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Age-and gender-related variations of liver diffusion metrics apparent diffusion coefficient (ADC) and diffusion derived vessel density (DDVD), and explanations with the known physiological T2 relaxation time variations among different volunteers' groups

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Abstract

Background Age-related liver diffusion metrics changes have been described. We aim to further clarify these questions: 1) whether an age-related reduction of liver perfusion can be observed by DDVD (diffusion derived vessel density) in older males; 2) whether there is a male female difference in liver perfusion; 3) whether liver ADC values and spleen ADC values are correlated. It is known that, physiologically, males' liver has a higher iron level (thus a shorter T2) than females' liver; pre-menopausal females have a lower liver iron level (thus a longer T2) than post-menopausal females. The observations of this study will be interpreted with the recently gained knowledge of the T2 contribution to diffusion metrics.

Methods Included in this healthy volunteer's study were 68 males (mean age:50.22 years, range: 25–70 years) and 43 females (mean age 45.56 years, range:20–71 years). DWI images with *b*-values of 0, 2, 10, 20, 60, and 600 s/mm^2 were acquired at 1.5T. DDVD were calculated with $b=0$, $b=2$, $b=10$, and $b=20$ s/mm^2 images. ADC were calculated with $b=0$, $b=2$, $b=60$ and $b=600$ s/mm^2 images.

Results There was a statistically significant age-related decline of liver DDVD values for females ($p=0.024$). A similar trend was observed for males, though statistical significance was not achieved ($p=0.113$). Liver DDVD values were all higher in females than in males ($p<0.001$). There was a statistically significant age-related decline of liver ADC values both for males ($ADC_{(b0b600)}$, $p=0.009$) and for females ($ADC_{(b0b600)}$, $p=0.016$). Liver ADC values and spleen ADC values were positively correlated ($ADC_{(b0b600)}$, $r=0.33$ for males and 0.31 for females, $p<0.05$). When the spleen ADC

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was used to normalize the liver ADC, then the age-related trend was largely removed, both for males and for females ($p > 0.05$).

Conclusion Females have a larger liver perfusion volume than males. There is an age-related decrease of DDVD and ADC, both for males and females. Liver ADC values and spleen ADC values are positively correlated. These gender and age-related changes are unlikely mainly caused by the liver T2 relaxation time variations.

Clinical trial number Not applicable.

Keywords Aging, Diffusion-weighted imaging, Liver, Apparent diffusion coefficient, Diffusion derived vessel density

Introduction

Diffusion weighted imaging (DWI) has been commonly applied to the liver and to the spleen, particularly in the oncological setting. For quantification, ADC (apparent diffusion coefficient) is already widely implemented, while IVIM (intravoxel incoherent motion) imaging remains in research setting [1–5]. Another emerging useful DWI biomarker is 'diffusion derived vessel density' (DDVD) [6–9]. Diffusion measurement from a healthy spleen is sometimes used to normalize the measures of other abdomen organs such as the liver and pancreas [10, 11]. Understanding the gender- and age-related normative values of diffusion metrics is highly relevant for liver imaging. Huang et al. [12] reported that there was aged-related reduction of liver DDVD among females, but such a trend was not observed in males, probably due to the small sample size for males in that study. There was no age-related change of spleen DDVD both in males and in females [7, 13]. Spleen ADC has also been shown to be lower among older females than among younger females, but such a trend was not noted among males [13]. A few studies described that liver ADC and liver IVIM-D_{slow} were lower among males than females [12, 14, 15].

The liver and spleen both function as iron storage organs. There are age and gender differences in normative values of liver and spleen T2/T1rho relaxometry [16]. Schwenzer et al. [17] reported a negative correlation between age and liver T2* in females (r=-0.46) and in males (r=-0.30). Liver T2* and spleen T2* were highly correlated (r=-0.73). Yu et al. [18] reported an age-related decrease of spleen T1rho for both females and males. This trend was consistent with the T1rho of females' liver. Females also had higher liver and spleen T1rho values than males. Spleen T1rho and liver T1rho were positively correlated, and when spleen T1rho was used to normalize liver T1rho, the ratio of $T1rho_{liver}/T1rho_{spleen}$ largely removed the sex- and age-effect [18].

Considering the existing literature on age-and gender-related variations of liver MR relaxometry and diffusion metrics, in this study we aim to further clarify these questions: 1) whether an age-related reduction of liver perfusion can be observed by DDVD in older males; 2) whether there is a male female difference in liver DDVD values; 3) how the selection of b-value will affect the age- and gender-related liver ADC difference; 4) whether liver ADC values and spleen ADC values are correlated. The observations of this study will be interpreted with the recently gained knowledge of T2 contribution to diffusion metrics [19–22]. Recent studies suggest that, in addition to 'true diffusion', both ADC and IVIM parameters are also heavily affected by tissue T2 relaxation times [19–21]. A shorter T2 (< 60 ms) or a longer TE (echo time) or a longer diffusion time (an increase of the diffusion gradient separation time) increases the measure of a tissue's fast diffusion compartment (i.e., conceptually the perfusion compartment according to IVIM model) and depresses the measure of tissue's slow diffusion compartment [22–27], while a longer T2 (> 80 ms) increases the measure of tissue's slow diffusion compartment and depresses the measure of fast diffusion compartment [21, 22, 28–31].

Materials and methods

The healthy volunteer upper abdomen MRI data acquisition as observational studies was approved by the institutional ethical committee, and informed consent was obtained for all subjects. All the study participants were all known to be healthy at the MRI exam and at the 6-month follow-up after the exam, without liver, spleen, and other abdominal organ disease history, and not on any regular medication. MRI was conducted from Apr 22, 2019 to Dec. 6, 2022. For all scans, participants were asked to fast for 6 h before imaging. Study subjects were scanned twice during the same session as long as the study subject could tolerate being within the magnet and lied still, with the subjects' position and selected scan planes unchanged. The IVIM type of diffusion scan was based on a single-shot spin-echo type echo-planar sequence using a 1.5-T magnet (Achieva, Philips Healthcare, Best, Netherlands). SPIR technique (Spectral Pre-saturation with Inversion-Recovery) was used for fat suppression. Image data were acquired with respiratory-gating. The TR was 1600ms and the TE was 63ms, with one TR per respiratory cycle. Other parameters included slice thickness = 7 mm and inter-slice gap 1 mm, matrix = 124 × 97, FOV = 375 mm×302 mm, NEX = 2, number of slices = 7. The included slices were focused on the central part of the liver with largest axial

parenchyma. IVIM series images with 16 *b*-values of 0, 2, 4, 7, 10, 15, 20, 30, 46, 60, 72, 100, 150, 200, 400, 600 s/mm^2 were acquired. The IVIM data acquisition time was ~6–7 min for one scan, depending on the respiration cycle of the subjects. Only images with *b*-values of 0, 2, 10, 20, 60, and 600 s/mm^2 were used in this study. For all acquired MRI data, images with notable motion and artefacts were discarded. For the participants scanned twice, when the two scans for a subject were of good quality, the mean values of the two scans were adopted; while when one scan had good quality and the other scan had unacceptable quality, then the one scan with unacceptable quality was discarded and the scan with good quality was used. The data of 8 females and 5 males were excluded as both of their two scans had unacceptable quality. Finally included for analysis in this study were 68 males (mean age: 50.22 years, range: 25–70 years, among them 44 had two scans for analysis) and 43 females (45.56 years, range: 20–71 years, years, among them 26 had two scans for analysis). The original MRI data have been partially analysed and published earlier (Table 1). For these data, the current study is the first for liver ADC analysis and for the liver ADC and spleen ADC correlation analysis. For liver DDVD, the sample size for males increased from $n = 33$ in the study of Huang et al. [12]. to the current study of $n = 68$.

Image segmentation was performed using ITK-SNAP (http://www.itksnap.org) and data analysis was conducte d with MATLAB (MathWorks, Natick, MA, USA). By a radiologist with 10 years' experience in reading abdominal MRI (MHS), free-hand ROIs (regions-of-interest) were manually placed on $b = 0$ s/mm^2 image to cover a large portion of liver and spleen parenchyma while avoiding large vessels and then copied to the images of other *b*-values of this slice. To count for the potential inter-scan motion, the copied ROI on $b = 2$, $b = 10$, $b = 20$, $b = 60$ and/or $b = 600$ s/mm^2 images were additionally manually adjusted. ADC was calculated according to.

$$ADC_{2b} = \frac{\ln(S(b_1)/S(b_2))}{b_2 - b_1} \quad (1)$$

where *b2* and *b1* refers to $b = 600$ and $b = 0$ (or = 2, or = 60) s/mm^2 respectively, where S(*b1*) and S(*b2*) denote the image signal-intensity acquired at the *b*-factor value of $b = 0$ (or = 2, or = 60) and $b = 600$ s/mm^2, respectively.

The measurement of liver and spleen DDVD followed our earlier reports [12, 13]. ROI for liver and spleen parenchyma was segmented on the $b = 0$ s/mm^2 image (resulting in ROI area of area0) and then copied onto the $b = 2$, $b = 10$, and $b = 20$ s/mm^2 images (resulting in ROI area of area2, area10, area20 respectively). To count for the potential inter-scan motion, the copied ROI on $b = 2$, $b = 10$, $b = 20$ images were additionally adjusted. DDVD was calculated according to Eq. 2.

$$\begin{aligned}\mathrm{DDVD\,(b0b2)} &= \mathrm{Sb0/ROIarea0} \\ &\quad - \mathrm{Sb2/ROIarea2\,(arbitraryunit/pixel)}\end{aligned} \quad (2)$$

where ROIarea0 and ROIarea2 refer to the number of pixels in the selected region-of-interest (ROI) on $b = 0$ s/mm^2 and $b = 2$ s/mm^2 images, respectively. Sb0 refers to the measured sum of liver signal intensity within the ROI when $b = 0$ s/mm^2, and Sb2 refers to the measured sum of liver signal intensity within the ROI when $b = 2$ s/mm^2, thus Sb/ROIarea equates to the mean signal intensity within the ROI. Sb2 and ROIarea2 were additionally replaced by other low *b*-value diffusion image data ($b = 10$ and $b = 20$ s/mm^2), which resulted in two additional DDVD values for $b = 10$ and $b = 20$ s/mm^2 respectively.

For all analysis, the mean of all included slice measurements was regarded as the value of the examination, with the last step weighted by the percentage ROI area for each slice (i.e., assuming the sum pixel number of all ROIs for each subject being 100%, according to pixel number in each slice's ROI, a percentage was assigned for each slice). For statistical analysis, data were processed using GraphPad Prism (San Diego, CA, USA). Comparisons were performed using independent 2 sample t test or Mann-Whitney U test as appropriate, and tests were all two-sided. The significances of diffusion measures and ages were tested with Pearson correlation. A p value < 0.05 was considered statistically significant, > 0.1 as not significant, and between 0.05 and 0.1 as with a trend of significance.

Results

The age-related changes of liver DDVD and ADC, male female differences of DDVD and ADC, and the correlations between liver ADC and spleen ADC, are shown in Table 2; Figs. 1, 2, 3 and 4.

Table 1 Liver diffusion weighted imaging data analysed in earlier studies [12, 13] and analysed newly in the current study

	Liver [12]#		Spleen [13]#			Liver [*]		Spleen [*]
	DDVD	IVIM	IVIM	DDVD	ADC	DDVD	ADC	ADC
Males, no. of cases'	33	26	48	65	69	68	68	68
Females, no. of cases	37	36	20	32	34	34	43	43

#: Image data used in the study reported by Huang et al. [12]. and Yu et al. [13]. *: analyses in the current study. Note that, all the results described in this study were based on measurements conducted in the current study

Table 2 Liver diffusion MRI measurements of health males and healthy females

		Values	Slope	*r*	P_{Slope}
Males($n=68$)	$DDVD_{b0b2}$ #	10.789 ± 3.778 (2.976–20.016) 95% CI:9.864–11.679	-0.067	-0.204	0.113
	$DDVD_{b0b10}$ #	20.482 ± 4.820 (10.785–32.891) 95% CI:19.379–21.640	-0.053	-0.129	0.318
	$DDVD_{b0b20}$ #	24.428 ± 5.383(13.060-37.372) 95% CI:23.203–25.740	-0.052	-0.110	0.394
	$ADC_{(b0b600)}$ ¶	1.389 ± 0.938(1.118–1.586) 95% CI:1.368–1.412	-0.003	-0.316	0.009
	$ADC_{(b2b600)}$ ¶	1.250 ± 0.078(1.05–1.40) 95% CI:1.231–1.268	-0.001	-0.135	0.273
	$ADC_{(b60b600)}$ ¶	1.013 ± 0.085(0.799–1.233) 95% CI:0.992–1.033	-0.001	-0.19	0.12
	$ADCr_{(b0b600)\ liver/spleen}$	1.471 ± 0.231(1.061–2.296) 95% CI:1.417–1.529	0.0002	0.013	0.915
	$ADCr_{(b2b600)\ liver/spleen}$	1.457 ± 0.247(1.022–2.401) 95% CI:1.398–1.522	0.001	0.052	0.676
	$ADCr_{(b60b600)\ liver/spleen}$	1.334 ± 0.340(0.853–3.517) 95% CI:1.263–1.425	0.006	0.201	0.100
Females ($n=43$)	$DDVD_{b0b2}$ #	15.608 ± 5.267(7.612–27.559) 95% CI:14.129–17.213	-0.123	-0.343	0.024
	$DDVD_{b0b10}$ #	25.141 ± 5.598(15.399–36.519) 95% CI:23.455–26.981	-0.127	-0.333	0.029
	$DDVD_{b0b20}$ #	30.330 ± 6.126(18.998–40.386) 95% CI:28.607–32.128	-0.144	-0.343	0.024
	$ADC_{(b0b600)}$ ¶	1.331 ± 0.884(1.143–1.545) 95% CI:1.303–1.356	-0.002	-0.364	0.016
	$ADC_{(b2b600)}$ ¶	1.186 ± 0.087(0.967–1.369) 95% CI:1.160–1.210	-0.002	-0.310	0.043
	$ADC_{(b60b600)}$ ¶	1.012 ± 0.091(0.818–1.214) 95% CI:0.987–1.037	-0.002	-0.356	0.019
	$ADCr_{(b0b600)\ liver/spleen}$	1.436 ± 0.152(1.089–1.694) 95% CI: 1.394–1.483	0.002	0.177	0.256
	$ADCr_{(b2b600)\ liver/spleen}$	1.375 ± 0.159(1.108–1.813) 95% CI:1.324–1.419	0.002	0.200	0.200
	$ADCr_{(b60b600)\ liver/spleen}$	1.266 ± 0.173(0.997–1.786) 95% CI:1.214–1.319	0.003	0.261	0.091

Value represented: mean ± standard deviation (range), 95% CI. *r*: Pearson correlation r value. P_{slope}: P-value for the fitted slope ($P_{slope} < 0.05$ suggests the slope of increasing or decreasing along age-axis is significant). $ADCr = ADC_{liver}/ADC_{spleen}$, #: unit in arbitrary unit/pixel, ¶: unit in $\times 10^{-3}$ mm^2 /s

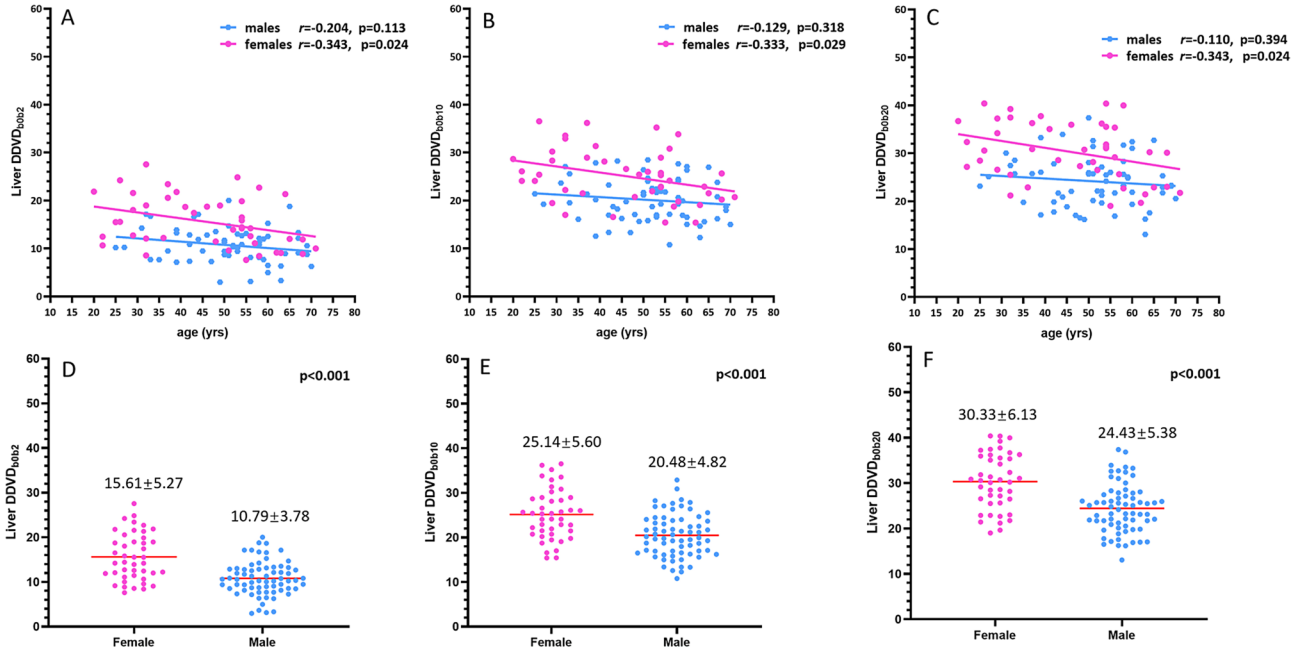

Fig. 1 A statistically significant age-related decline of liver DDVD value (unit in arbitrary unit/pixel) was noted for females ($p<0.05$). A similar trend was observed for males, though statistical significance was not achieved ($p=0.113$). DDVD values were higher in females than in males ($p<0.001$). $DDVD_{b0b2}$ was calculated with b = 0 and b = 2 s/mm^2 images, $DDVD_{b0b10}$ was calculated with b = 0 and b = 10 s/mm^2 images, $DDVD_{b0b20}$ was calculated with b = 0 and b = 20 s/mm^2 images

Figure 1A, B, C show, there was a statistically significant age-related decline of liver DDVD values for females. A similar trend was observed for males, though statistical significance was not achieved. For females, the Pearson *r* was comparable among $DDVD_{b0b2}$ (*r*=-0.343), $DDVD_{b0b10}$ (*r* = 0.333), and $DDVD_{b0b20}$ (*r* = 0.343); while for males, the correlation strength was $DDVD_{b0b2}$ (*r*=-0.240) > $DDVD_{b0b10}$ (=-0.129) > $DDVD_{b0b20}$ (*r* = -0.110). Fig. 1D, E, F, show $DDVD_{b0b2}$, $DDVDb_{0b10,}$ and $DDVD_{b0b20}$ values were all significantly higher in females than in males, suggesting significantly higher liver perfusion volume among females.

Figure 2 shows, there was a statistically significant age-related decline of liver ADC values both for males

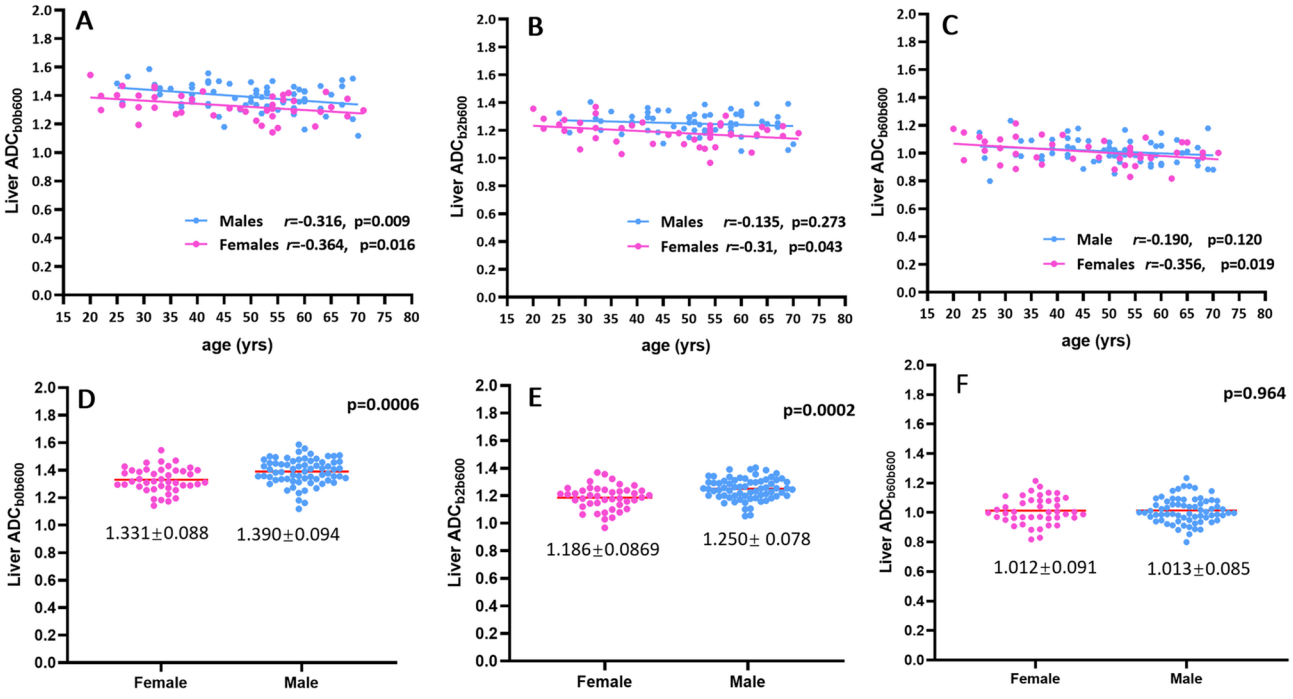

Fig. 2 A statistically significant age-related decline of liver ADC value (unit in $\times 10^{-3}$ mm^2/s) was noted both for males and females. Males had a higher value than females for $ADC_{(b0b600)}$ and $ACD_{(b2b600)}$. On the other hand, for $ADC_{(b60b600)}$, males and females derived very comparable ADC values. (note: the trends shown in this figure had been double confirmed by a second reader)

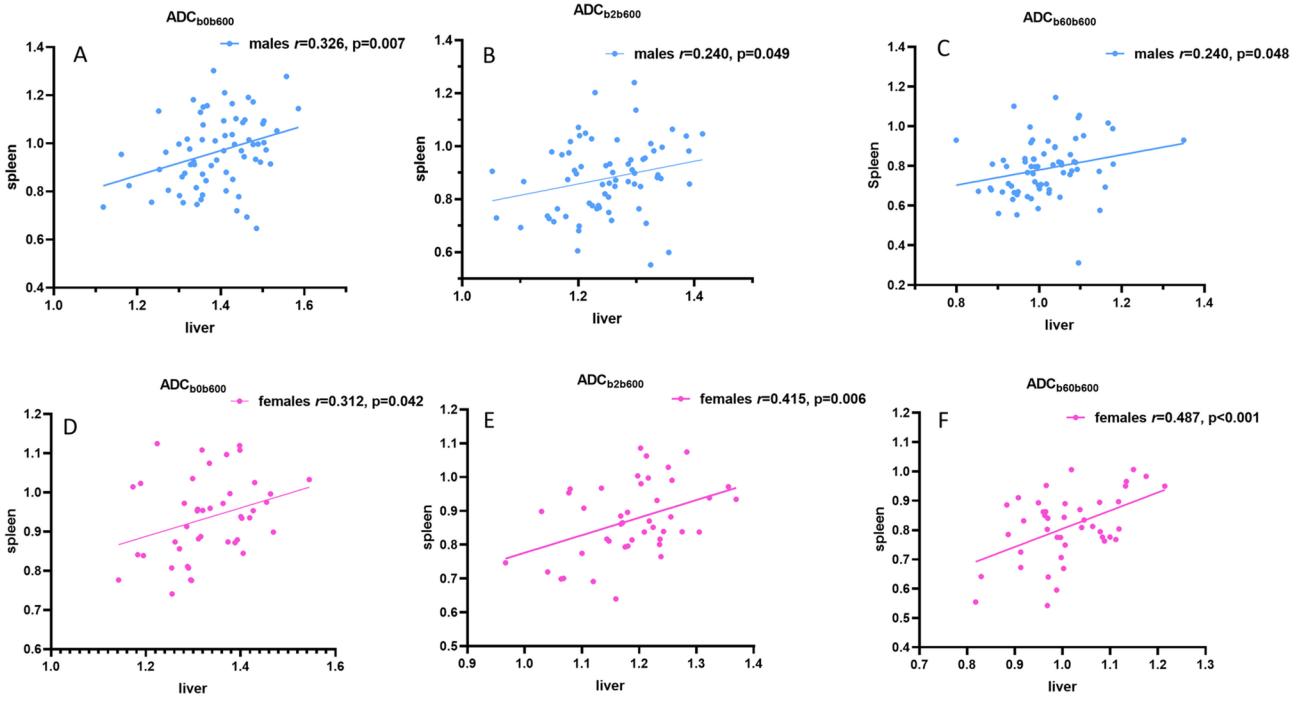

Fig. 3 Liver ADC values and spleen ADC values (unit in $\times 10^{-3}$ mm^2/s) were positively correlated, with the correlation tended being stronger for females than for males (**B**, **C**, **E**, **F**)

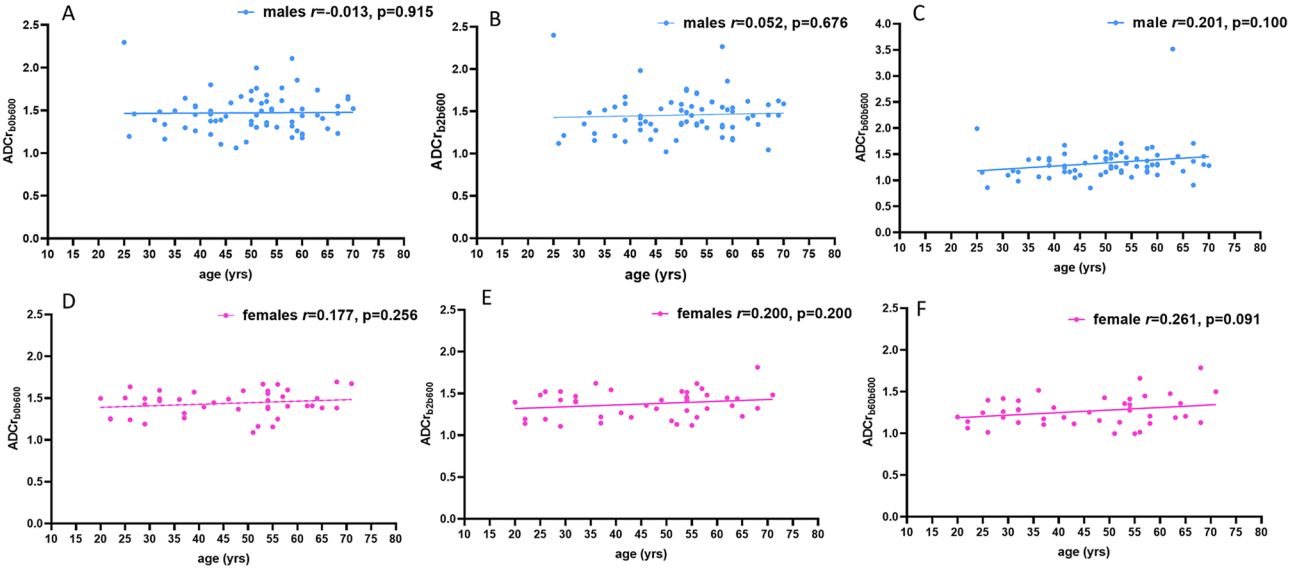

Fig. 4 When the spleen ADC was used to normalize the liver ADC (unit in $\times 10^{-3}$ mm^2/s), then the age-related trend was largely removed. For all ratios of ADC_{liver}/ADC_{spleen} of females (**D**, **E**, **F**) and the ratio of $ADC_{liver}/ADC_{spleen(b60b600)}$ for males (**C**), a weak trend was seen that ADC_{liver}/ADC_{spleen} was greater at the older age

(Fig. 2A) and for females (Fig. 2A, B, C). The correlation strength was comparable between males and females in Fig. 2A. Fig. 2D, E, F show males have a higher value than females for $ADC_{(b0b600)}$ and $ACD_{(b2b600)}$. On the other hand, for $ADC_{(b0b600)}$, males and females derived very comparable values.

Figure 3 shows that liver ADC values and spleen ADC values were positively correlated. Moreover, the correlations were stronger for females than for males. Fig. 4 shows, when the spleen ADC was used to normalize the liver ADC, then the age-related trend was largely removed, both for males and for females. For all ratios of ADC_{liver}/ADC_{spleen} of females (Fig. 4D, E, F) and the ratio of $ADC_{liver}/ADC_{spleen(b60b600)}$ for males (Fig. 4C), age was weakly positively correlated ADC_{liver}/ADC_{spleen}, i.e., a trend was seen that ADC_{liver}/ADC_{spleen} was greater at the older age.

Discussion

This study systematically evaluated gender- and age-related DDVD and ADC changes, and also compared $ADC_{(b0b600)}$, $ADC_{(b2b600)}$, and $ADC_{(b60b600)}$. $ADC_{(b0b600)}$ would have contained more perfusion information than $ADC_{(b60b600)}$. This study shows there was a statistically significant age-related decline of DDVD values for females, such a trend was also noted for males though statistical significance was not achieved. Liver DDVD was higher in females than in males and being more so among younger subjects. There was a statistically significant age-related decline of $ADC_{(b0b600)}$ values both for men and for women. Liver ADC values and spleen ADC values were positively correlated, and when the spleen ADC was used to normalize the liver ADC, then the age-related trend was largely removed. In this discussion, we try to explain what factors might have contributed to these age-related diffusion metrics changes, particularly with the known gender- and age-related T2 relaxation differences [17]. In healthy subjects there are iron storage depots in the liver, spleen, and bone marrow. In healthy subjects without diet iron deficiency, liver iron concentration rises sharply in men toward the end of the adolescent growth spurt in the late teens and reached maximum before 40 years old. In women, liver iron concentration remains relatively low until after the fourth decade of life, after which they exhibit a steep rise. Maximum levels observed in women after menopause are approximately two thirds of those for men of comparable age [32]. In addition to liver T2 shortening caused by the liver iron deposition [17, 33], since blood has longer T2 than that of liver parenchyma, a lower blood volume in males' liver also contributes to the shorter T2 in males' liver (see discussion in [21] and the paragraph below). Liver T1rho, which is related to T2, is notably longer in females than in males [34]. It has been noted that shorter T2 values correlate to higher ADC values in the liver [21, 35]. Physiological age-related decrease in liver blood flow has been well documented using a variety of technical methods including histology, dye dilution, and indicator clearance [36, 37]. In our earlier analysis, we demonstrated the DDVD of females' liver decrease with aging, however, no such a trend was noted for the DDVD of males' liver [12]. In the current study, with increased sample size, a trend weaker than that of females was shown that DDVD of males' liver also decreased with aging. Though we still could not

achieve statistical significance in this study, the trend for $DDVDb_{b020}$ was close to being significant ($p = 0.113$). However, for male's liver $DDVDb_{b020}$, the decrease with aging further weakened (Pearson r=-0.204 for $DDVD_{b0b2}$, r=-0.129 $DDVD_{b0b10}$, r=-0.110 $DDVD_{b0b20}$). This supports the concept that, applying a very low second b-value for DDVD calculation increases the sensitivity for measuring tissue perfusion by DDVD [8, 31]. Following the increase of second b-value for DDVD calculation, the contribution from diffusion and T2 effect also increase [38]. The age-related DDVD reduction was stronger for females, which could be due to that females have a menopause which has a fundamental impact on physiology. Estrogen has an effect on vascular smooth muscle and endothelial cells, with estrogen administration promoting vasodilatation [39]. Liver DDVD measure for females were larger than those of males (Fig. 1). This is also consistent with our earlier report that spleen DDVD measures for females were larger than those of males [7]. On the aspect of gender difference in perfusion volume, with catheter an indocyanine green infusion technique, Møller et al. [40] described that whole body total blood volume (ml) and the cardiac output (ml/min) per kg lean body mass were higher in healthy females than in healthy males. More recently, Nickander et al. [41] reported that healthy females have higher myocardial perfusion, blood volume and extracellular volume in the heart compared to males. However, as shown in Fig. 1, the male female difference is likely to diminish for very old populations. Note that, when TR/TE is 1500/60 ms (with respiration-triggered imaging) and the second b-value is 2 s/mm^2, the T2 effect is unlikely to cause major over- or under-estimation for liver DDVD [42]. If we consider that younger men have a shorter liver T2 than younger women, then the T2 difference between liver and spleen T2 values may slightly depress the DDVD difference between the gender groups, as shorter T2 promote higher perfusion measure [20, 21]. Similarly, the age-related differences in liver DDVD are also unlikely to be caused by the T2 effect. Actually, the T2 effect may depress age-related difference in DDVD, as shorter T2 among older population may promote the DWI-derived perfusion measure [24, 28].

According to IVIM theory, $ADC_{(b0b600)}$ is a composite measure of both perfusion compartment and slow diffusion compartment, and $ADC_{(b60b600)}$ more reflects diffusion compartment [21]. Since males have a shorter liver T2 than females, and shorter T2 can promote perfusion measure [21, 43], this explains that Fig. 2 shows $ADC_{(b0b600)}$ was higher in males than females, and the male female difference diminished for $ADC_{(b60b600)}$. We have earlier reported lower IVIM-D_{slow} in men than in women [12]. Lavdas et al. [14] also reported slightly lower ADC in females when $b = 0$ s/mm^2 was incorporated for ADC calculation, while ADC is lower in males than in females when the first b-value for ADC calculation was 150 s/mm^2. Metens et al. [15]. used the first b-value of 150 s/mm^2 to calculate liver ADC and reported lower ADC in males than in females. Note that, when the first b-value is 150 s/mm^2 to calculate liver ADC, ADC is effectively equivalent to IVIM-D_{slow}. Therefore, the recently gained knowledge of T2's contribution to ADC may help to explain the initially puzzling results for male female difference in liver ADC. Since the shorter T2 in older subjects' liver and spleen would promote ADC [21], the age-related decrease of liver ADC is unlikely due to T2 effect. T1rho relaxometry studies also showed lower liver and spleen T1rho among older subjects which may not relate to the iron level factor [18, 34], reflecting biochemical changes among the older subjects. True vessel volume reduction as shown by histology studies and DDVD measures will at least partially contribute to the age-related reduction of ADC [12, 36, 37].

The study on the correlation between spleen ADC and liver ADC was motivated by our recent observation that spleen T1rho and liver T1rho were positively correlated, and when spleen T1rho was used to normalize liver T1rho, the ratio of $T1rho_{liver}/T1rho_{spleen}$ largely removed the gender- and age-effect [18]. The current study confirmed that spleen ADC and liver ADC are positively correlated (Fig. 3). It has been reported that the liver iron level and spleen iron level are positively correlated. Schwenzer et al. [17] reported liver T2* and spleen T2* had a strong correlation with $r = 0.73$. The correlation between spleen T1rho and liver T1rho was around $r = 0.6$, being modestly strong. While earlier work showed age-related deductions of both liver T2* and T1rho measures [17, 34], the age-related reduction of T1rho may be independent of the age-related reduction of T2* associated with iron level increasing [32]. In another study, a modestly strong correlation was noted for liver DDVD and spleen DDVD (r = around 0.6 [7]). The current study shows the correlation of liver ADC and spleen ACD was weaker than those of T2*/T1rho relaxometry and DDVD correlation. This study also shows, for females' ADC (Fig. 4D, E, F) and for males' $ADC_{(b60b600)}$ (Fig. 4C), the age-related reduction changed to a slight increasing trend after liver ADC is normalized by spleen ADC, which in effective suggests that age-related ADC reduction was faster for spleen ADC than for liver ADC. Since it was noted that there was an age-related reduction of liver DDVD but an age-related reduction of spleen DDVD was not observed [13, 14], thus for the spleen, the age-related reductions of both $ADC_{(b0b600)}$ and $ADC_{(b60b600)}$ are dominated by slow diffusion reduction. $ADC_{(b0b600)}$ contained information with perfusion and slow diffusion, and $ADC_{(b60b600)}$ is dominated by slow diffusion. Note that the correlation between liver ADC and spleen ADC was stronger in females than in males. This is not surprising,

as we noted higher heterogenicity in males' spleen ADC than females' spleen ADC [13].

There are many limitations to this study. It can be argued that the sample size in this study was small. However, we expect that while more samples may lead to some of the trends becoming statistically more significant, it is unlikely the trend directions will be altered. In this study, to explain the relative contribution of perfusion and slow diffusion, we presented $DDVD_{b0b20}$. However, $DDVD_{b0b20}$ is not advocated as we use a very low second *b*-value to measure perfusion information [38]. On the other hand, we did not use even higher first *b*-value, such as $b = 150$ s/mm^2, to calculate ADC, but we consider that the trends have already been demonstrated by a comparison between ADC_{b0b600} data and $ADC_{b60b600}$ data. The data were collected at 1.5T. Compared with the measures at 1.5T, 3.0T is associated with a lower measure of slow diffusion and a higher measure of perfusion [27, 44]. Liver ADC value is lower at 3.0T than at 1.5 T [45, 46]. Absolute liver and spleen DDVD values are substantially higher at 3.0T than at 1.5T, however the ratio of $DDVD_{liver}/DDVD_{spleen}$ is only slightly higher at 3.0T than at 1.5T [31, 42]. Another limitation is that we do not have data for pediatric and adolescent populations. Though we did not provide intra- and inter-observer variability data, we do not expect this to be an issue as the ROI placements are reasonably straightforward. Moreover, we did have an additional reader measured the data shown in Fig. 2 and derived same trends.

To summarize, this study shows there is an age-related decrease of liver DDVD both for males and females. Liver DDVD values for females are larger than those of males. However, the male female difference in liver perfusion volume is likely to decrease for very old populations. There is an age-related decrease of ADC values both for males and for females. Liver ADC values and spleen ADC values are positively correlated. We suggest that these variations are unlikely mainly caused by the T2 effect associated with liver iron level variations. In fact, if the T2 effect could be eliminated, the gender- and age-related changes of liver diffusion metrics would be more pronounced in many aspects. We expect the findings of this study will be relevant for diffusion metrics as biomarkers of liver diseases classification, as well as for understanding physiology related to gender differences and aging for the liver.

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Author contributions
Contributions: (I) Conception and design: YXJ Wáng (II) Administrative support: MH Sun, YXJ Wáng (III) Provision of study materials or patients: YY Deng, H Huang, (IV) Collection and assembly of data: YY Deng, H Huang, (V) Data analysis and interpretation: YY Deng, MH Sun, YXJ Wáng (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Data availability
The data used in the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval was granted by the Ethics Committee of the Third People's Hospital of Shenzhen, and all participants provided informed consent. All methods were carried out according to relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Taouli B, Koh DM. Diffusion-weighted MR imaging of the liver. Radiology. 2010;254:47–66.
2. Wang YX, Wang X, Wu P, Wang Y, Chen W, Chen H, Li J. Topics on quantitative liver magnetic resonance imaging. Quant Imaging Med Surg. 2019;9:1840–90.
3. Jang KM, Kim SH, Hwang J, Lee SJ, Kang TW, Lee MW, Choi D. Differentiation of malignant from benign focal Splenic lesions: added value of diffusion-weighted MRI. AJR Am J Roentgenol. 2014;203:803–12.
4. Wang YX, Huang H, Zheng CJ, Xiao BH, Chevallier O, Wang W. Diffusion-weighted MRI of the liver: challenges and some solutions for the quantification of apparent diffusion coefficient and intravoxel incoherent motion. Am J Nucl Med Mol Imaging. 2021;11:107–42.
5. Bian W, Huang Q, Zhang J, Li J, Song X, Cui S, Zheng Q, Niu J. Intravoxel incoherent motion diffusion-weighted MRI for the evaluation of early spleen involvement in acute leukemia. Quant Imaging Med Surg. 2024;14:98–110.
6. Xiao BH, Huang H, Wang LF, Qiu SW, Guo SW, Wáng YXJ. Diffusion MRI derived per area vessel density as a surrogate biomarker for detecting viral hepatitis B-Induced liver fibrosis: A Proof-of-Concept study. SLAS Technol. 2020;25:474–83.
7. Zheng CJ, Huang H, Xiao BH, Li T, Wang W, Wáng YXJ. Spleen in viral Hepatitis-B liver fibrosis patients May have a reduced level of per unit micro-circulation: non-invasive diffusion MRI evidence with a surrogate marker. SLAS Technol. 2022;27:187–94.
8. Li XM, Yao DQ, Quan XY, Li M, Chen W, Wáng YXJ. Perfusion of hepatocellular carcinomas measured by diffusion-derived vessel density biomarker: higher hepatocellular carcinoma perfusion than earlier intravoxel incoherent motion reports. NMR Biomed. 2024;37:e5125.
9. Ni CX, Lin RL, Yao DQ, Ma FZ, Shi YT, 5, He YY, Song Y, Yang G, Wáng RF. YXJ. Magnetic resonance diffusion-derived vessel density (DDVD) as a valuable tissue perfusion biomarker for Isocitrate Dehydrogenase genotyping in diffuse gliomas. BMC Medical Imaging, 2025;25:79.
10. Do RK, Chandarana H, Felker E, Hajdu CH, Babb JS, Kim D, Taouli B. Diagnosis of liver fibrosis and cirrhosis with diffusion-weighted imaging: value of

normalized apparent diffusion coefficient using the spleen as reference organ. AJR Am J Roentgenol. 2010;195:671–6.
11. Song JS, Kwak HS, Byon JH, Jin GY. Diffusion-weighted MR imaging of upper abdominal organs at different time points: apparent diffusion coefficient normalization using a reference organ. J Magn Reson Imaging. 2017;45:1494–501.
12. Huang H, Zheng CJ, Wang LF, Che-Nordin N, Wáng YXJ. Age and gender dependence of liver diffusion parameters and the possibility that intravoxel incoherent motion modelling of perfusion component is constrained by diffusion component. NMR Biomed. 2021;34:e4449.
13. Yu WL, Ma FZ, Huang H, Xiao BH, Li XM, Wáng YXJ. Age and gender differences of normative values of spleen diffusion MRI parameters. Rofo. 2025;197:535–45.
14. Lavdas I, Rockall AG, Castelli F, Sandhu RS, Papadaki A, Honeyfield L, Waldman AD, Aboagye EO. Apparent diffusion coefficient of normal abdominal organs and bone marrow from Whole-Body DWI at 1.5 T: the effect of sex and age. AJR Am J Roentgenol. 2015;205:242–50.
15. Metens T, Ferraresi KF, Farchione A, Moreno C, Bali MA, Matos C. Normal hepatic parenchyma visibility and ADC quantification on diffusion-weighted MRI at 3 T: influence of age, gender, and iron content. Eur Radiol. 2014;24:3123–33.
16. Wáng YXJ. Gender-specific liver aging and magnetic resonance imaging. Quant Imaging Med Surg. 2021;11:2893–904.
17. Schwenzer NF, Machann J, Haap MM, Martirosian P, Schraml C, Liebig G, Stefan N, Häring HU, Claussen CD, Fritsche A, Schick F. T2* relaxometry in liver, pancreas, and spleen in a healthy cohort of one hundred twenty-nine subjects-correlation with age, gender, and serum ferritin. Invest Radiol. 2008;43:854–60.
18. Wáng YXJ, Yu WL, Deng M. Age- and sex-related variations of normal spleen T1rho and the more stable liver T1rho to spleen T1rho ratio. Rofo. 2024 Oct 22. English. https://doi.org/10.1055/a-2428-7409
19. Wáng YXJ, Aparisi Gómez MP, Ruiz Santiago F, Bazzocchi A. The relevance of T2 relaxation time in interpreting MRI apparent diffusion coefficient (ADC) map for musculoskeletal structures. Quant Imaging Med Surg. 2023;13:7657–66.
20. Xiao BH, Wáng YXJ. Different tissue types display different signal intensities on b = 0 images and the implications of this for intravoxel incoherent motion analysis: examples from liver MRI. NMR Biomed. 2021;34:e4522.
21. Wáng YXJ. An explanation for the triphasic dependency of apparent diffusion coefficient (ADC) on T2 relaxation time: the multiple T2 compartments model. Quant Imaging Med Surg. 2025;15:3779–91.
22. Lemke A, Laun FB, Simon D, Stieltjes B, Schad LR. An in vivo verification of the intravoxel incoherent motion effect in diffusion-weighted imaging of the abdomen. Magn Reson Med. 2010;64:1580–5.
23. Jerome NP, d'Arcy JA, Feiweier T, Koh DM, Leach MO, Collins DJ, Orton MR. Extended T2-IVIM model for correction of TE dependence of pseudo-diffusion volume fraction in clinical diffusion-weighted magnetic resonance imaging. Phys Med Biol. 2016;61:N667–80.
24. Führes T, Riexinger AJ, Loh M, Martin J, Wetscherek A, Kuder TA, Uder M, Hensel B, Laun FB. Echo time dependence of biexponential and triexponential intravoxel incoherent motion parameters in the liver. Magn Reson Med. 2022;87:859–71.
25. Iima M, Nobashi T, Imai H, Koyasu S, Saga T, Nakamoto Y, Kataoka M, Yamamoto A, Matsuda T, Togashi K. Effects of diffusion time on non-Gaussian diffusion and intravoxel incoherent motion (IVIM) MRI parameters in breast cancer and hepatocellular carcinoma xenograft models. Acta Radiol Open. 2018;7:2058460117751565.
26. Wu D, Zhang J. Evidence of the diffusion time dependence of intravoxel incoherent motion in the brain. Magn Reson Med. 2019;82:2225–35.
27. Riexinger AJ, Martin J, Rauh S, Wetscherek A, Pistel M, Kuder TA, Nagel AM, Uder M, Hensel B, Müller L, Laun FB. On the field strength dependence of Bi- and triexponential intravoxel incoherent motion (IVIM) parameters in the liver. J Magn Reson Imaging. 2019;50:1883–92.
28. Ma FZ, Wáng YXJ. T2 relaxation time elongation of hepatocellular carcinoma relative to native liver tissue leads to an underestimation of perfusion fraction measured by standard intravoxel incoherent motion magnetic resonance imaging. Quant Imaging Med Surg. 2024;14:1316–22.
29. Mazaheri Y, Hötker AM, Shukla-Dave A, Akin O, Hricak H. Effect of intravascular contrast agent on diffusion and perfusion fraction coefficients in the peripheral zone and prostate cancer. Magn Reson Imaging. 2018;51:120–7.
30. Stabinska J, Thiel TA, Zöllner HJ, Benkert T, Wittsack HJ, Ljimani A. Investigation of diffusion time dependence of apparent diffusion coefficient and intravoxel incoherent motion parameters in the human kidney. Magn Reson Med. 2025;93:2020–8.
31. Ju ZG, Leng XM, Xiao BX, Sun MH, Huang H, Hu GW, Zhang G, Sun JH, Zhu MSY, Guglielmi G, Wáng YXJ. Influences of the second motion probing gradient b-value and T2 relaxation time on magnetic resonance diffusion derived 'vessel density' (DDVD) calculation: the examples of liver, spleen, and liver simple cyst. Quant Imaging Med Surg. 2025;15:74–8.
32. Wáng YXJ. Physiological variation of liver iron concentration May not be dominantly responsible for the liver T1rho variations associated with age and gender. Quant Imaging Med Surg. 2021;11:1668–73.
33. Liu CY, Noda C, van der Geest RJ, Triaire B, Kassai Y, Bluemke DA, Lima JAC. Sex-specific associations in multiparametric 3 T MRI measurements in adult livers. Abdom Radiol (NY). 2023;48:3072–8.
34. Wáng YXJ, Deng M, Lin J, Kwok AWL, Liu EKW, Chen W. Age- and Gender-Associated liver physiological T1rho dynamics demonstrated with a clinically applicable Single-Breathhold acquisition. SLAS Technol. 2018;23:179–87.
35. Wáng YXJ, Ma FZ. A tri-phasic relationship between T2 relaxation time and magnetic resonance imaging (MRI)-derived apparent diffusion coefficient (ADC). Quant Imaging Med Surg. 2023;13:8873–80.
36. Wynne H, Cope L, Mutch E, Rawlins M, Woodhouse K, James OFW. The effect of age upon liver volume and apparent liver blood flow in healthy man. Hepatology. 1989;9:297–301.
37. Fiel MI, Deniz K, Elmali F, Schiano TD. Increasing hepatic arteriole wall thickness and decreased luminal diameter occur with increasing age in normal livers. J Hepatol. 2011;55:582–6.
38. Ma FZ, Xiao BH, Wáng YXJ. MRI signal simulation of liver DDVD (diffusion derived 'vessel density') with multiple compartments diffusion model. Quant Imaging Med Surg. 2025;15:1710–8.
39. Tostes RC, Nigro D, Fortes ZB, Carvalho MH. Effects of Estrogen on the vascular system. Braz J Med Biol Res. 2003;36:1143–58.
40. Møller S, Henriksen JH, Bendtsen F. Central and noncentral blood volumes in cirrhosis: relationship to anthropometrics and gender. Am J Physiol Gastrointest Liver Physiol. 2003;284:G970–9.
41. Nickander J, Themudo R, Sigfridsson A, Xue H, Kellman P, Ugander M. Females have higher myocardial perfusion, blood volume and extracellular volume compared to males - an adenosine stress cardiovascular magnetic resonance study. Sci Rep. 2020;10:10380.
42. Wáng YXJ, Li CY, Yao DQ, Tang SN, Xiao BH. The impacts of time of echo (TE) and time of repetition (TR) on DDVD (diffusion-derived 'vessel density') measurement: examples of liver, spleen, and hepatocellular carcinoma. Quant Imaging Med Surg. 2025;15:3771–8.
43. Wáng YXJ. Mutual constraining of slow component and fast component measures: some observations in liver IVIM imaging. Quant Imaging Med Surg. 2021;11:2879–87.
44. Li YT, Cercueil JP, Yuan J, Chen W, Loffroy R, Wáng YX. Liver intravoxel incoherent motion (IVIM) magnetic resonance imaging: a comprehensive review of published data on normal values and applications for fibrosis and tumor evaluation. Quant Imaging Med Surg. 2017;7:59–78.
45. Cui Y, Dyvorne H, Besa C, Cooper N, Taouli B. IVIM Diffusion-weighted imaging of the liver at 3.0T: comparison with 1.5T. Eur J Radiol Open. 2015;2:123–8.
46. Zhu J, Zhang J, Gao JY, Li JN, Yang DW, Chen M, Zhou C, Yang ZH. Apparent diffusion coefficient normalization of normal liver: will it improve the reproducibility of diffusion-weighted imaging at different MR scanners as a new biomarker? Med (Baltim). 2017;96:e5910.

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