Age-Related Variations in Hepatic Biosynthesis of Phosphatidylcholine: A Study of Choline Metabolism with Perfused Rat Liver (44154)

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Abstract. **This study investigated the age-related increase in phospholipid secretion previously described in perfused rat livers. The hypothesis of this study was that the increased secretion is mainly due to an enhanced hepatic biosynthesis of phosphatidylcholine (PC). Specifically, we evaluated the contribution to this increase of the major hepatic pathway of phosphatidylcholine formation (i.e., the conversion of choline into phosphatidylcholine *via* cytidine diphosphate [CDP]-choline).**

The measurements of [^{3}H]choline incorporation into phosphatidylcholine and its precursors in liver and bile throughout the 2-hr duration of the experiments showed significant differences in the amount of newly synthesized labeled PC secreted in the bile produced by adult and young rat livers. However, the present findings do not support the idea that the age-related increase in phosphatidylcholine hepatic secretion was due only to a strong increase in phosphatidylcholine synthesis by *via* CDP-choline.

Conversely, they suggest that future research should be directed towards the mechanisms regulating the diacylglycerol metabolism in the hepatocytes, as the alteration of the splitting ratio of hepatic diacylglycerol flow could lead to an age-related increase in conversion of diacylglycerol into phosphatidylcholine, rather than into triacylglycerol. This, in turn, may decrease the availability of triacylglycerol for hepatic very low density lipoprotein (VLDL) assembly and contribute to altered VLDL synthesis, as previously observed in the aging process. [P.S.E.B.M. 1997 Vol 216]

Typical age-related alterations in lipid metabolism in male Wistar rats include variations in the distribution of plasma lipoproteins (1, 2), and enhanced secretion of phospholipids into bile (3, 4).

In a previous study, we reported that perfusion of livers taken from rats of different ages showed age-related differences in lipoprotein lipid output. These were mostly attributable to a higher proportion of phospholipids and a lower proportion of triacylglycerols in the secreted lipoproteins (4). Moreover, we found an age-related increase in biliary phospholipid secretion, both in concentration and total output, notwithstanding the decreased bile flow in livers from older donors (3–5). This increased hepatic secretion of phosphatidylcholine (PC), the major phospholipid in biliary and lipoprotein output from the liver, did not apparently deplete liver content of PC or other phospholipid classes (3).

The age-related increase in both the secretion and concentration of biliary phospholipids has not been explained definitively. It has been proposed that it may derive from a compromised microtubule network in older animals, which induces a misdirected secretion of phospholipids from the sinusoidal to the canalicular pole of the hepatocyte, but this contrasts with the finding that hepatic secretion of phospholipid shows an age-related increase both in lipoprotein (sinusoidal) and bile (canalicular) compartments (4). It was also suggested that the increase in phospholipid secretion with aging may derive from changes in bile acid composi-

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This investigation was partially supported by the Progetto Finalizzato Invecchiamento of the National Research Council of Italy (Grant 95.00000.PF40. Inv 95.3.000).

Received September 25, 1996. [P.S.E.B.M. 1997, Vol 216]
Accepted May 2, 1997.

0037-9727/97/2161-0044$10.50/0

tion. However, Ferland *et al.* (6) reported that the increased secretion of hydrophobic bile acids in old rats was too small to account for the variations in phospholipid output. Therefore, alterations in the secretion of biliary phospholipids appeared to be linked to the aging process (5), by a mechanism that is not well understood.

This study aimed to investigate the possibility that the enhanced secretion of hepatic PC depends on an age-related increase in the rate of PC synthesis. The rate of synthesis was evaluated by the incorporation of [^{3}H]choline added to the medium of perfused livers into lipids secreted into bile and remaining in the livers. The source of biliary PC is a preformed hepatic pool. *De novo* synthesis contributes only 3%–5% of PC secretion (7), and is smaller in a perfused liver (8).

The difficulty of an exact evaluation of PC synthesis lies in the fact that there are five separate pathways of PC synthesis differently expressed in the various tissues and organs (9). In rat liver, the main synthetic route is the phosphorylation of the choline precursor followed by its conversion into cytidine diphosphate (CDP)-choline and transformation into PC by reaction with diacylglycerol (10, 11). Perfusion with labeled choline, as performed in this study, allowed us to trace this major pathway of the liver, but not the less-relevant stepwise methylation of phosphatidylethanolamine (10, 12).

Biosynthesis of PC, *via* CDP-choline, depends on the activity of the rate-limiting enzyme CTP-phosphocholine cytidyltransferase (13). Diacylglycerol, (common precursor of both phospholipid and triacylglycerol), and fatty acids are recognized to be modulating factors of these syntheses (13, 14). In particular, a low fatty acid availability and a low rate of diacylglycerol synthesis promote conversion into phospholipids destined for bile secretion and membrane turnover (15). This metabolic status prevails under conditions of stress, starvation, and diabetes (16).

Materials and Methods

Choline chloride [methyl-^{3}H] and L-3-phosphatidylcholine, 1-palmitoyl-2-[1-^{14}C] linoleyl-phosphatidylcholine were purchased from Amersham, Life Science (Milan, Italy). All chemicals used were of analytical grade and were obtained from Farmitalia Carlo Erba (Milan, Italy). The enzymatic kits for the determination of choline, triacylglycerol, and cholesterol (free and total) levels were purchased from Boehringer Mannheim Italia (Milano, Italy). Adult and young male Wistar rats (aged 15.2 ± 1.2 and 4.5 ± 0.6 months; weight 747 ± 55 and 448 ± 51 g, respectively) purchased by Charles River Italia (Calco-Como, Italy) were housed at 25°C under day length (12 hr) and allowed free access to food (standard pellet diet) and water for at least 2 weeks before the experiments.

Liver Perfusion. Rats were anaesthetized with thiopental sodium salt (Farmotal, Grison Pharma, Rome, Italy). The surgical procedure and liver perfusions were performed as previously described (17, 18). After a 5-min washout with Krebs-Ringer bicarbonate buffer (pH 7.4), livers (weight 16.9 ± 2.9 and 11.9 ± 0.7 g in adult and young rats, respectively) were perfused for 2 hr with a volume of median five times the liver weight, using a recirculating system. The medium contained one-third freshly prepared bovine erythrocytes and two-thirds Krebs-Ringer bicarbonate buffer (pH 7.4) together with 4% bovine serum albumin (BSA) and 0.1% glucose, and was recirculated at a flow rate of 1 ml/min/g liver. The perfusion apparatus (Disa, Milan, Italy) was kept at 37°C throughout the experiment. The viability of each liver in the course of perfusion experiments was demonstrated by standard parameters (i.e., aspartate amino-transferase level, O_2 utilization, pH values, and hemolysis degree) (17, 18). These did not show significant differences between adult and young livers.

Experimental Protocol. After a 30-min collection of basal bile, 20 pmol of [^{3}H] choline chloride were added to the perfusion medium of the livers. Then, four samples of bile were collected at 30-min intervals for 120 min (identified as Bile 1, Bile 2, Bile 3, and Bile 4, respectively), when perfusion was stopped. Small samples of medium were taken out at 5, 10, 15, 20, 30, 60, 90, and 120 min after labeled choline infusion, for determination of radioactivity. Liver, medium and bile samples were extracted as described below in Analytical Procedures. Extracts were kept at −20°C until analyzed. At the end of perfusion experiment, 30 ml of medium, free from erythrocytes, were ultracentrifuged at 110,000 *g* at 5°C in a 60-Ti rotor, with a Beckman ultracentrifuge for isolation of β-lipoproteins (20-hr run, density adjusted to 1.050 g/ml) and α-lipoproteins (24-hr run, density adjusted to 1.22 g/ml).

Analytical Procedures. Choline present in liver, bile, and medium samples was separated from other metabolites (mainly betaine, phosphorylcholine, and CDP-choline) or PC by an extraction procedure reported by Sundler *et al.* (10). This method applied to liver extracts gives three phases: aqueous phase (AqP), containing choline and its water soluble metabolites; ethanol-water phase (EthP), containing PC precursors; and the lipid phase (LipP), containing phospholipids (i.e., PC and sphingomyelin). In practice, choline and betaine are found mostly in the AqP, while the PC precursors (PC-P), phosphorylcholine and CDP-choline, are almost exclusively recovered in the EthP.

The extraction method described by Sundler (10) for liver was validated in bile and medium by adding to them known amounts of radiolabeled choline and PC. Radioactivity associated with EthP in extracts of these different samples was always negligible. Extraction of these rat bile samples, spiked with a known amount of [^{14}C]PC showed that 0.3%, 3.2%, and 94% (total recovery [^{14}C]PC 97.5%) of added radioactivity was recovered in AqP, EthP, and LipP phases, respectively. Addition of [^{3}H]choline to rat bile indicated recoveries of 82%, 11%, and 2% (total recovery [^{3}H]choline 95%) in AqP, EthP, and LipP phases, respectively. Similar recoveries were found with medium samples.

An aliquot of 10 ml of the perfusion medium, collected at the end of each perfusion experiment, was fractionated according the method above described. Small aliquots, taken at different times throughout the perfusion, were used for radioactivity counting.

The amount of choline enzymatically determined in the AqP, EthP, and LipP obtained from the livers, bile, and medium extractions was converted into PC precursors and PC concentration, by the appropriate stoicheiometric factors (19). Untreated or concentrated aliquots of aqueous phase were used for determination of choline. The samples of EthP and LipP for enzymatic determination of PC (20) were prepared as follows: an aliquot of extract was taken to dryness under N_2. The residues were resuspended with a volume of sodium taurocholate 2.8 m*M* dissolved in 0.15 *M* NaCl and a volume of isopropanol. Solutions were analyzed following the directions given by manufacturer of the kit. It has been estimated that the concentration of phosphorylcholine in liver is approximately 40 times higher than that of CDP-choline (11, 19). Thus, the specific activity of the EthP of hepatic extracts was calculated using an average molecular weight derived from phosphorylcholine and CDP-choline in the ratio 40:1.

The incorporation of [^{3}H]choline into phospholipids such as PC and sphingomyelin was evaluated from the radioactivity associated with these phospholipids in liver lipid extracts after separation by TLC. More than 98% of radioactivity was found to be associated with PC. Radioactivity associated with sphingomyelin was barely detectable.

Results

Effects of Aging on the Recovery and Distribution of Radioactivity in Liver Perfusion Medium after [^{3}H]Choline Infusion. Radiolabeled choline was taken up by livers at rates that depended on the age of donor rats. Radioactivity recovered in the perfusion medium of livers from adult and young animals at 5 min from infusion of [^{3}H]choline was 54% ± 18% and 43% ± 27% of administered dose, respectively.

Time courses of the disappearance of radioactivity from the medium (expressed as dpm/dose/g liver) are reported in Figure 1. The young donor group showed a much faster uptake of radiolabeled choline in the first 10 min. After this time, livers of adult donors showed significantly lower values of radioactivity in the perfusion medium from 20 to 120 min. Fractionation of the perfusion medium at the end of the experiments showed that EthP had negligible values of radioactivity. Therefore, the contribution from this phase was

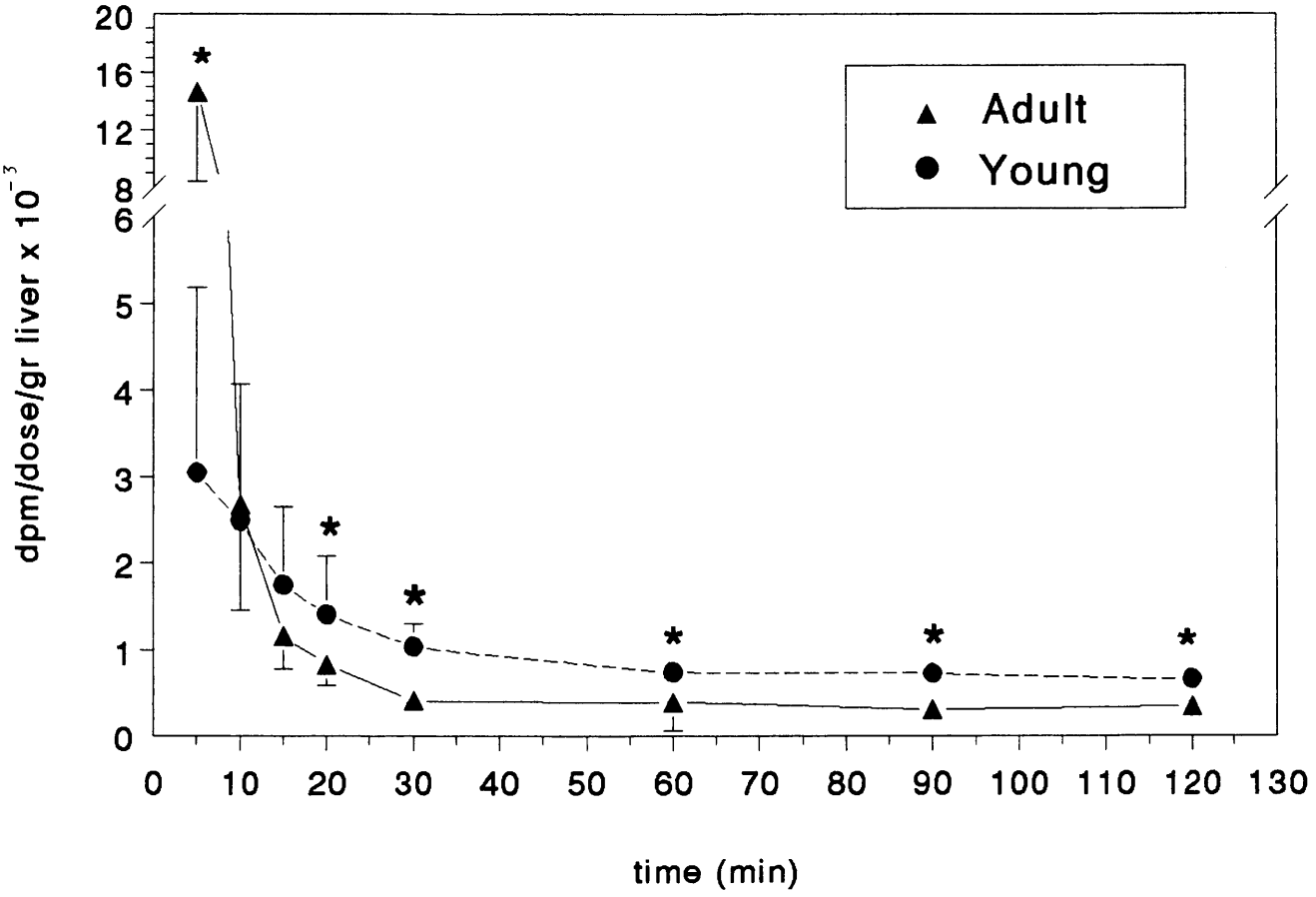

Figure 1. Clearance of radioactivity from medium. Radioactivity in the medium of rat perfused livers from adults (▲) and young (●) at various times after injection of [^{3}H]choline. Each point is the mean ± SD from six and seven experiments in adult and young group, respectively. Significance limit, adult versus young rats: *$P < 0.05$.

omitted from calculations. Distribution of radioactivity (with respect to the total dose injected) in the AqP and LipP extracts did not show any significant difference between the two groups. However, the perfusion medium of livers from adult donors usually showed a higher incorporation of labeled choline in LipP. Most of total radioactivity was found in AqP (about 94% and 96% in adult and young groups, respectively), while 6.0% ± 5.2% and 3.9% ± 2.6% was found in LipP of adult and young rats, respectively.

Labeled PC secreted by perfused livers into the perfusion medium was present both in VLDL (β-lipoproteins) and HDL (α-lipoproteins), but its proportion differed in the two groups (Fig. 2). In particular, α-lipoproteins secreted by adult livers had a significantly higher content of labeled PC than young livers (45.8 ± 16.4 and 23.0 ± 8.6 dpm/dose/g liver · 10^{-6} in adult and young group, respectively; $n = 7$; $p < 0.05$).

Effects of Aging on PC Synthesis in the Perfused Liver after [^{3}H]Choline Infusion. The hepatic concentration of choline (AqP) was 0.53 ± 0.15 and 0.68 ± 0.12 μmol/g liver in the adult and young groups, respectively. The radioactivity recovery in AqP of hepatic extracts at the end of experiments was 3.00 ± 0.89 and 1.76 ± 0.17 dpm/dose/g liver · 10^{-3}, with a significant difference ($p < 0.005$) between the groups.

At the same time (i.e., 2 hr after [^{3}H]choline infusion), the radioactivity and concentration of PC-P and PC were evaluated in EthP and LipP of liver extracts (Table I). A lower proportion of radioactivity was recovered in EthP and LipP extracts of adult livers. This indicated a higher incorporation of [^{3}H]choline into PC-P and PC in livers of younger donors compared with adult group. At the end of the experiments, the amount of [^{3}H]PC-P newly synthesized per unit of liver weight was, in fact, about halved in adult livers with respect to young livers (0.110 ± 0.054 pmol/g liver/2 hr, $n = 6$, and 0.212 ± 0.057 pmol/g liver/2 hr, $n = 7$; $P < 0.01$, adult and young livers, respectively).

Values for the total liver concentration of PC-P did not show any age-related difference (1480 ± 420 and 1530 ± 170 pmol/g liver in adult and young livers, respectively, with a ratio adult/young of 1.03). Concentrations of [^{3}H]PC-P derived from new synthesis showed differences, but these were not significant (2.46 ± 0.98, $n = 6$, in adult and 3.26 ± 0.75, $n = 7$, pmol/liver in young, with a ratio between adult and young of 0.75).

Total newly hepatic synthesized [^{3}H]PC, measured at the end of the perfusion from radioactivity counts in LipP, was 10.37 ± 3.59 and 7.19 ± 2.86 pmol/liver in livers from adult and young donors, respectively, with a ratio adult/young of 1.44, while this ratio for synthesized [^{3}H]PC expressed per unit of liver weight was 0.69, being 0.468 ± 0.215 and 0.674 ± 0.443 pmol/g liver/2 hr in adult and young, respectively (Table I). Despite the larger liver mass, total hepatic concentration of PC showed an age-related

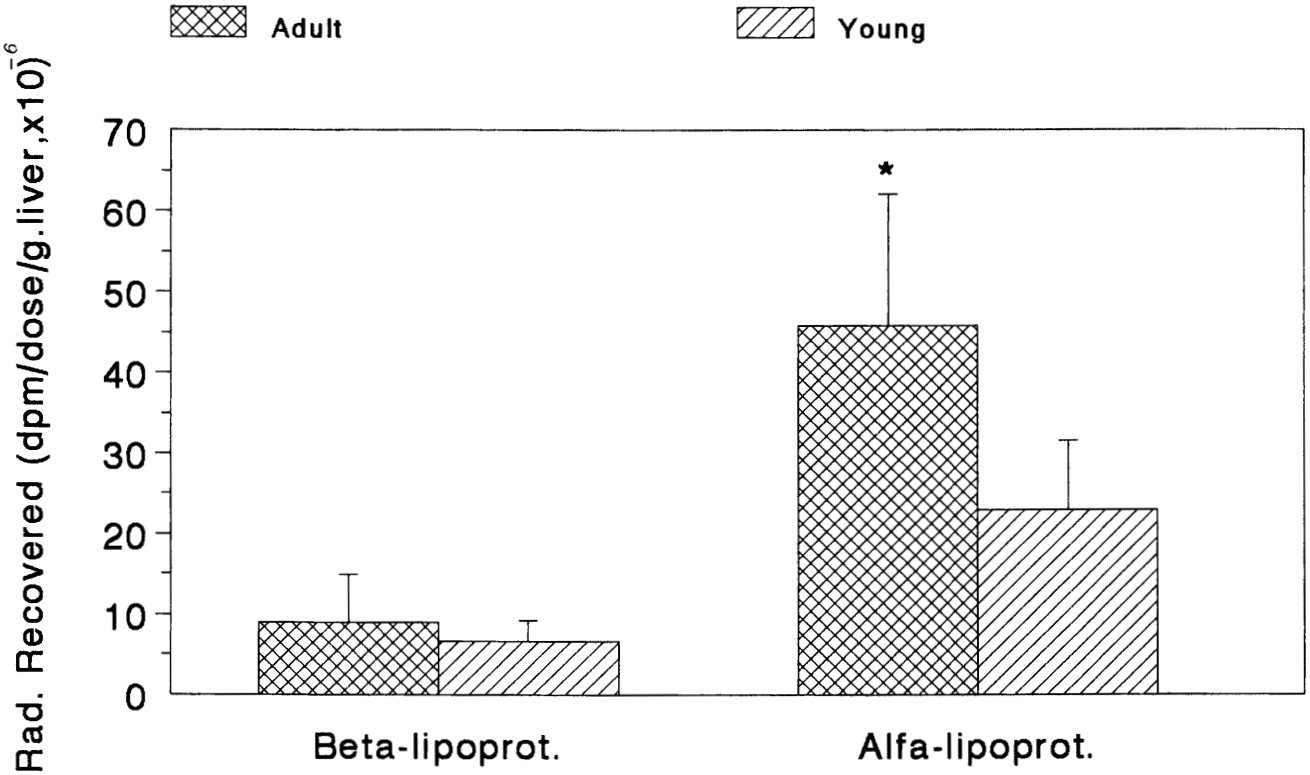

Figure 2. Newly synthesized phosphatidylcholine (PC) secretion in lipoprotein. Recovery of newly synthesized [^{3}H]PC in β = (density < 1.050 g/ml) and α = (density 1.050–1.220 g/ml) lipoprotein fractions from medium of perfused livers from adult and young rat donors. Significance limit, adult versus young rats: *$P < 0.05$.

Table I. Total and Newly Synthesized Concentrations of Phosphatidylcholine Precursors (Phosphorylcholine and CDP-Choline: PC-P) and Phosphatidylcholine (PC) in Livers from Adult and Young Rats 2 hr after [^{3}H]Choline Infusion

	Phosphatidylcholine precursors *(Ethanol phase of liver extracts)*				
	Total (pmol/g liver)	Radioact. recovered (dpm/dose/g liver × 10^{-3})	S.A.[a] (dpm/pmol × 10^{-6})	Synthesis of [^{3}H]PC-P (pmol/g liver/2 hr)	(pmol/liver)
Adult	1,480 ± 420	3.11 ± 1.70[b]	0.037 ± 0.019[b]	0.110 ± 0.054[c]	2.46 ± 0.98
Young	1,530 ± 170	5.91 ± 1.99	0.061 ± 0.015	0.212 ± 0.057	3.26 ± 0.75
	Phosphatidylcholine *(Lipid phase of liver extracts)*				
	Total (pmol/g liver)	Radioact. recovered (dpm/dose/g liver × 10^{-3})	S.A.[a] (dpm/pmol × 10^{-6})	Synthesis of [^{3}H]PC-P (pmol/g liver/2 hr)	(pmol/liver)
Adult	13,690 ± 6,610	3.81 ± 1.69	0.049 ± 0.018	0.468 ± 0.215	10.37 ± 3.59
Young	22,280 ± 12,000	6.08 ± 3.99	0.044 ± 0.016	0.674 ± 0.443	7.19 ± 2.86

Note. Radioactivity recovery and specific activity of [^{3}H]PC-P and [^{3}H]PC are reported. Values are mean ± SD of six and seven perfusion experiments for adult and young group, respectively.
[a] S.A. = specific activity.
[b] Adult versus young group: $P < 0.05$.
[c] Adult versus young group: $P < 0.01$.

decrease in older rats in comparison with younger animals (13690 ± 6610 and 22280 ± 12000 pmol/g liver with an adult/young ratio of 0.61).

As a consequence of these changes, the specific radioactivity of PC-P, in the adult group was about one-half the value in young group, with significant differences between the two groups, while the specific activity of PC was very similar in the two groups (Table I).

Effects of Aging on the Recovery and Distribution of Radioactivity in Biliary Phospholipid after Infusion of [^{3}H]Choline. Values of bile flow, PC secretion, and PC concentration in basal and bile samples collected at 30-min intervals following [^{3}H]choline addition to the medium are reported in Table II. Bile flow was significantly lower in the adult than in young group, but the PC concentration in bile was about five times higher in the adult rats. However, notwithstanding the slower rate to bile secretion, the total secretion of PC was significantly increased in the adult animals (Table II).

The amounts of total radioactivity were significantly lower in bile from adult as compared with young livers (Table III). Furthermore, the distribution of radioactive compounds in bile extracts showed that only AqP and LipP contained measurable amounts of labeled components. Radioactivity associated with EthP (i.e., PC-P) was barely detectable in all bile samples. Consequently, it was omitted from the calculations of the radioactivity distribution in bile extracts (Table III). Most of radioactivity was recovered in the AqP with only a small proportion of radioactivity recovered in LipP in both groups. Approximately 2%–4% and less than 1% of total dose was associated with biliary lipids in the adult and young groups, respectively. Values for radioactivity found in LipP, and expressed as dpm/dose/g liver, were about twice as high in bile from adult livers as in that collected from young livers. Individual values found in

Table II. Bile Flow and Biliary Secretion and Concentration of Phosphatidylcholine, Collected during Perfusion Experiments with Adult (15.2 ± 1.2-month-old) and Young (4.5 ± 0.6-month-old) Rat Livers

	Bile flow (µl/hr/g liver)	PC output in bile (pmol/g liver/hr)	PC concentration in bile (pmol/µl)
Basal			
Adult	10.91 ± 5.17[a]	45.9 ± 18.1	5.17 ± 1.66[b]
Young	18.97 ± 7.89	33.2 ± 9.4	1.98 ± 0.56
Bile 1			
Adult	9.98 ± 2.17[a]	47.5 ± 24.6[c]	5.41 ± 2.16[b]
Young	13.31 ± 2.70	12.7 ± 5.9	1.05 ± 0.61
Bile 2			
Adult	8.83 ± 2.44[a]	43.5 ± 24.4[c]	4.96 ± 1.43[b]
Young	11.06 ± 2.30	12.2 ± 4.7	0.89 ± 0.48
Bile 3			
Adult	6.21 ± 2.87[a]	19.4 ± 5.9[c]	4.41 ± 1.40[b]
Young	10.06 ± 3.56	5.6 ± 3.6	0.77 ± 0.41
Bile 4			
Adult	4.95 ± 2.02[a]	8.6 ± 5.3	2.78 ± 0.86[b]
Young	8.67 ± 3.16	5.5 ± 2.2	0.66 ± 0.23

Note. Results are mean ± SD of six or seven perfusion experiments in adult and young group, respectively.
[a] Adult versus young group: $P < 0.05$.
[b] Adult versus young group: $P < 0.01$.
[c] Adult versus young group: $P < 0.001$.

bile samples are reported in Table III. These data indicate that adult livers secreted higher amounts of newly synthesized [^{3}H]PC into bile. Total [^{3}H]PC secreted in bile during the 2 hr following [^{3}H]choline addition was 0.043 ± 0.022 pmol ($n = 6$) and 0.018 ± 0.008 pmols ($n = 7$), $P < 0.05$, in the adult and young groups, respectively. The [^{3}H]PC secreted in the single samples of bile (Table III) was significantly higher in adult compared with young group in

Table III. Radioactivity Recovered in Bile and Lipid Phase of Bile Extracts in the four Bile Samples Collected at 30-min Intervals, after Infusion of [^{3}H]Choline, during Perfusion of Livers from Adult and Young Rat Donors

	Rad. recovery in total bile (dpm/dose/g liver × 10^{-5})	Rad. recovery in lipid phase (dpm/dose/g liver × 10^{-5})	Neosynthesized [^{3}H]PC in bile (pmol/g liver/2 hr × 10^{-3})	S.A.[a] of biliary PC (dpm/pmol)
Adult				
Bile 1	15.69 ± 5.67[b]	0.33 ± 0.28	0.382 ± 0.332	1.67 ± 0.89[c]
Bile 2	24.48 ± 12.86[b]	0.51 ± 0.22[c]	0.646 ± 0.332[b]	3.58 ± 1.63[c]
Bile 3	17.78 ± 5.59[c]	0.47 ± 0.20[b]	0.674 ± 0.210[c]	4.65 ± 3.91[b]
Bile 4	13.61 ± 4.99[c]	0.59 ± 0.25[b]	0.686 ± 0.242[d]	3.07 ± 1.51[b]
Young				
Bile 1	24.27 ± 7.08	0.19 ± 0.15	0.250 ± 0.186	18.43 ± 10.48
Bile 2	81.07 ± 53.27	0.24 ± 0.13	0.294 ± 0.164	33.49 ± 24.41
Bile 3	39.00 ± 13.37	0.23 ± 0.16	0.340 ± 0.190	18.32 ± 11.88
Bile 4	30.17 ± 7.43	0.21 ± 0.11	0.270 ± 0.162	6.52 ± 10.22

Note. Data of the biliary secretion of newly synthesized [^{3}H]phosphatidylcholine (PC) and its specific activity are also shown. Results are mean ± SD of six and seven perfusion experiments in adult and young group, respectively.
[a] S.A. = specific activity.
[b] Adult versus young group: $P \leq 0.05$
[c] Adult versus young group: $P \leq 0.01$
[d] Adult versus young group: $P \leq 0.005$

Bile 2, 3, and 4. However, the specific activity of biliary PC was significantly lower in the adult in comparison to the young group, because of the higher total concentration of PC.

Discussion

We have reported in a previous paper that perfused rat livers showed an age-related increase in secretion of high-density lipoproteins (α-lipoproteins) containing a significantly higher content of phospholipids (4). Our present data indicate further that these differences in the secretion of lipoprotein PC are also paralleled by changes in the secretion of newly synthesized PC.

Furthermore, the present *in vitro* experiments confirm previous *in vivo* and *in vitro* observations that the aging process is associated with a decreased bile flow and an increased biliary phospholipid output in rats (3–6). However, they do not support the hypothesis that the age-related increase in hepatic secretion of PC is due to an enhanced production of PC *via* the CDP pathway.

Perfused livers of adult donors secreted a significantly higher amount of newly synthesized PC into bile compared with young livers (Table III). Total amount of biliary [^{3}H]PC secreted in 2 hr by adult livers was more than twice that found in young livers (0.043 ± 0.022 and 0.018 ± 0.008 pmols in adult and young, respectively; ratio: 2.38). Taking into account the higher liver weight, the total content of newly synthesized [^{3}H]PC of adult livers was only 1.34 times higher than in the young group (10.40 ± 3.59 and 7.74 ± 2.86 pmol/liver in adult and young, respectively; ratio: 1.34). Thus, in bile of older rats a higher proportion of hepatic newly synthesized labeled PC is secreted with respect to bile of younger group. Unfortunately, these experiments did not allow us to evaluate the contribution from minor pathways (i.e., *S*-adenosyl-methionine methylation).

The specific activity of biliary PC secreted by adult livers was much lower than that of young livers. This suggests that the age-related increase in biliary phospholipid secretion is supported by both newly synthesized PC and intrahepatic pools of PC.

Livers, independently of their age, actively incorporated [^{3}H]choline into hepatic PC. The aliquot of radioactivity incorporated into sphingomyelin was, as expected, hardly detectable (9). At the end of the experiments with adult livers, the radioactivity found in [^{3}H]PC is about half that in young livers, as indicated by significantly lower specific activities of hepatic [^{3}H]PC-P (Table I). This may be attributed to faster incorporation of [^{3}H]choline into [^{3}H]PC-P and/or to lower transformation of [^{3}H]PC-P to [^{3}H]PC in the young rats. However, irrespective of the mechanisms involved, the present data suggest that, for each gram of liver, the lower transformation of [^{3}H]choline into [^{3}H]PC-P corresponds with a higher amount of radiolabeled CDP-choline transferred to diacylglycerol for the formation of [^{3}H]PC in the adult group, at least for the time interval studied. Consequently, newly synthesized [^{3}H]PC in adult livers is about 70% of that of young livers, while the specific activity is about the same in the two groups. In other words, these results suggest that a higher proportion of [^{3}H]PC-P is transformed into PC in adult livers than in younger livers.

In other words, combined data obtained on bile and liver indicate that, in the elderly, the newly synthesized PC alone cannot account for increased secretion of PC, and that liver uses part of its constitutive content to maintain this increased secretion, perhaps changing the availability of hepatic pool destined for bile secretion (7). This could lead, in the long term, to both a depletion of PC liver pools and a decreased availability of lipids for VLDL production.

The data in the literature on age-related variations in

hepatic phospholipid and PC content are controversial and cannot be totally attributed to the heterogeneity of the different membrane fractions analyzed. In total liver membrane extraction, Murawasky *et al.* (21) reported that hepatic phospholipids increased, and other studies have reported both to have been unchanged (3, 22), while decreased values have been reported in both plasma membranes and microsomes (23, 24). However, data on the composition of lipid classes show that the amount of PC in the liver of older rats is decreased in hepatic membrane (3) and microsomes (25), even when the relative proportion of PC in membranes is increased in plasma membrane (23) and microsomes (25), with respect to all phospholipids.

In summary, although this study does not allow definitive explanation about the origin of the increased secretion of PC into bile in aging, it does allow some interesting hypotheses on the age-related alterations of lipid metabolism.

Present data do not support the hypothesis that the increased secretion of PC, seen both at the canalicular and at the sinusoidal pole of the hepatocytes in aging, is due to an upregulation of choline cytidyltransferase, the key enzyme in the main biosynthetic pathway of PC in liver. Conversely, in a speculative way, they suggest the existence of an age-related modification in the splitting ratio of hepatic diacylglycerol flow between PC and triacylglycerol synthesis. The biliary phospholipid secretion rate increases with age, but it is uncoupled with bile acids and cholesterol secretion changes. Also, food restriction, while exerting a beneficial effect on survival and bile formation, does not affect biliary phospholipid output (6). Furthermore, livers from older rats showed a slightly decreased, rather than an enhanced, rate of hepatic PC synthesis. Therefore, we hypothesize that some of the many age-related changes observed in liver membranes (23–25) might be caused by an increased recruitment of membrane palmitoyl-linoleyl-PC for its canalicular secretion. In turn, the decreased fluidity of hepatic membranes in older animals (24) may reflect the reduced content of unsaturated molecular species of PC (1, 22), preferentially used for biliary secretion (26), and accompanied by an increased proportion of phosphatidyl-ethanolamine (26).

Alterations in secretion and composition of very low density lipoprotein (VLDL) may occur in aging (4, 27). The secretion of VLDL particles depends on the capability of the liver in the assembly of apoliprotein B and lipid components, mostly triacylglycerol and PC. In some metabolic states (i.e., diabetes, stress, starvation), diacylglicerols (common precursor for phospholipid and triacylglycerol) are mainly converted to phospholipids (15, 16). A pathway similar to that observed under stress conditions (16) may decrease the availability of triacylglycerols necessary for VLDL synthesis in aging. In turn, this may contribute to the synthesis of smaller VLDL particles having a reduced content of triacylglycerol and an increased amount of cholesteryl esters, exactly as we observed in a previous study in the rat (4). It is interesting that an age-related reduction of triacylglycerol production in humans has also been hypothesized (28). Future investigations direct addressing the study of the age-related diacylglycerol flow in hepatocytes will help to clarify these processes.

We gratefully acknowledge the technical assistance of Mrs. Tiziana Marinelli.

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