschy. 1989. Interaction of aging and dietary fat in the regulation of low density lipoprotein transport in the hamster. *J. Lipid Res.* **30:** 559–569.
25. Angelin, B., K. Hershon, and J. D. Brunzell. 1987. Bile acid metabolism in hereditary forms of hypertriglyceridemia: evidence for an increased synthesis rate in monogenic familial hypertriglyceridemia. *Proc. Natl. Acad. Sci. USA.* **84:** 5434–5438.
26. Marchesini, G., V. Bua, A. Brunori, G. Bianchi, P. Pisi, A. Fabbri, M. Zoli, and E. Pisi. 1988. Galactose elimination capacity and liver volume in aging man. *Hepatology.* **8:** 1079–1083.
27. Heubi, J. E., S. Burtstein, M. A. Sperling, D. Gregg, M. T. R. Subbiah, and D. E. Matthews. 1983. The role of human growth hormone in the regulation of cholesterol and bile acid metabolism. *J. Clin. Endocrinol. Metab.* **57:** 885–891.
28. Ahlberg, J., B. Angelin, I. Björkhem, and K. Einarsson. 1977. Individual bile acids in portal venous and systemic blood serum of fasting man. *Gastroenterology.* **73:** 1377–1382.
29. Street, J. M., D. J. H. Trafford, and H. L. J. Makin. 1983. The quantitative estimation of bile acids and their conjugates in human biological fluids. *J. Lipid Res.* **24:** 491–511.
30. Nuber, R., H. Maucher, and E. F. Stange. 1990. Size exclusion chromatography for extraction of serum bile acids. *J. Lipid Res.* **31:** 1517–1522.
31. Van der Werf, S. D. J., A. W. M. Huijbregts, H. L. M. Lamers, G. P. Van Berge Henegouwen, and J. H. M. Van Tongeren. 1981. Age-dependent differences in human bile acid metabolism and 7α-dehydroxylation. *Eur. J. Clin. Invest.* **11:** 425–431.
32. Nagengast, F. M., S. D. J. Van der Werf, H. L. M. Lamers, M. P. C. Hectors, W. C. A. M. Buys, and J. H. M. Van Tongeren. 1988. Influence of age, intestinal transit time and dietary composition on fecal bile acid profiles in healthy subjects. *Dig. Dis. Sci.* **33:** 673–678.
33. Tint, G. S., G. Salen, and S. Shefer. 1986. Effect of ursodeoxycholic acid and chenodeoxycholic acid on cholesterol and bile acid metabolism. *Gastroenterology.* **91:** 1007–1018.
34. Reihnér, E., I. Björkhem, B. Angelin, S. Ewerth, and K. Einarsson. 1989. Bile acid synthesis in humans: regulation of hepatic microsomal cholesterol 7α-hydroxylase activity. *Gastroenterology.* **97:** 1498–1505.
35. Vlahcevic, Z. R., D. M. Heuman, and P. B. Hylemon. 1991. Regulation of bile acid synthesis. *Hepatology.* **13:** 590–600.