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The Effect of Age upon Liver Volume and Apparent Liver Blood Flow in Healthy Man

HILARY A. WYNNE, LANCE H. COPE, ELAINE MUTCH, MICHAEL D. RAWLINS, KENNETH W. WOODHOUSE AND OLIVER F. W. JAMES

Departments of Medicine (Geriatrics), Clinical Pharmacology and Radiology, University of Newcastle Upon Tyne, Newcastle Upon Tyne, United Kingdom

The aim of this study was to determine the effect of aging upon liver volume and apparent liver blood flow in healthy man. Sixty-five subjects between 24 and 91 years of age were recruited. Liver volume was quantitated by a gray scale B ultrasound scan method. Apparent liver blood flow was determined from the plasma clearance of indocyanine green, based on an assumption of no change in hepatic extraction of the dye with age.

A significant negative correlation was observed between age and both liver volume and apparent liver blood flow ($p < 0.001$), whether expressed in absolute terms or per unit body weight. Similarly, a significant negative correlation was observed between apparent liver blood flow per unit volume of liver (liver perfusion) and age ($p < 0.005$).

The reduction in liver volume, apparent liver blood flow and perfusion may at least partly account for the decline in the clearance of many drugs undergoing liver metabolism which has been noted to occur with aging in man.

The systemic elimination of a number of drugs metabolized by hepatic microsomal monooxygenase enzymes (MMOs) has been shown to decline with age in man (1, 2). The age-related fall of the specific activities of these enzymes in rats, first reported by Kato et al. (3) and subsequently confirmed by many other workers, has led to the assumption that similar changes occur in aged humans and may thus account for decreased drug elimination. We have recently measured the *in vitro* specific activities of a number of hepatic MMOs in histologically normal human liver specimens, many obtained from subjects with normal liver function tests undergoing routine cholecystectomy, and have found no age-related decline (4). Furthermore, in nonhuman primates, liver microsomal NADPH cytochrome C (P-450) reductase (5) and benzo[α]pyrene hydroxylase activities are unchanged with aging (6). These findings have therefore brought into question the above assumption. We have therefore hypothesized that changes in liver mass and liver blood flow in the absence of a reduction in the specific activities of MMOs might explain at least some of these apparent contradictions.

Postmortem studies have shown that liver mass falls with advancing age in man (7, 8), but these findings cannot necessarily be extrapolated to healthy individuals. *In vivo* liver volume can be accurately measured by ultrasonography (9, 10), and this technique has been used in young and old subjects to confirm a lower liver volume in the elderly (11). Splanchnic blood flow is also reputed to fall with age, but this is based on the results of a study which was never intended for this purpose (12), using a substrate (bromosulfophthalein) which is inappropriate (13). *In vivo* investigations of the relationship of liver volume to liver blood flow have not studied the effect of aging upon both of these parameters in normal subjects over a wide age range (14).

We have therefore performed a systematic investigation of the changes in both liver volume and apparent liver blood flow in healthy subjects over a wide age range.

SUBJECTS AND METHODS

Subjects. Sixty-five healthy volunteers (33 females) between the ages of 24 and 91 years were recruited from among colleagues and from local social organizations for the elderly. All gave informed, written consent and the study had the approval of the Newcastle Health Authority Ethical Committee. No subject was suffering from hepatic, renal, respiratory or cardiac disorders as assessed by full clinical history and examination. In addition, all subjects had normal blood count and film; serum bilirubin, alkaline phosphatase, aminotransferase and plasma proteins; creatinine, and electrolytes. Only two were taking any medication (one ibuprofen and one piroxicam).

Apparent Liver Blood Flow. Subjects were studied following a 4-hr fast and after a 30-min period of recumbency. An initial blood sample was taken from a 19-gauge cannula inserted into an antecubital vein. Indocyanine green (ICG) (Hyson, Westcott and Dunning, Baltimore, MD), 0.5 mg per kg, was injected intravenously over 20 sec into a contralateral forearm vein. Heparinized blood (5 ml) was withdrawn at 2-min intervals for 14 min and centrifuged at 3,000 × *g* for 20 min, and the plasma was removed. ICG concentrations were determined by absorption spectrophotometry (Pye Unicam PU8800) at a wavelength of 800 nm (15). Plasma clearance of ICG (Cl_{ICG}) was calculated by dividing the dose by the area under the

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Address reprint requests to: O. F. W. James, M.D., The University of Newcastle Upon Tyne, Department of Medicine, Floor 4, Clinical Block, The Medical School, Framlington Place, Newcastle Upon Tyne, United Kingdom NE2 4HH.

plasma concentration time curve (AUC) (determined by the trapezoidal rule):

$$Cl_{ICG} = \frac{Dose}{AUC}$$

and apparent liver blood flow was estimated as:

$$\text{Apparent liver blood flow} = \frac{Cl_{ICG}}{0.74(1 - HCT)}$$

where HCT is the hematocrit, determined by Coulter Counter (erythrocyte count × mean corpuscular volume) and 0.74 is the mean extraction ratio of ICG obtained by direct measurement in normal subjects (15–20).

Liver Volume. Liver volume was estimated by an ultrasound technique, modified from that of Carr et al. (21) using a static gray scale B scanner (Diasonics 2.5-MHz transducer) while subjects were supine. All scans were performed on fasted subjects in the early afternoon and during full inspiration. The first longitudinal scan was taken as far to the right of the midline as good acoustic contact made possible. Subsequent images were obtained by moving the transducer gantry in 1-cm intervals to the subject's left until the whole liver had been scanned. Serial horizontal liver scans were obtained in order to calculate the distance between the most lateral (right) longitudinal scan and the edge of the liver. Liver volume was calculated from the sum of the area of each of the longitudinal liver images, to which was added a computed volume (V) for the liver lateral to the first longitudinal image which was assumed to be paraboloid:

$$V = \tfrac{2}{3}(A \times h)$$

where A is the area of the first image and h is the horizontal distance from the first image to the edge of the liver (Fig. 1). The images were recorded on videotape for subsequent calculation of the liver area using a computer graphics tablet. Six subjects underwent scanning on two separate occasions in order to assess the reproducibility of measurement. The mean variation was 6.5% (range: 2.6 to 13%). Liver volume derived by ultrasound method was also compared to values obtained by computed tomography which was calculated by summating the cross-sectional areas of liver, on contiguous 1-cm slices, through the upper abdomen. Values obtained by the two methods, in six patients, showed good correlation (r = 0.98).

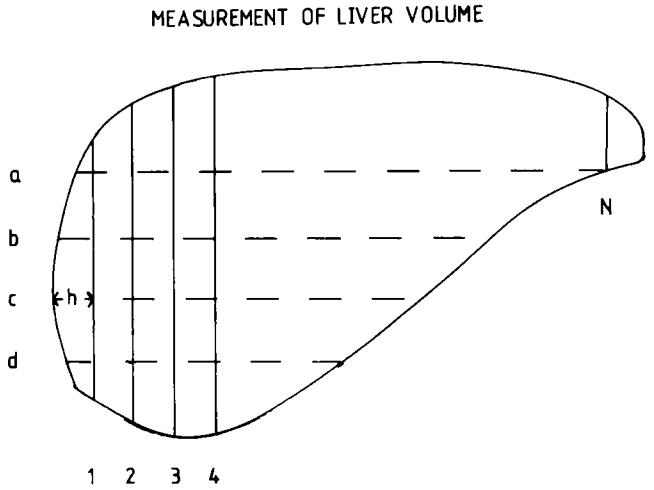

FIG. 1. Diagrammatic representation of the ultrasonic measurement of liver volume. Liver volume was calculated from the sum of the area of numbered serial longitudinal scans taken at 1-cm intervals, to which was added a computed value, ⅔(A × h), where A was the area of Image 1 and h was the horizontal distance from Image 1 to the edge of the liver.

Statistical Methods. The correlations between age and liver volume, apparent liver blood flow and liver perfusion (ml apparent blood flow per ml liver) were assessed by calculating Kendall's rank order correlation coefficient (T). The significance of differences in percentage change from mean values of these parameters in males and females was tested by the t test for independent samples. Age groupings were compared by analysis of variance followed by the Scheffe's test.

RESULTS

Liver volume (Fig. 2) declined with age, whether expressed in absolute values ($T = 0.381$, $p < 0.001$) or in proportion to body weight ($T = 0.440$, $p < 0.001$). Apparent liver blood flow (Fig. 3) also diminished with age both in absolute terms ($T = 0.451$, $p < 0.001$) and in

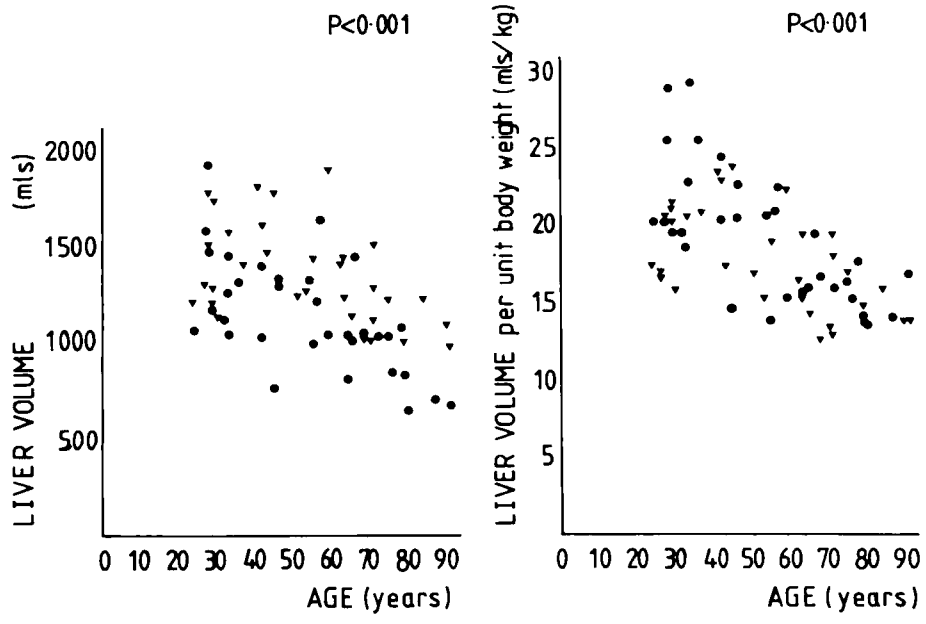

FIG. 2. Correlation between age and liver volume (expressed as ml and as ml per kg body weight) in subjects studied. ▼ = males; ● = females.

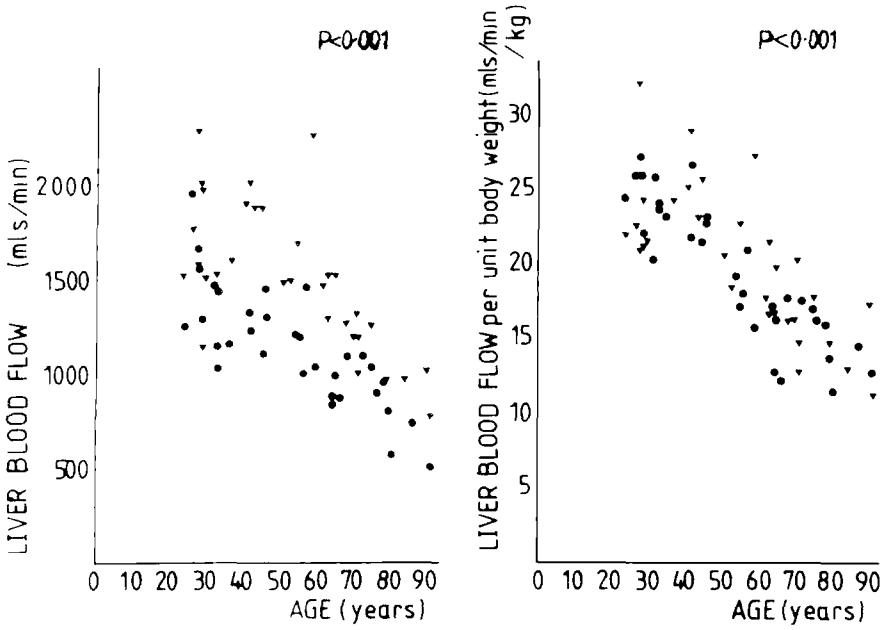

FIG. 3. Correlation between age and apparent liver blood flow (expressed as ml per min and as ml per min per kg body weight) in the subjects studied. ▼ = males; ● = females.

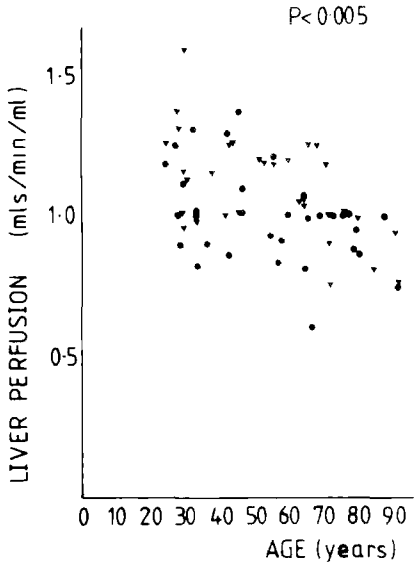

FIG. 4. Correlation between age and apparent liver blood flow per unit volume of liver (liver perfusion) (expressed as ml per min per ml liver) in the subjects studied. ▼ = males; ● = females.

proportion to body weight ($T = 0.617$, $p < 0.001$). Apparent liver blood flow per unit volume of liver (liver perfusion, Fig. 4) declined with age ($T = 0.274$; $p < 0.005$). There was a significant correlation, independent of age, between liver volume and apparent liver blood flow ($r = 0.809$, $p < 0.01$). Values for liver volume, liver blood flow and liver perfusion at age 24 and 91 years, calculated from linear regression analysis, are shown in Table 1. Results for males and females are shown separately. Although the percentage falls in each parameter were numerically greater in females than males, these differences did not reach statistical significance. Mean values obtained for liver volume, liver blood flow and liver perfusion, at age less than 40 years, at 40 to 64 years and at 65 years and over, are shown in Table 2.

TABLE 1. **Values of liver volume, apparent liver blood flow, and liver perfusion at ages 24 and 91 years estimated by linear regression analysis**

	24 years	91 years	% fall
Liver volume (ml)			
Males	1,524	1,102	28
Females	1,415	789	44
Males + females	1,474	934	37
Liver volume (ml/kg body wt)			
Males	20.7	14.5	30
Females	23.8	13.6	43
Males + females	23.6	14.0	41
Apparent liver blood flow (ml/min)			
Males	1,864	1,126	40
Females	1,546	645	58
Males + females	1,717	807	53
Apparent liver blood flow (ml/min/kg body wt)			
Males	25.3	14.5	43
Females	25.5	11.5	55
Males + females	25.7	13.5	47
Liver perfusion (ml/min)			
Males	1.24	1.02	18
Females	1.11	0.88	21
Males + females	1.18	0.94	20

DISCUSSION

The fall in estimated liver volume with advancing age, obtained using the ultrasound method described here, is in good general agreement with that obtained by Calloway et al. (8) in a large postmortem study, although our values (in ml) are some 16% lower than those found in the cadavers (in gm). Although Grainger et al. (20) reported ICG kinetics derived from plasma concentrations measured between 5 and 22 min to be biexponential, our plasma concentration data fitted well to a single exponential function. This is consistent with work pre-

TABLE 2. Mean values of liver volume, apparent liver blood flow and liver perfusion in relation to age in males and females

	<40 years	40–64 years	>65 years
Liver volume			
Males (ml)	1,406 ± 71[a]	1,502 ± 71[b]	1,133 ± 51[c,g]
Females (ml)	1,339 ± 86[d]	1,151 ± 72[e]	940 ± 70[f]
Males (ml/kg)	19.5 ± 0.7	19.4 ± 0.9	15.3 ± 0.6[g]
Female (ml/kg)	23.2 ± 1.2	19.0 ± 0.9[g]	16.1 ± 0.5[g]
Liver blood flow			
Males (ml/min)	1,699 ± 105	1,727 ± 89	1,211 ± 66
Females (ml/min)	1,401 ± 91	1,170 ± 59	869 ± 62[g]
Males (ml/min/kg)	23.4 ± 0.9	22.3 ± 0.9	16.2 ± 0.6[g]
Females (ml/min/kg)	24.1 ± 0.5	19.5 ± 0.8[g]	14.8 ± 0.5[g]
Liver perfusion			
Males (ml/min/ml liver)	1.22 ± 0.05	1.15 ± 0.02	1.07 ± 0.04
Females (ml/min/ml liver)	1.06 ± 0.05	1.04 ± 0.05	0.95 ± 0.04

Males: [a]n = 10; [b]n = 11; [c]n = 11.
Females: [d]n = 10; [e]n = 12; [f]n = 11.
Values = means ± S.E.
[g] $p < 0.05$ by ANOVA.

viously reported by the same group (22), as well as others (23). The lower intravenous dose employed by Grainger et al. may have accentuated an artifact introduced by the presence of an impurity in the commercial ICG preparation (24). Although a spectrophometric assay has been shown to be nonspecific, detecting the unidentified metabolite or impurity, values for plasma clearance of indocyanine green are similar when calculated from data obtained up to 15 min and measured by spectrophotometry or HPLC assay (23, 24). In this study, apparent liver blood flow was calculated after assuming an hepatic extraction ratio of 0.74 for all subjects, a mean value obtained after direct measurement in six studies in younger normal subjects (15–20). ICG is actively and rapidly excreted into bile without undergoing biotransformation. Its clearance is limited by liver blood flow and by the capacity of the hepatic active transport mechanisms, and the delivery of ICG to the liver is rate limiting at low doses such as those used here (25). Age-related changes in the hepatic metabolism of bromosulfophthalein, the organic anion dye which is used to investigate liver function, have been noted. Thus, studies in both man (13) and rats (26) support a fall in relative storage capacity but no change in transport rate maximum during continuous dye infusion. Although the effect of age upon the hepatic extraction ratio of ICG in normal humans has not been examined, studies of age-related changes in the kinetics of ICG in rats suggest a fall in the maximal removal rate from immaturity to young adulthood but stability from adulthood to senescence (27). In this study we have assumed that, as in rats, there is no change in hepatic extraction ratio of ICG in humans with aging, and thus that the fall noted in ICG clearance represents solely a fall in liver blood flow rather than impairment of uptake or transport of the dye into hepatocytes. Although confirmation of this by direct measurement in healthy humans of varying ages is limited by ethical constraints, it is required in order to remove any uncertainty. Should a change in hepatic extraction ratio of ICG occur with advanced age, values of apparent blood flow derived from ICG clearance would clearly need subsequent modification.

Recent work has shown that interindividual variations in liver volume are related to variations in antipyrine clearance (28). Schnegg and Lauterberg (29) recently suggested that the decline in clearance with advancing age of such drugs as aminopyrine (30), antipyrine (31) and caffeine (29), all metabolized by MMOs, might be attributable to loss of the functioning mass of hepatocytes. The classic study of Vestal et al. (31) showed a fall of 18.5% in the systemic clearance of antipyrine between young males (mean age: 32.9 years) and elderly males (mean age: 68.7 years). Swift et al. (10) showed a decline in antipyrine clearance of 26% between the third and the ninth decade. Our estimate of a 28% reduction in liver volume in relation to body weight in males and a 12% fall in estimated liver perfusion between subjects under age 40 and those over age 65 which mirrors pharmacokinetic findings suggests that morphological changes in the liver have considerable influence upon changes in drug metabolism. The fall in free phenytoin and in antipyrine clearance per unit volume of liver, however (10, 11), as well as the marked change in ratios of urinary antipyrine metabolites noted in elderly subjects (32), suggest that other factors such as decreased hepatic enzyme activities or an impairment of certain metabolic pathways may be important, particularly for low-extraction drugs, including certain oxidized benzodiazepines (33). The age-related reduction in liver blood flow that we have noted is similar in degree to and may largely explain reported falls in the systemic clearance of highly extracted drugs such as triazolam, chlormethiazole and propranolol (34–36), although alterations in intrinsic clearance may also contribute (37, 38).

Although absolute values for liver volume and blood flow were lower in females, values for both sexes were similar when expressed per unit body weight. Greater percentage falls in both liver volume and liver blood flow were noted in females than males with aging, but these differences were not statistically significant. A similar fall in liver perfusion was noted in both sexes. These observations, therefore, fail to explain the greater age-related reduction in metabolic clearance of some oxidized benzodiazepines noted in male then in female subjects (39).

All subjects taking part in the present study were healthy, ambulant and living independently in the community. These results may well be an underestimate of the decline in liver blood flow which could occur in patients with multiple degenerative diseases, receiving multiple prescribed medications—the subjects who form the large majority of patients over age 75 years (40).

A decline in liver volume and liver blood flow with aging may be a major component of age-related alterations in the liver, leading to the fall in clearance of many of the drugs whose pharmacokinetics have been found to be altered with age.

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