Moderate Long-Term Physical Activity Improves the Age-Related Decline in Bile Formation and Bile Salt Secretion in Rats

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Abstract. **This study examined the effect of moderate long-term exercise, begun soon after weaning, on the age-related decline in bile formation and the secretory rate maximum (SRm) of taurocholate (TC), the major bile salt (BS) in rats. Eight-month-old sedentary (S) female Sprague-Dawley rats showed a significant decrease in basal and TC-stimulated bile formation when compared with 2.5-month-old S controls. As in younger rats, decreased biliary phospholipid (PL) output was associated with the TC SRm, but this change appeared much more rapidly in S animals, which also exhibited significant increased plasma lipids and higher TC concentrations in plasma and liver at the end of TC infusion. Exercise significantly improved bile flow (BF), including the bile salt-dependent and -independent fractions under basal and TC-stimulated conditions in 8 month-old rats. Although all the biliary parameters evaluated were improved, maximal BF and PL secretion values were affected the most and were virtually not different from those obtained in younger S animals. Exercise also significantly lowered the age-related elevation of plasma PL. Thus, moderate long-term exercise exerts a beneficial effect on hepatobiliary function and the BS SRm, an effect that may be attributed in part to increased availability of a biliary PL pool previously implicated in regulation of the BS SRm.** [P.S.E.B.M. 1994, Vol 206]

Several hepatobiliary functions have been reported to decline during aging (1–6). Although the biliary secretion of bile salts (BS) has been shown to be normal or slightly reduced in the basal state during aging, the capacity of old rats to handle high levels of BS and other organic anions has been demonstrated to be significantly declined (2–4, 7, 8). Similarly, the liver capacity for adaptation to nutritional or clinical stress have been shown to decrease with age (9–11). The degree of capability of the aging rat liver to respond to various exogenous stressors is important since decreased handling as well as decreased threshold resistance to BS and drugs may be associated with a higher frequency of hepatotoxicity.

Caloric restriction is an effective means of retarding the aging process in rodents (2, 12, 13), including hepatobiliary function (2). In these studies, caloric restriction was achieved through food reduction (without malnutrition). Another means of influencing energy balance is through caloric expenditure by increased physical activity. Thus, long-term physical activity has been proposed as a mean to prevent or ameliorate some pathologies and may also increase longevity (14, 15). However, there are very few reports in the literature on the effect of exercise on hepatobiliary function (16–18), particularly in aging. Furthermore, most of these studies were performed with exhausting exercise, which is associated with meta-

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bolic alterations due to oxidative stress. We, therefore, investigated the effect of caloric expenditure from moderate long-term physical activity on the age-related decline in bile formation as well as maximal BS transport and secretion in rats.

Materials and Methods

Female Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) were obtained at weaning and housed individually. They were maintained under constant temperature (22°C) and lighting (7:00 PM to 7:00 AM darkness) with free access to food (a semisynthetic diet) and water prior to the experiment. Soon after weaning, the rats were randomly divided in three groups. One group (of 10 rats) was subjected to moderate physical exercise on a treadmill at 18 m/min for 60 min 5 days per week until the age of 8 months (Group E). Sedentary (S) controls were maintained for the time intervals under study (2.5 and 8 months, respectively). The 8-month-old S and E groups were kept under per fed conditions.

At 2.5 and 8 months, all rats were anesthetized with pentobarbital (45 mg/kg body wt, ip), and, after laparotomy, the common bile duct was cannulated with a PE-10 catheter. Body temperature was maintained throughout the experiment at 37°C, using a rectal probe and thermostatically-controlled heat lamp. Basal bile formation was evaluated from the first 30 min of bile collection (two periods, 15 min each). Handling of a high BS load was evaluated in four animals from each group. In these rats, a PE-50 catheter was inserted in the right internal jugular vein. BS administration was begun after 90 min of bile collection to substantially deplete the endogenous BS pool (19). Infusion of 4.5% albumin in 0.9% NaCl (BS vehicle) for 30 min preceded the infusion of taurocholate (TC) sodium salt (Calbiochem; Boehringer Corp., La Jolla, CA) in albumin solution for 90 min at an increasing rate of 1.0, 1.5, and 2.0 μmole/min/100 g body wt (each for 30 min). One μCi of ^{14}C-labeled TC per mmole of cold BS solution was mixed with the infusion. Bile was collected throughout the BS infusion in 10-min aliquots, and the 15 min preceding TC infusion was considered as the preinfusion period. Following bile collection, blood samples were obtained from the abdominal aorta, and the gastrocnemius muscle was harvested. Livers were perfused with 0.9% NaCl, excised, and weighed, and a 10% homogenate was prepared in 1 m*M* NaHCO3 buffer, using a Polytron R homogenizer at maximal speed for 5 sec. Plasma, bile, and liver homogenates were stored at −80°C until analyzed.

In the gastrocnemius muscle, citrate synthase activity was assessed by Srere's method, using 5,5,dithiobis-2-nitrobenzate (20). Total cholesterol (CL), phospholipid (PL), and high density lipoprotein (HDL)-CL were quantitated in plasma obtained from the retroorbital plexus one day prior to bile collection, as described by Levy *et al.* (21).

BF was measured gravimetrically, assuming a density of 1 g per ml. Total endogenous BS were assessed enzymatically as described elsewhere (22), using 3-α-hydroxysteroid deshydrogenase (Sigma Chemical Co., St. Louis, MO). TC secretion was determined from the radioactivity secreted in bile and the sp act of TC infused to avoid measuring endogenous BS. Radioactivity distribution was determined in aliquots of bile, liver homogenate or blood, using a Beckman liquid scintillation counter equipped with an external standard, as described previously (23). Total biliary PL was analyzed by measuring inorganic phosphate according to the Bartlett method (24) after digestion of all biliary components in perchloric acid as described by Galliard *et al.* (25). Biliary CL was measured with a CL oxidase kit (Boehringer-Mannheim, Montreal, Quebec, Canada) following biliary CL extraction by the method of Bligh and Dyer (26) because some biliary components interfere with this assay. The bile salt-independent fraction (BSIF) of BF was evaluated, in the basal period as well as in the choleretic phase during TC infusion, by extrapolation of the linear relationship between BS secretion and BF to zero BS in each group (27, 28). The choleretic potency of BS was measured by calculating the slope of this correlation. The amounts of CL and PL secreted in association with exogenous BS administration were evaluated by calculating the actual increase in lipid secretion (period value minus basal value of lipids) per exogenous BS secretion.

The data were statistically evaluated by analysis of variance (ANOVA). When the values were found to be significant, comparisons between groups were made using Duncan's test. *P* values lower than 0.05 were considered significant.

Results

Body and Liver Weight. As expected, 8-month-old E rats demonstrated a statistically significant decrease (17%) in body weight versus the age-matched S group (327.2 ± 13.2 vs 392.3 ± 18.5 g, respectively). Aging significantly increased liver weight while exercise maintained it at a level similar to that in younger rats (8.40 ± 0.51, 11.48 ± 0.66, and 9.19 ± 0.40 for 2.5-month-old rats, 8-month-old S rats, and 8-month-old E rats, respectively). However, the percent liver to body weight ratio remained similar in the 8-month-old groups with or without physical activity, and this ratio was statistically lower than in the younger animals (4.88 ± 0.23, 2.93 ± 0.11, and 2.82 ± 0.82 for 2.5-month-old rats, 8-month-old S rats and 8-month-old E rats respectively).

Citrate Synthase Activity. Citrate synthase ac-

Table I. Effect of Aging and Physical Activity on Basal Bile Flow and Biliary Secretion

	Bile flow (μl/min/g liver)	Secretion rate (nmole/min/g liver)			Lipid to bile salt molar ratio (mmole/mole bile salts)	
		Bile salts	Cholesterol	Phospholipids	Cholesterol	Phospholipids
2.5-Month-old sedentary rats	1.16 ± 0.08	24.38 ± 2.01	0.40 ± 0.07	3.23 ± 0.81	18.08 ± 3.08	151.35 ± 36.73
8-Month-old sedentary rats	0.98 ± 0.06[b]	24.41 ± 2.58	0.25 ± 0.05[a]	2.00 ± 0.65	9.08 ± 1.16[a]	85.14 ± 26.70
8-Month-old exercised rats	1.27 ± 0.07	31.59 ± 4.52	0.31 ± 0.06	3.66 ± 0.81	12.03 ± 1.00	145.09 ± 24.77

Note. Results are the means of the first 30 min of bile collection. Data are means ± SEM obtained from 4–10 animals per group.
[a] Significantly different ($P < 0.05$) from 2.5-month-old sedentary rats.
[b] Significantly different ($P < 0.05$) from 8-month-old exercised rats.

tivity was increased significantly in 8-month-old E rats (23.08 ± 1.58 [n = 9] vs 15.76 ± 1.63 μmole/min/g tissue in 8-month-old S controls [n = 7]), indicating the effectiveness of physical training in E group.

Bile Formation. *Bile formation during basal period.* Table I enumerates the BF, BS, CL, and PL secretion rates (BSSR, CLSR, and PLSR, respectively), as well as the CL and PL to BS molar ratios. The 8-month-old S group showed a significant reduction of CLSR and the CL to BS molar ratio. BF, PLSR and the molar PL to BS ratio were also reduced but not significantly. However, the BSSR was not affected in comparison with younger 2.5-month-old rats. In the 8-month-old E group, all parameters examined were

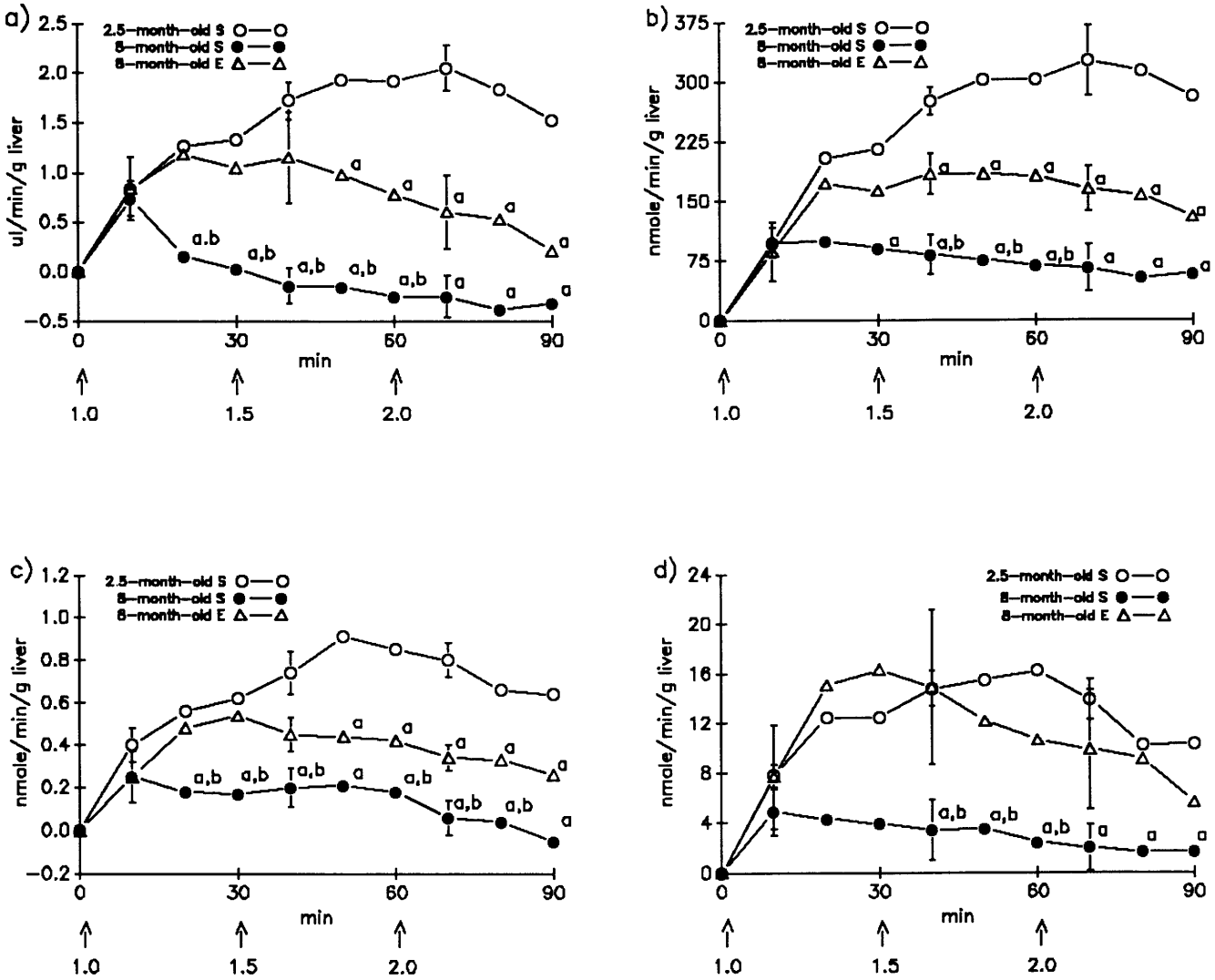

Figure 1. Effect of aging and moderate exercise on changes in bile flow (1a), bile salt secretion rate (1b), cholesterol secretion rate (1c), and phospholipid secretion rate (1d) during infusion of three 30-min step-wise increasing doses (1.0, 1.5, and 2.0 μmole/min/100 g body wt) of taurocholate (TC) (see arrows). Values represent the mean of four animals. SEM are deleted for clarity. [a]Significantly different ($P < 0.05$) from 2.5-month-old rats. [b]Significantly different ($P < 0.05$) from 8-month-old exercised rats.

Table II. Effect of Aging and Moderate Physical Exercise on the Taurocholate (TC) Secretory Rate Maximum (SRm) and TC-Induced Biliary Secretion

	TC SRm (nmole/min/g liver)	Maximal bile flow (μl/min/g liver)	Maximal lipid secretion (nmole/min/g liver)	
			Cholesterol	Phospholipids
2.5-Month-old sedentary rats	346.22 ± 42.64	3.42 ± 0.25	1.44 ± 0.14	20.29 ± 1.14
8-Month-old sedentary rats	119.60 ± 32.85[a]	1.89 ± 0.25[ab]	0.58 ± 0.10[a]	7.54 ± 2.07[ab]
8-Month-old exercised rats	214.00 ± 12.91[a]	2.78 ± 0.19	0.89 ± 0.13[a]	21.43 ± 6.46

Note. Results are means ± SEM of four animals per group.
[a] Significantly different ($P < 0.05$) from 2.5-month-old sedentary rats.
[b] Significantly different ($P < 0.05$) from 8-month-old exercised rats.

not significantly different from those in younger rats, and BF was significantly improved relative to the 8-month-old S controls. Improvement of BF by exercise was associated with increases in both bile salt-dependent fraction (BSDF) of BF (0.39 ± 0.17 vs 0.52 ± 0.31 μl/min/g liver for 8-month-old S and 8-month-old E, respectively) and BSIF (0.62 ± 0.11 vs 0.78 ± 0.24 μl/min/g liver for 8-month-old S and 8-month-old E, respectively).

Bile formation during TC infusion. Figure 1 depicts the changes in BF, BSSR, CLSR, and PLSR values during TC infusion in step-wise increasing doses. In 2.5-month-old rats, BF was augmented with escalating infusion doses of TC until the last 20 min of infusion at which time it started to decline. In the 8-month-old S group, BF rose during the first 10 min of infusion and progressively decreased thereafter to below the basal value. In the E group, it increased for 30 min and then declined but remained higher than baseline. The change in BF during the first 10 min of infusion was similar in all groups, but was significantly lower thereafter in 8-month-old S rats than in the younger animals and 8-month-old E group. Similarly, the decline in BF in the 8-month-old E group after 50 min of infusion was significantly lower than in younger controls (Fig. 1a). The trend in BSSR (Fig. 1b) was similar to that seen with BF except that the decrease after reaching the SRm was not as drastic as the reduction in BF, especially in both 8-month-old groups (S and E). However, the BSSR was significantly different in the three groups after 40 min of infusion. CLSR (Fig. 1c) was similar to BF, rising with the increase in infusion time and falling after reaching a maximum secretory rate. Similarly, PLSR also increased with the length of infusion time until it reached a maximum, but the PLSR decrease appeared prior to the decline in BSSR in all groups (Fig. 1d).

Table II summarizes the TC SRm as well as the maximal BF, CL, and PL secretion values obtained in experiments in which TC was infused in step-wise increasing doses. In 8-month-old S rats, the TC SRm was significantly reduced (65%) as compared with younger animals and this was associated with significant decreases in maximal BF, CL, and PL secretion (45%, 60%, and 63%, respectively). In E rats, the SRm remained significantly lower than in younger animals (38%) but was higher (although not significantly) than in 8-month-old S controls. The SRm increase in E rats was also associated with a rise in maximal BF, CL, and PL secretion, maximal BF and PL secretion being significantly higher than in the 8-month-old S controls.

The mean BSIF and apparent choleretic activity of TC calculated from data obtained in the choleretic periods during TC infusion (see Materials and Methods) are shown in Table III. Although BSIF was reduced by 38% in the 8-month-old S group and increased by 23% in 8-month-old E rats as compared with the younger controls, resulting in an 83% increase versus the 8-month-old S group. Choleretic activity on the other hand was elevated by 36% in the 8-month-old S group and was only slightly reduced (14%) in the E group as compared to the 2.5-month-old controls. However, the

Table III. Effect of Aging and Moderate Physical Activity on Bile Salt-Independent Fraction (BSIF) and Choleretic Activity of Bile Salts Following Taurocholate (TC) Infusion

	BSIF (μl/min/g liver)	% of total bile flow	Apparent choleretic activity of TC (μl/μmole BS)	Coefficient of regression (r^*)
2.5-Month-old sedentary rats	1.31 ± 0.10	47.3	5.94 ± 0.37	0.95
8-Month-old sedentary rats	0.92 ± 0.28	49.5	8.08 ± 2.25	0.85
8-Month-old exercised rats	1.68 ± 0.29	64.9	5.13 ± 1.60	0.75

Note. Correlations were made between bile flow and bile salt secretory rate for samples obtained before the decreased secretion of these parameters as explained in Materials and Methods. Results are means ± SEM of four animals per group.
* Significant at $P < 0.05$.

Table IV. Percent Recuperation and Distribution of Taurocholate ^{14}C (TC) in Plasma, Liver, and Bile Following TC Infusion in Aging and Exercised Rats

	Blood	Bile	Liver	Total
2.5-Month-old sedentary rats	7.9 ± 0.7	79.4 ± 3.7	9.2 ± 0.8	96.5 ± 2.6
8-Month-old sedentary rats	39.3 ± 8.4[ab]	11.5 ± 4.7[ab]	29.7 ± 11.7[ab]	80.5 ± 4.3
8-Month-old exercised rats	10.1 ± 3.5	29.8 ± 5.5[a]	54.0 ± 1.6[a]	93.9 ± 2.7

Note. Data are means ± SEM obtained at the end of the experiment from four animals per group.
[a] Significantly different ($P < 0.05$) from 2.5-month-old sedentary rats.
[b] Significantly different ($P < 0.05$) from 8-month-old exercised rats.

differences between means of the three groups for BSIF and the choleretic activity did not reach the significance level at $P < 0.05$.

Table IV depicts the distribution of TC in blood, bile, and liver at the end of the infusion experiment. Of the radioactivity injected, 80.5% to 96.5% was found in these three compartments. In the younger rats, most of it was secreted in bile. However, in the 8-month-old S group, the highest radioactivity was present in blood, followed by the liver. On the other hand, in E rats, over 50% of radioactivity was evident in the liver at the end of infusion but the amount seen in blood was similar to that in younger animals and significantly lower than in 8-month-old S controls.

Plasma lipid content. As shown in Table V, total plasma CL doubled in the 8-month-old S group, despite no significant change in HDL-CL. PL concentration was augmented by 65%. In E rats, total CL was also increased, although not significantly, versus the younger controls, but this elevation was associated with a significant rise in HDL-CL, and, thus, the HDL-CL to total CL ratio was not significantly different from that in younger animals but significantly higher than in the 8-month-old S group. PL concentration in the E group was also not significantly different from that in the younger controls but significantly lower (36%) than in 8-month-old S rats.

Discussion

The objective of this investigation was to evaluate the effect of moderate long-term exercise on the age-related decline in bile formation. The study showed that aging for 8 months in rats significantly reduced CLSR, slightly but not significantly decreased BF and PLSR, while it did not modify BSSR under basal physiological conditions. In TC-stimulated bile formation, the TC SRm as well as the maximal secretion rates of BF, CL, and PL were all significantly reduced. Long-term moderate exercise resulted in BF, BSSR, CLSR, and PLSR values in the basal period similar to those in younger (2.5-month-old) rats. On the other hand, in TC-stimulated bile formation, the TC SRm, and maximal CL secretion remained significantly lower than in younger animals but higher than in 8-month-old S controls while maximal BF and PL secretion were similar to that in younger S rats. Therefore, moderate long-term exercise improved the age-related decline in bile formation both under basal and TC-stimulated conditions in aging animals.

These data on the age-related changes on basal and TC-stimulated bile formation are in agreement with those reported previously, showing a decrease in TC excretion in 12- and 24-month-old animals (3, 4, 8). Although BSSR was similar in young 2.5-month-old and 8-month-old rats in the basal period, TC infusion in the 8-month-old group revealed a defect in handling higher BS load, and this was clearly reflected in a lower SRm and associated bile composition. The decrease in BF was accompanied by a decline in BSDF and BSIF fractions. The factors controlling the SRm of BS are numerous, but a hypothesis has been advanced which suggests that the SRm is reached after depletion of the PL pool available for BS secretion (29). In 8-month-old rats, it is apparent that the supply of biliary PL is limited in the basal period, and this was represented by lower PLSR despite normal BSSR. In addition, plasma PL and HDL-CL were increased which suggests that plasma PL and CL were not available for biliary secretion. Since more than 30% of bil-

Table V. Plasma Lipid Content in Control and Exercised Rats

	Total cholesterol (T-CL) (m*M*)	HDL-Cholesterol (HDL-CL) (m*M*)	HDL-CL to T-CL ratio	Phospholipids (m*M*)
2.5-Month-old sedentary rats	2.01 ± 0.23	1.25 ± 0.09	0.66 ± 0.18	1.81 ± 0.13
8-Month-old sedentary rats	4.07 ± 0.50[a]	1.88 ± 0.63	0.50 ± 0.18[b]	2.99 ± 0.16[ab]
8-Month-old exercised rats	3.59 ± 0.45	2.62 ± 0.88[a]	0.81 ± 0.06	2.21 ± 0.04

Note. Data are means ± SEM obtained from 8–10 animals per group.
[a] Significantly different ($P < 0.05$) from 2.5-month-old sedentary rats.
[b] Significantly different ($P < 0.05$) from 8-month-old exercised rats.

iary PL is of extracellular origin mainly HDL-PL (30), it is possible that aging reduced HDL uptake by the liver and consequently reduced the availability of PL for biliary secretion. The data on radioactivity distribution at the end of TC infusion further support this hypothesis. It was shown that, with high concentration of plasma BS, there is an increase in BS binding to lipoprotein (31), and, therefore, a defect in lipoprotein uptake in the 8-month-old rats would result in less TC uptake and an increase in the amount of TC remaining in plasma, as seen in Table IV.

Long-term moderate exercise improved BF both under basal and TC-stimulated conditions. These results are in agreement with the increased bile formation reported in young rats following 6 weeks of voluntary exercise, an effect that has been proposed to be related to hyperphagia following exercise (32). The results obtained in this study, however, suggest that additional mechanisms must be implicated, since the 8-month-old rats used were maintained in per fed conditions. In our study, although bile formation remained normal in the 8-month-old E rats (similar to that in younger rats) under basal conditions, the capacity to excrete TC was decreased compared with the younger group, but it was ameliorated in relation to 8-month-old S rats. Improvement of bile formation by exercise in age-matched (8-month-old) animals resulted in better generation of BSDF, but particularly of BSIF which was similar to that in younger rats. Although the mechanisms implicated in BSIF generation are not well characterized, Ballatori and Truong (33, 34) suggest that glutathione (GSH) mediates about half of BSIF generation. Acute exercise is known to modulate hepatic GSH levels (16, 17, 35) and thus, it is possible that GSH secretion in exercise might have been responsible for the improvement in BSIF. BS have previously been shown to alter BSIF (28), and, since alterations in BS secretion have been suggested to affect organic anion secretion (17), such as of GSH, improvement of TC secretion by exercise may also contribute to improved BSIF generation. Exercise also improved biliary lipid secretion, and it is interesting that despite normal biliary PL secretion in the basal period, the decrease in PL secretion was still more rapid and the TC SRm still significantly lower than in younger animals. This may indicate that the availability of PL for BS secretion was still limited and, therefore, depleted by the lower amount of BS secreted in 8-month-old rats. This suggestion is supported by the data on plasma PL in these animals which showed an increase compared with younger rats. However, these changes were less marked than in non-E animals, and, similarly, the moderate beneficial effect of exercise on the TC SRm may be associated with relatively higher PL availability for BS secretion. The distribution of radioactivity in the exercised group showed that TC uptake was similar to that in younger animals. However, exercised rats stored much more TC in the liver, suggesting that exercise did not ameliorate as much the capacity of TC excretion. In addition to decreased availability of PL, another possibility is that the reduced SRm in exercised versus younger animals may be associated with the incapacity of exercise to correct an age-associated decrease in maximum BS secretory capacity. Whatever the mechanism, the improvement of bile formation observed in this study by increasing energy expenditure resembles the previously-demonstrated beneficial effect of caloric restriction on the same biliary parameters (2) suggesting that a net decrease in energy balance itself results in better biliary secretion.

In conclusion, this investigation provides evidences of improved age-related decline in bile formation and BS excretion in rats with long-term moderate exercise. Concomitant with the effects of exercise on bile formation, there were significant changes in plasma PL. It remains to be established whether these events are related.

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