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ORIGINAL RESEARCH

Age-associated alterations in cholesterol homeostasis: evidence from a cross-sectional study in a Northern Italy population

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Marco Bertolotti[1]
Chiara Mussi[1]
Elisa Pellegrini[1]
Alessandro Magni[2]
Marina Del Puppo[2]
Silvia Ognibene[1]
Lucia Carulli[1]
Claudia Anzivino[1]
Enrica Baldelli[1]
Paola Loria[1]
Nicola Carulli[1]

[1]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; [2]Department of Health Sciences, University of Milano Bicocca, Monza, Italy

Background: The modifications of cholesterol metabolism associated with aging are ill-defined. The objective of this study was to define age-associated alterations of the different metabolic pathways controlling cholesterol homeostasis by analyzing circulating sterols.

Methods: We analyzed serum samples collected from 201 adult (75 male, 126 female) subjects within the epidemiological MICOL study (Multicentrica Italiana Colelitiasi). The age range was 38–79 years; 103 had evidence of gallstones. The concentrations of the different sterols, recognized as markers of the main pathways of cholesterol homeostasis, were analyzed by gas chromatography–mass spectrometry, including lathosterol (synthesis), campesterol and sitosterol (absorption), and 7α-hydroxy-4-cholesten-3-one (degradation to bile acids).

Results: A significant direct correlation was detected between age and cholesterol levels (r=0.34, P<0.01). The lathosterol/cholesterol ratio was lower in older age quartiles (P<0.05 by analysis of variance), with an inverse correlation between the lathosterol/cholesterol ratio and age (r=–0.32, P<0.01). Such correlation was particularly evident in females. The campesterol/cholesterol and sitosterol/cholesterol ratios were inversely correlated with aging in control, but not in gallstone patients. The levels of 7α-hydroxy-4-cholesten-3-one were not correlated with age.

Conclusion: These data show a reduction of cholesterol synthesis with aging which is associated with increased circulating cholesterol levels. The finding might be related to a reduced metabolic need for cholesterol in advancing age, leading to a downregulation of the main mechanisms of cholesterol intake in the liver. A different age-related behavior was observed in gallstone-free versus gallstone patients regarding cholesterol absorption. The possible implications in terms of the pharmacological management of hypercholesterolemia in the elderly remain to be defined.

Keywords: aging, cholesterol metabolism, cholesterol synthesis, gallstone disease, cardiovascular risk

Introduction

The influence of aging on cholesterol homeostasis in humans is poorly defined. A number of epidemiological studies suggest that serum cholesterol levels tend to increase in adult age, but subsequently decrease in the very elderly.[1] On the other hand, the impact of serum cholesterol as a cardiovascular risk factor is debated[2] and the use of cholesterol-lowering agents in old age is extremely controversial.[3–6]

Whereas the prevalence of cholesterol gallstone disease increases with advancing age,[7] the underlying mechanisms are not well understood, and the presence of metabolic alterations themselves as predisposing factors of gallstone disease is also a matter of debate.[8,9]

Correspondence: Marco Bertolotti
Division of Geriatric Medicine, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Nuovo Ospedale Civile di Modena, via Giardini 1355, Modena, Italy
Tel +39 059 3961802
Fax +39 059 3961335
Email marco.bertolotti@unimore.it

Clear-cut evidence on specific alterations of cholesterol metabolism associated with old age is presently missing, mainly due to the complexity of the studies of clinical pathophysiology and, consequently, to the limited number of observations. Data in human males, however, have shown a reduction in the turnover of low density lipoprotein (LDL)[10] and other reports seem to suggest that a reduction in the conversion of cholesterol to bile acid takes place.[11–13]

Likewise, the presence of specific metabolic alterations in gallstone disease has been postulated in the past.[14,15] Once again, the rather limited number of patients and tissue samples in individual reports makes it extremely difficult to achieve definite conclusions.

In the last few decades, the analysis of circulating levels of cholesterol precursors, plant sterols, and hydroxylated sterols as metabolic markers has allowed the investigation of the individual steps of cholesterol balance (synthesis, absorption, degradation to bile acids) in broader populations.

Here, we attempted to provide a systematic investigation on the effects of aging on the different metabolic steps of cholesterol homeostasis; this was made possible thanks to an epidemiological study conducted in Northern Italy, which allowed the collection of clinical information and serum samples in a large community population. Alterations related with gallstone disease were also investigated.

Subjects and methods

Study population

The Multicentrica Italiana Colelitiasi (MICOL) project was a population-based, cross-sectional study carried out in Italy. Complete details on the study protocol have been published elsewhere.[16] The project plan included two cross-sectional surveys; the first began in 1985 and was completed in 1988 and the second survey was carried out on the same subjects 10 years later in order to estimate the incidence of gallstone disease and its natural history. Seven of the original operative units, including the Unit of Modena, were able to complete the second survey.

The re-examination took place between 1995 and 1998.[17] Subjects with gallstones or a history of cholecystectomy for gallstones at the first survey were recorded.

Consistently with the first survey, the screening protocol included upper abdominal ultrasound, physical examination, fasting blood specimen collection, and administration of a questionnaire, which was administered by each operative unit.

In the present paper, we analyzed serum samples from 201 subjects over a rather wide age range (38–79 years); 75 of them were males, 126 females; 103 had clinical evidence of gallstones and the remaining 98 were treated as control subjects; samples were collected within the Modena cohort in the 1995–1998 re-examination phase and stored at −80°C thereafter until analyzed. Additional relevant clinical information, including serum lipid levels and presence/absence of gallstone disease, was collected from the inherent database. The levels of serum lipids, including total cholesterol, were measured by standard automated analysis on samples obtained in the morning.

The study has been conducted in conformity with the most recent version of the Declaration of Helsinki and has been approved by the Ethical Committee of the Province of Modena.

Analysis of circulating lathosterol, plant sterols, and hydroxysterols

Quantitative evaluation of lathosterol and plant sterols (campesterol and beta-sitosterol) in serum was performed by gas chromatography–mass spectrometry (GC-MS) as previously described.[18] After the addition of deuterated lathosterol (0.5 μg) and 5α-cholestane (0.5 μg) as internal standards to 0.1 mL serum samples, alkaline hydrolysis was carried out and sterols extracted with petroleum ether. The organic phase was evaporated to dryness under a stream of nitrogen and sterols converted into trimethylsilyl ethers with trimethylsilylimidazole: piperidine (1:1) before analysis by GC-MS.

Serum levels of 7α-hydroxy-4-cholesten-3-one (C4) were analyzed as previously described.[13,19] After the addition of 19-hydroxycholesterol (250 ng) as the internal standard to 0.5 mL of serum, the sample was diluted with physiological solution and C4 was extracted with chloroform/methanol (2:1, v:v). Purification by solid phase extraction with a silica cartridge column (Supelclean LC-Si; Supelco Inc., St Louis, MO, USA) was performed; the oxysterol fraction was evaporated and sterol converted into trimethylsilyl ethers by the addition of 50 μL of trimethylsilylimidazole: piperidine (1:1) at room temperature for 20 minutes. After evaporation, the derivatives were dissolved in hexane. Aliquots (1–2 μL) of the silylated mixtures were injected for GC-MS analysis.

GC-MS analysis was carried out using a Thermo Finnigan GC-Q instrument (Waltham, MA, USA), as previously described.[18,19]

In previous reports, the lathosterol/cholesterol ratio has shown a strict correlation with whole body cholesterol synthesis, measured with the direct balance method, under different experimental conditions,[20] and therefore, is widely accepted as an index of cholesterol synthesis. Likewise, the sitosterol/cholesterol and campesterol/cholesterol ratios were

considered as markers of intestinal cholesterol absorption, according to the literature.[21]

The absolute serum concentration of C4 was used as a marker of bile acid synthesis and of the activity of cholesterol 7α-hydroxylase, the limiting enzyme of the metabolic pathway.[19,22]

Statistical analysis

Whenever appropriate, continuous variables were expressed as the mean value and SD.

The patients were stratified into age quartiles; the significance of the differences of continuous variables between age quartiles was performed by one-way analysis of variance (ANOVA). The difference between individual groups was further evaluated by means of Bonferroni post hoc analysis.

Linear correlation analysis was performed using the least squares method.

The significance of the differences between gallstone-free and gallstone patients was evaluated by Student's *t*-test for independent data.

Significance was accepted at the $P<0.05$ level.

Statistical analysis was performed by means of SPSS software (v17 for Windows; SPSS Inc., Chicago, IL, USA) on a PC-IBM compatible platform.

Results

A direct correlation was detected between age and serum levels of total cholesterol, as shown in Figure 1 ($r=0.34$, $P<0.01$). A significant between-group difference was also detected by one-way ANOVA (Table 1). When the significance was further tested by Bonferroni's test, a difference was detected between the youngest quartile and all other age groups, but not between the oldest ones; this, together with the visual inspection of the data presented in Figure 1, suggests a trend for serum cholesterol to plateau when approaching the oldest age groups.

The correlation between serum cholesterol and age was observed in both gallstone and gallstone-free subjects (respectively, $r=0.28$ and $r=0.46$, $P<0.01$ for both; data not shown).

Such correlation was particularly evident in female subjects (n=126; $r=0.43$, $P<0.01$), whereas in males, a trend was present, but did not reach statistical significance (n=75; $r=0.19$, $P<0.1$; data not shown).

Figure 2 illustrates the relationship between the lathosterol/cholesterol ratio, an index of cholesterol synthesis, and age. In the whole population (upper panel) a significant inverse correlation is present ($r=-0.32$, $P<0.01$). One-way ANOVA confirmed the presence of a significant difference among different age groups and Bonferroni's test once again showed a statistical significance only between the younger age group and the remaining ones (Table 1).

As shown in the figure (middle and lower panel), the inverse correlation was detected regardless of the presence or absence of gallstone disease.

As observed with serum cholesterol, such correlation was highly significant in females ($r=-0.47$, $P<0.01$), whereas in male subjects, while the negative trend was present, it

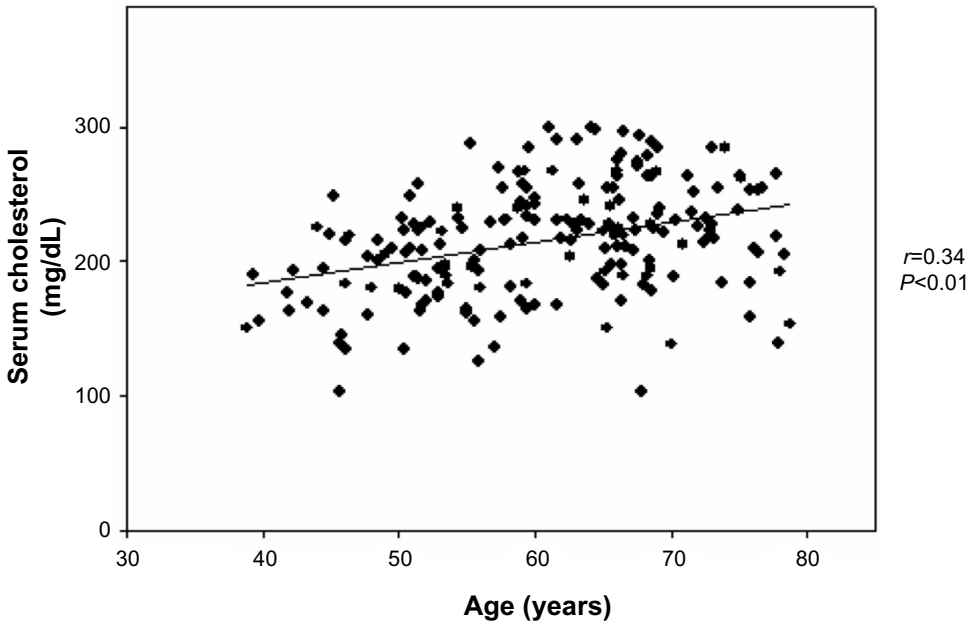

Figure 1 Correlation between serum cholesterol levels and age.
Notes: Individual data points are shown for 201 subjects; $r=0.34$; $P<0.01$, linear regression analysis.

Table 1 Serum levels of total cholesterol and other sterols in the different age quartiles[a]

Age range	Cholesterol* (mg/dL)	Latho/Ch* (μg/100 mg)	C4 (μg/dL)	Campe/Ch (μg/100 mg)	Sito/Ch (μg/100 mg)
38–53	191±32**	122±54**	1.16±1.48	192±119	391±235
53–62	217±42	87±34	1.01±1.08	171±94	378±213
62–68	229±41	86±48	1.19±1.49	142±72	306±271
68–79	226±38	78±40	0.99±0.68	164±133	373±302

Notes: [a]Data are presented as mean value and SD; *$P<0.01$ among age quartiles, one-way analysis of variance; **$P<0.05$ versus all remaining groups, Bonferroni's test.
Abbreviations: Latho/Ch, lathosterol to cholesterol ratio; C4, 7α-hydroxy-4-cholesten-3-one; Campe/Ch, serum campesterol to cholesterol ratio; Sito/Ch, serum sitosterol to cholesterol ratio.

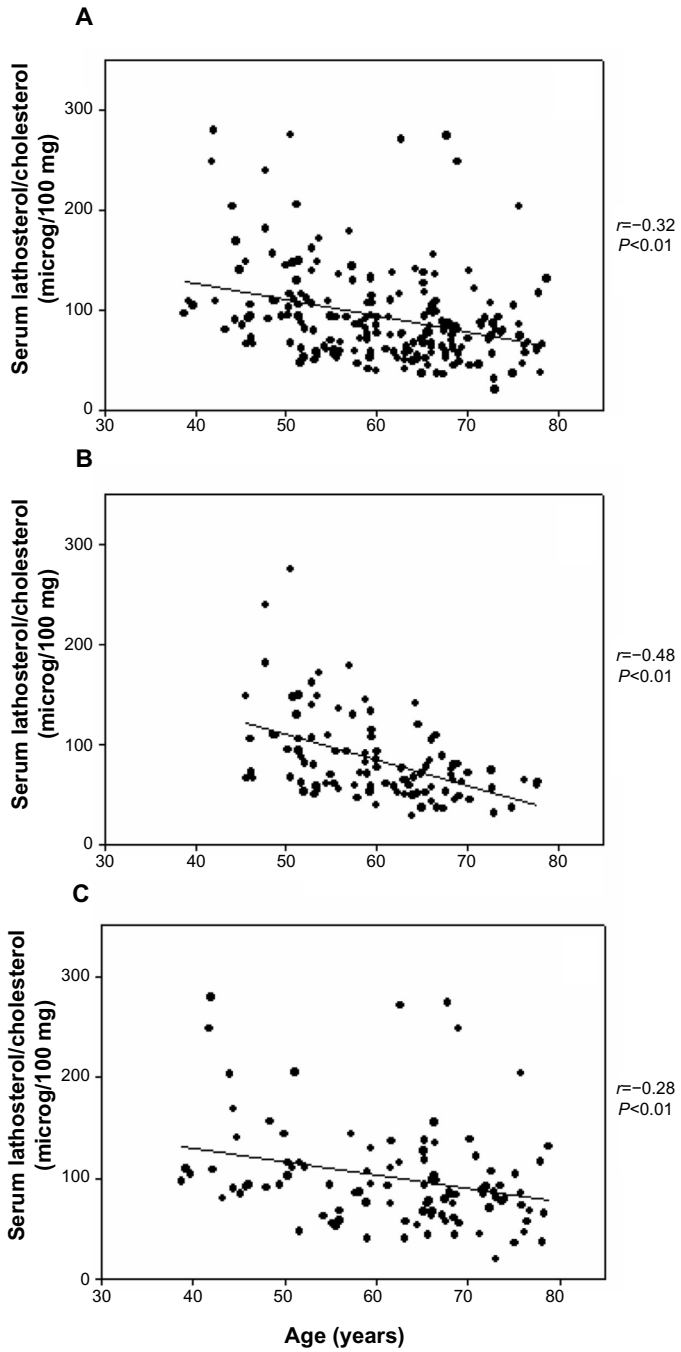

Figure 2 Correlation between serum lathosterol to cholesterol ratio, a marker of cholesterol synthesis, and age. (**A**) Individual data points are shown for all subjects (n=201) of the studied cohort; $r=-0.32$; $P<0.01$, linear regression analysis; (**B**) data points are shown for gallstone-free subjects (n=98); $r=-0.48$; $P<0.01$, linear regression analysis; (**C**) data points are shown for gallstone patients (n=103); $r=-0.28$; $P<0.01$, linear regression analysis.

did not reach statistical significance (r=–0.20, P<0.1; data not shown).

According to previous evidence, a decrease in the markers of bile acid synthesis may have been expected with aging.[11–13] Instead, no correlation was detected between age and serum C4, as shown in Table 1 and Figure 3.

A peculiar behavior was observed with regards to the markers of intestinal cholesterol absorption as related to age. A trend toward an inverse correlation was detected in the whole patient group, which did not reach statistical significance (respectively, r=–0.13 for the campesterol/cholesterol ratio and r=–0.09 for the sitosterol/cholesterol ratio, data not shown). When the population was subdivided into the two groups according to the presence or absence of gallstones, distinct patterns were observed. As shown in Figure 4, a significant inverse correlation between age and the campesterol/cholesterol ratio was present in gallstone-free subjects whereas no correlation was observed in gallstone patients. A similar trend was observed for the sitosterol/cholesterol ratio (r=–0.23 in controls, P<0.05; r=–0.009 in gallstone patients, P<0.1) (data not illustrated).

Finally, the levels of the different metabolic biomarkers were compared in gallstone and gallstone-free subjects. The serum lathosterol/cholesterol ratio was significantly higher (P<0.05) in gallstone subjects compared to controls (respectively, 101±51 versus [vs] 85±42 µg/100 mg). The levels of serum C4 tended to be higher in gallstone subjects, even if the difference did not reach statistical significance (1.24±1.30 vs 0.93±1.12 µg/dL, P=0.07) mainly because of the high degree of dispersion of the experimental data. No difference was detected in the markers of cholesterol absorption: campesterol/cholesterol ratio: 169±118 vs 165±96 µg/100 mg; sitosterol/cholesterol ratio: 361±256 vs 363±213 µg/100 mg (P>0.1, data not illustrated).

Discussion

The aim of the present paper was to clarify the alterations of cholesterol metabolism associated with aging, utilizing a large cross-sectional population of non-hospitalized subjects, most of whom can be considered healthy except for the presence of gallstone disease.

First of all, a significant correlation was detected between age and serum cholesterol, even if the trend tended to plateau in the higher age quartiles. The finding is in agreement with previous evidence from Ericsson et al,[10] where the LDL catabolic rate was found to be significantly decreased, consistently with reduced LDL turnover. In our population, aging was also inversely correlated with the lathosterol/cholesterol ratio, suggesting a reduction of whole body (and presumably hepatic) cholesterol synthesis. As far as we know, this is the first report documenting a reduction of cholesterol synthesis with ongoing age; a recent study carried out in a Northern Europe population also showed an increase in serum cholesterol and failed to show any changes in the markers of cholesterol synthesis.[23] Additional evidence from another Northern Europe cohort of very old age (up to 85 years) showed a tendency for serum cholesterol levels to

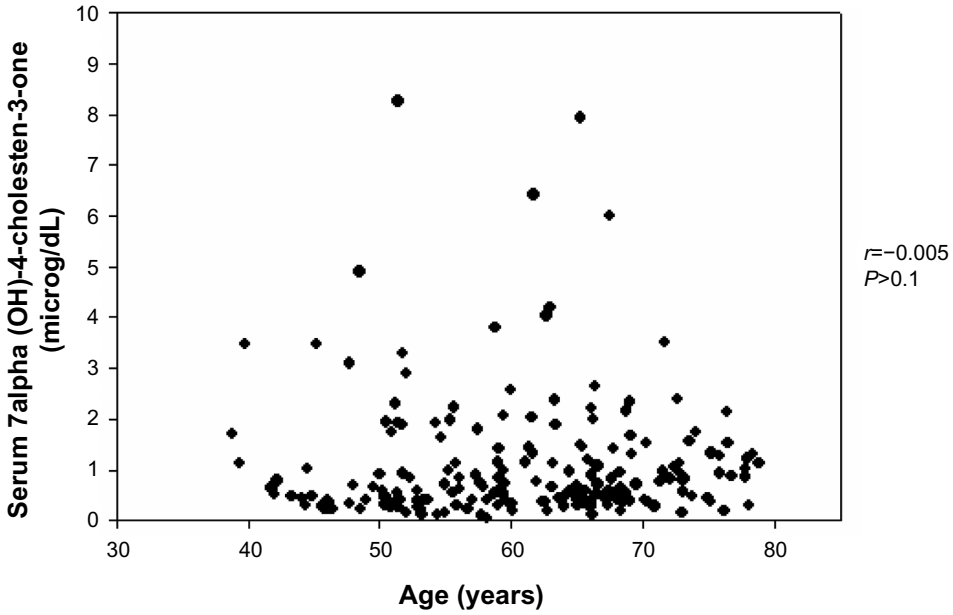

Figure 3 Correlation between serum levels of 7α-hydroxy-4-cholesten-3-one, a marker of cholesterol degradation to bile acids, and age.
Note: Individual data points are shown for 201 subjects; r=–0.005; P>0.1.

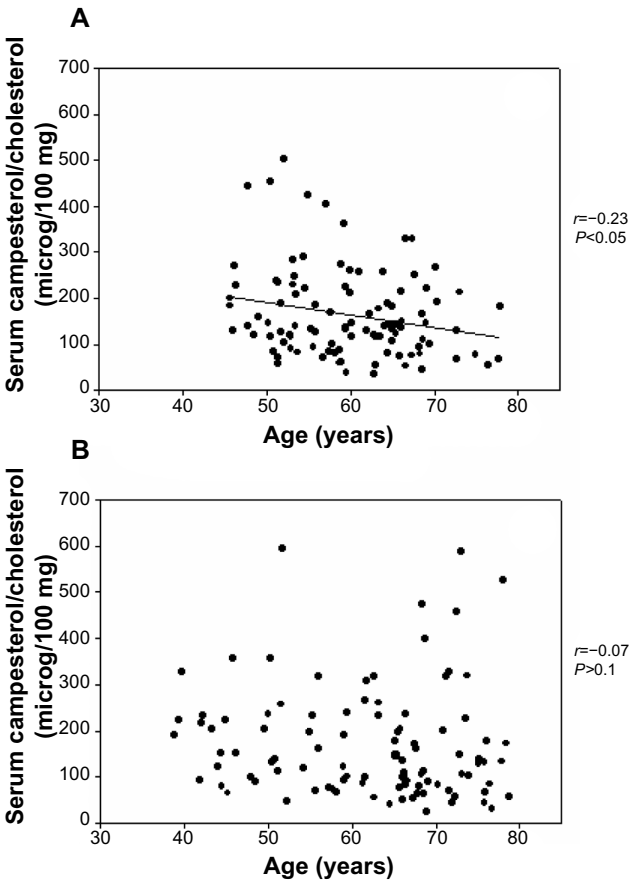

Figure 4 Correlation between serum campesterol-to-cholesterol ratio, a marker of cholesterol absorption, and age. (**A**) Individual data points are shown for gallstone-free subjects (n=98); r=–0.23; $P<0.05$, linear regression analysis; (**B**) data points are shown for gallstone patients (n=103); r=–0.07; $P>0.1$.

plateau, and even to decrease in women, with no significant changes in lathosterol levels.[24]

It is generally recognized that cholesterol synthesis and LDL uptake in most experimental conditions are coordinately regulated and undergo strict homeostatic control, where the liver plays a central role;[25] such regulation involves molecular sensors of cellular sterol content, the sterol regulatory element binding proteins (SREBPs) and SREBP-cleavage activating protein (SCAP).[26] The data of the present paper cannot elucidate the pathways undergoing the observed changes in cholesterol homeostasis. Nonetheless, our findings, taken together with evidence from the literature, are consistent with the view of a reduction in the metabolic need of cholesterol with aging; this might explain on one hand the reduction of LDL uptake (and consequently, the increase of serum cholesterol levels), and on the other, the reduced expression and/or activity of the key enzymes of cholesterol synthesis. This is in agreement with the reduction of hydroxymethylglutaryl-CoA reductase expression observed in aged rats.[27] The involvement of nuclear receptor-mediated signaling pathways in this regard is plausible, as described in an experimental mouse model.[28]

When segregating our data by sex, the correlation with serum cholesterol and with the lathosterol/cholesterol ratio was highly significant in females; in males, a distinct trend was observed with either parameter but did not reach significance. The finding might be related to the smaller number of observations; however, it cannot be excluded that sex-specific alterations might take place, as suggested by the different age-related profiles of plasma cholesterol.[12,16]

According to previous reports in the literature, both from our group and from other authors,[11–13] a negative correlation between age and bile acid synthesis might have been expected. However, no such trend was observed. A similar finding was noted by Gälman et al.[23] We have no clear explanation for this discrepancy; it has to be underlined that the subjects in the present study were randomly selected from the general population, whereas the patients studied in our previous reports[12,13] were hospitalized. In addition, as pointed out,[23] the population of the older studies was much smaller.

The profile of the serum markers of cholesterol absorption was peculiar: a significant inverse correlation with age was observed in gallstone-free subjects but not in gallstone patients, making the correlation non-significant in the whole cohort. This finding is in partial agreement with the already mentioned reports, where a decrease in markers of cholesterol absorption was described with aging[23,24] and fits with the view of a reduction in cholesterol requirement.

The role of intestinal cholesterol absorption in the pathogenesis of gallstone disease is debated (reviewed by Portincasa et al).[29] The present data are consistent with a trend toward a decrease in cholesterol absorption with aging, which does not take place in gallstone patients. Due to the design of the study, such data, however, cannot allow extensive speculations in terms of pathogenesis. It has to be further considered that the markers of cholesterol absorption were similar in the two groups.

Our findings also show a slight but significant increase in the markers of cholesterol synthesis and an increase, though not reaching statistical significance, of bile acid production in gallstone patients.

The role of perturbations of cholesterol homeostasis as predisposing factors for gallstone formation is debated. Together with the regulatory role of the physical–chemical properties of the recirculating bile acid pool,[30] it has been postulated that opposing changes of cholesterol production and degradation might increase biliary cholesterol saturation and predispose to stone formation,[14] even if this was not

confirmed by enzyme activity analysis in the liver tissue.[15] Further, more recent evidence has demonstrated an increase, rather than a decrement, in metabolic markers of bile acid synthesis.[31,32] This has been interpreted as a manifestation of subclinical bile acid malabsorption, which in peculiar subsets might favor the supersaturation of bile. The present data are largely consistent with the literature; the proportional changes in serum lathosterol and C4 are similar to those described by Muhrbeck et al,[31] supporting the possibility that in epidemiological-size cohorts, gallstone disease may associate with metabolic perturbations with a likely pathogenetic significance. The possible role for such alterations in individual subjects, however, can hardly be defined.

The potential implications of the alterations observed with aging deserve consideration.

The management of hypercholesterolemia in elderly subjects is controversial; whereas the benefits of statin treatment are evident also in older age,[4,33] direct evidence in the very elderly is scarce, and mostly, treatment with lipid-lowering agents may associate with important adverse events due to the different pharmacokinetic and pharmacodynamic responses of the individual patient.[34] Furthermore, in elderly people, the correlation between circulating cholesterol and cardiovascular disease is weaker,[2] emphasizing the possible role of other biological components, including inflammation, as risk determinants in this population.

Epidemiological evidence has even suggested that low plasma cholesterol levels, as well as low levels of cholesterol synthesis and absorption markers, may bear a negative prognostic significance.[24] In particular, serum non-high-density lipoprotein cholesterol was found to bear a protective effect in elderly subjects with good functional status.[35] More recent evidence showed an inverse correlation between serum cholesterol and non-cardiovascular mortality.[36] Indeed, patterns of low cholesterol levels may associate with a comorbidity and frailty profile.[37] On the other hand, it appears clear that spontaneous and drug-induced low cholesterol levels (so-called passive and active cholesterol lowering) may associate with different clinical pictures and prognosis.[24]

Furthermore, the relationship between plasma cholesterol and cognitive function is complex and as yet incompletely understood.[38]

This considered, the treatment decision is obviously articulated and accurate consideration of the risk–benefit ratio, together with the multi-dimensional evaluation of comorbidity and frailty status needs to be carried out.[39] Being aware of a possible decrease in cholesterol synthesis and/or absorption in old age should not necessarily prevent health care providers from treating with statins or ezetimibe, respectively, if the balance between the benefit–risk profile and the functional status warrants this so. Useful information might derive from the identification of older patients as "hyperabsorbers" or "hypersynthezisers", which would potentially orient toward more specific treatment approaches.[40] In the absence of such evaluation as routine analysis, careful clinical judgment will orient the management choice.

Author contributions

MB designed the study, performed the statistical analysis and drafted the manuscript. CM helped with the analysis and the writing. EP, SO, and LC performed sample collection and helped in the writing. AM, MDP, CA, and EB helped in sample collection and performed the experimental analysis. PL and NC collaborated in the design of the study, supervised analysis, and manuscript drafting. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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