

# Association of total testosterone, free testosterone, bioavailable testosterone and sex hormone-binding globulin with hepatic steatosis and the ratio of aspartate aminotransferase to alanine aminotransferase

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**Abstract.** Several articles have shown the inverse association between total testosterone (TT) or sex hormone-binding globulin (SHBG) and hepatic steatosis. No articles report associations of TT, SHBG, free testosterone (FT), and bioavailable testosterone (BioT) with aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratios. Therefore, we investigated the associations of TT, FT, BioT and SHBG with hepatic steatosis and AST/ALT ratios. A total of 218 men were enrolled. We diagnosed hepatic steatosis by ultrasound. TT and SHBG showed a reduced risk for hepatic steatosis when analyzed with or without adjusting for age, smoking, alcohol consumption and physical activity. Compared with the lowest quartile, the ORs for hepatic steatosis in the third and fourth quartiles (0.32 [95% CI: 0.14–0.75] and 0.27 [95% CI: 0.10–0.73], respectively) of SHBG were significantly lower after adjustments. The OR for hepatic steatosis in the fourth quartile of TT (0.41 [95% CI: 0.17–0.95]) was significantly lower than in the lowest quartile after adjustments. The mean AST/ALT ratios in men with hepatic steatosis were lower than those without hepatic steatosis (0.83 and 1.04, respectively), due to the elevated ALT levels in hepatic steatosis groups. Furthermore, TT and SHBG were positively associated with AST/ALT ratios with and without adjustments. In conclusion, higher TT and SHBG levels in men are associated with the reduced risk of hepatic steatosis and elevated AST/ALT ratios, independent of age, smoking, alcohol consumption and physical activity.

**Key words:** Sex hormone-binding globulin, Total testosterone, Hepatic steatosis, Alanine aminotransferase

**HEPATIC STEATOSIS**, or fatty liver, is characterized by massive accumulation of lipids in hepatocytes [1]. Nonalcoholic fatty liver disease (NAFLD) refers to the accumulation of excess lipids in the liver that is not caused by excessive use of alcohol, and may progress from steatosis to steatohepatitis and cirrhosis [2, 3]. Sex hormone-binding globulin (SHBG) is primarily synthesized and secreted by the liver into the bloodstream

where it binds sex steroids and regulates their bioavailability at tissue levels [4]. Therefore, it is reasonable to speculate that hepatic production of SHBG and subsequent serum levels may be influenced by hepatic steatosis. SHBG overexpression in hepatic steatosis models decreases liver fat accumulation [5]. It has also been reported that increased intrahepatic fat content is associated with decreased SHBG levels [6]. Several epidemiological studies have highlighted the relationship between low SHBG levels and hepatic steatosis. Higher SHBG levels are strongly associated with decreases in liver fat [7]. In a cohort of subjects with diabetes mellitus, the degree of NAFLD also shows a negative association with SHBG levels [8, 9]. Furthermore, a significant associa-

Submitted Mar. 2, 2018; Accepted May 19, 2018 as EJ18-0095

Released online in J-STAGE as advance publication Jun. 16, 2018

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tion has been observed between hepatic fat accumulation and circulating SHBG levels in men and premenopausal women [10].

Several studies have highlighted the association of testosterone and hepatic steatosis. Testosterone may affect key regulatory lipogenic enzymes to protect against hepatic steatosis in mice [11]. Hepatic steatosis occurs in castrated rats fed a high fat/low carbohydrate diet, which suggests that testosterone deficiency may contribute to the severity of hepatic steatosis [12], and testosterone treatment for 18 weeks has been shown to reduce liver fat in obese men with obstructive sleep apnea [13]. In addition, studies have shown that testosterone suppresses the development of hepatic steatosis [14, 15], and that testosterone protects against NAFLD [16, 17]. Clinically, low serum total testosterone (TT) levels are independently associated with NAFLD [18]. In contrast, one study has reported that testosterone promotes NAFLD development [19].

NAFLD is a common cause of chronic liver disease. It is characterized by asymptomatic, mild elevations of the serum liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [20]. Subjects with NAFLD usually had an AST to ALT ratio of less than 1:1 [21, 22]. No articles reported association of TT, free testosterone (FT), bioavailable testosterone (BioT), and SHBG with hepatic steatosis and the ratio of AST/ALT. We enrolled 218 men to detect the serum levels of TT and SHBG and calculated the levels of FT and BioT. Hepatic steatosis was diagnosed using abdominal ultrasonography by experienced sonographers. Liver enzymes ALT and AST were recorded at the hospital. The associations of TT, FT, BioT, and SHBG with hepatic steatosis and the ratio of AST/ALT were investigated.

## Materials and Methods

### Subjects

This study was conducted in the Second Affiliated Hospital of Shantou University Medical College. In total, 218 male participants (ages ranged from 38 to 80 years), who underwent a health physical between August 2015 and May 2016 and who agreed to participate in the study, were recruited. This study was approved by the Ethical Committee of Shantou University Medical College and by the Ethics Committee of the Second Affiliated Hospital of Shantou University Medical College. Written informed consent was obtained from all participants. Trial registration number is SUMC-2015-26.

Anthropometric characteristics (height and weight), ALT, AST and albumin were recorded at the Second Affiliated Hospital. Weight and height were used to calculate body mass index (BMI; kg/m<sup>2</sup>). Waist circumference was measured at the umbilicus level while the subject was in a standing position. Information on life-style factors (smoking, alcohol consumption and physical activity) was assessed with questionnaires. Physical activity levels were obtained by asking the participants about their average frequency of physical activity: rarely/never, 1 to 3 times per month, 1 to 2 times per week, and more than 3 times per week [23]. Smoking status was classified as never, former, or current [23]. Alcohol drinking status included never and current drinker [24]. Of the 218 male participants, the numbers of subjects with and without hepatic steatosis were 86 and 132, respectively. Among the 86 hepatic steatosis subjects, 29 subjects were current drinkers. Alcohol consumption of these subjects was less than 30 g/day through communication by phone. Therefore, the 86 hepatic steatosis subjects were identified as NAFLD.

### Serum measurements of sex hormones

Testosterone is mainly classified into three fractions in the blood circulation. About half of circulating testosterone is bound to SHBG, and another half to albumin. Only 0.5%–3% of the testosterone remains in the free, non-protein-bound form, and is referred to as FT [25]. FT and albumin-binding testosterone can be readily used by the tissues of the body and are defined as BioT.

Fasting blood samples were collected between 08:00 and 10:00 h. Blood samples were centrifuged and aliquots of serum were frozen at –80°C until analysis. Serum TT and SHBG levels were measured by chemiluminescent immunoassay (Beckman Coulter Inc., CA, USA) using a Beckman DXI 800 Analysis System (Fullerton, CA, USA). The levels of FT and BioT were calculated from the TT, SHBG and albumin levels by using a simple formula and a calculator widely available on the web (<http://www.issam.ch/freetesto.htm>) [26].

### Diagnosis of NAFLD

The diagnosis of hepatic steatosis was based on abdominal ultrasonography with a 3.5-MHz transducer (HDI 5000, Philips, Bothell, WA, USA). Ultrasound examinations were performed by experienced sonographers who were blinded to the subject's clinical and laboratory data. The ultrasonographic criteria used to diagnose hepatic steatosis included the presence of high-

**Table 1** Anthropometric and hormonal characteristics between men with and without hepatic steatosis

Variables	No Hepatic steatosis		Hepatic steatosis		<i>p</i> -value
	<i>N</i>	mean $\pm$ SD	<i>N</i>	mean $\pm$ SD	
Age (years)	132	54.08 $\pm$ 10.66	86	49.62 $\pm$ 7.89	<0.001
BMI (kg/m <sup>2</sup> )	100	22.89 $\pm$ 2.60	77	26.02 $\pm$ 2.46	<0.001
Waist (cm)	120	85.32 $\pm$ 7.07	81	92.31 $\pm$ 6.49	<0.001
ALT (U/L)	123	25.68 $\pm$ 13.78	84	35.55 $\pm$ 21.52	<0.001
AST (U/L)	123	23.63 $\pm$ 7.52	84	25.55 $\pm$ 8.75	0.094
AST/ALT	123	1.04 $\pm$ 0.36	84	0.83 $\pm$ 0.27	<0.001
TT (nmol/L)	127	14.01 $\pm$ 4.30	83	12.71 $\pm$ 3.93	0.027
FT (pmol/L)	125	235.37 $\pm$ 71.65	83	246.02 $\pm$ 75.31	0.305
BioT (nmol/L)	125	5.88 $\pm$ 1.82	83	6.19 $\pm$ 1.86	0.236
SHBG (nmol/L)	125	44.61 $\pm$ 19.37	83	36.30 $\pm$ 28.22	0.013

Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TT, total testosterone; FT, free testosterone; BioT, bioavailable testosterone; SHBG, sex hormone-binding globulin.

level echoes from the hepatic parenchyma (bright), stronger echoes in the hepatic parenchyma than in the renal parenchyma, blurred liver vessel structure and narrowing of the lumen of the hepatic veins.

### Statistical analysis

Differences in means between hepatic steatosis and non-hepatic steatosis were tested by the independent samples *t*-test. Univariate logistic regression analysis was used to assess the risk factors for hepatic steatosis with or without adjustment. TT, FT, BioT and SHBG were categorized into quartiles. The associations of TT, FT, BioT, SHBG quartiles and hepatic steatosis were also analyzed using univariate logistic regression. The lowest quartiles of TT, FT, BioT and SHBG were used as the reference category. Multivariable linear regression analysis was conducted for assessing contributions of TT, FT, BioT and SHBG to AST/ALT ratios. AST/ALT ratios were used as dependent variables, and TT, FT, BioT, SHBG, age, smoking, alcohol consumption and physical activity were used as independent variables. For all analyses, two models were evaluated: model 1 was without any adjustments; model 2 was adjusted for age, smoking, alcohol consumption and physical activity. Values are presented as mean  $\pm$  standard deviation. A *p* < 0.05 was regarded as statistically significant. All analyses were carried out with SPSS 20.0 statistical package software (SPSS Inc., Chicago, IL, USA).

## Results

### Baseline characteristics and hepatic steatosis

Table 1 shows the variable characteristics of the study population with and without hepatic steatosis. The numbers of men with and without hepatic steatosis were 86 and 132, respectively. Compared to men without hepatic steatosis, men with hepatic steatosis were younger, and had significantly higher mean BMI and waist circumference, and lower TT and SHBG levels. In addition, men with hepatic steatosis had significantly higher mean ALT levels than those without hepatic steatosis, but mean AST levels showed no difference. The mean AST/ALT ratios in men with and without hepatic steatosis were 0.83 and 1.04, respectively. Therefore, the AST/ALT ratios in men with hepatic steatosis were lower than those without hepatic steatosis, because of the elevated ALT levels. However, FT and BioT levels between the two groups were not significantly different.

### Associations of TT, FT, BioT and SHBG with hepatic steatosis

Table 2 shows the associations between TT, FT, BioT, SHBG and hepatic steatosis. BMI and waist circumference were risk factors for hepatic steatosis. TT and SHBG were protective factors for hepatic steatosis, and the significant associations remained after adjusting for age, smoking, alcohol consumption and physical activity.

**Table 2** Univariate logistic regression analyses for hepatic steatosis

	Model 1		Model 2	
	OR (95%CI)	<i>p</i> -value	OR (95%CI)	<i>p</i> -value
Age	0.95 (0.92–0.98)	0.001		
BMI (kg/m <sup>2</sup> )	1.66 (1.41–1.96)	<0.001		
Waist (cm)	1.17 (1.11–1.23)	<0.001		
TT (nmol/L)	0.93 (0.86–0.99)	0.030	0.91 (0.85–0.98)	0.012
FT (pmol/L)	1.00 (0.99–1.01)	0.304	1.00 (0.99–1.00)	0.824
BioT (nmol/L)	1.10 (0.94–1.28)	0.236	0.99 (0.83–1.17)	0.877
SHBG (nmol/L)	0.98 (0.96–0.99)	0.014	0.98 (0.96–0.99)	0.013

Model 1: crude odds ratios; model 2: adjusted for age, smoking, alcohol consumption and physical activity. Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; TT, total testosterone; FT, free testosterone; BioT, bioavailable testosterone; SHBG, sex hormone-binding globulin.

**Table 3** Odds ratios of hepatic steatosis according to quartiles for TT, FT, BioT and SHBG

	<i>n</i>	Model 1		Model 2	
		OR (95%CI)	<i>p</i> -value	OR (95%CI)	<i>p</i> -value
TT (nmol/L)					
Q1 (<10.06)	41	1.00 (Ref)		1.00 (Ref)	
Q2 (10.06–12.73)	60	0.64 (0.28–1.42)	0.266	0.64 (0.28–1.47)	0.292
Q3 (12.73–15.89)	50	0.58 (0.25–1.35)	0.208	0.55 (0.23–1.31)	0.180
Q4 (>15.89)	59	0.45 (0.20–1.03)	0.058	0.41 (0.17–0.95)	0.038
FT (pmol/L)					
Q1 (<179.37)	40	1.00 (Ref)		1.00 (Ref)	
Q2 (179.37–226.13)	51	1.23 (0.52–2.95)	0.637	1.12 (0.46–2.77)	0.802
Q3 (226.13–281.93)	54	1.93 (0.82–4.51)	0.130	1.33 (0.53–3.34)	0.540
Q4 (>281.93)	63	1.37 (0.60–3.14)	0.462	0.86 (0.35–2.17)	0.760
BioT (nmol/L)					
Q1 (<4.45)	37	1.00 (Ref)		1.00 (Ref)	
Q2 (4.45–5.53)	51	1.01 (0.42–2.44)	0.988	0.84 (0.33–2.16)	0.720
Q3 (5.53–6.84)	56	1.29 (0.55–3.04)	0.565	0.86 (0.34–2.21)	0.755
Q4 (>6.84)	63	1.58 (0.68–3.64)	0.288	0.92 (0.36–2.37)	0.869
SHBG (nmol/L)					
Q1 (<29.00)	59	1.00 (Ref)		1.00 (Ref)	
Q2 (29.00–38.40)	60	0.74 (0.36–1.52)	0.407	0.81 (0.39–1.70)	0.574
Q3 (38.40–56.33)	51	0.27 (0.12–0.61)	0.002	0.32 (0.14–0.75)	0.009
Q4 (>56.33)	38	0.21 (0.83–0.54)	0.001	0.27 (0.10–0.73)	0.010

Model 1: crude odds ratios; model 2: adjusted for age, smoking, alcohol consumption and physical activity. Abbreviations: OR, odds ratio; CI, confidence interval; TT, total testosterone; FT, free testosterone; BioT, bioavailable testosterone; SHBG, sex hormone-binding globulin.

Table 3 shows the associations between quartiles of TT, FT, BioT, SHBG and hepatic steatosis as determined by univariate logistic regression analysis. The OR (odds

ratio) for hepatic steatosis in the fourth quartile of TT (0.45 [95% CI: 0.20–1.03]) was lower than in the lowest quartile, and nearly reached the significant difference

**Table 4** Multiple regression analyses with AST/ALT ratios as dependent variables

Independent variable	Model 1			Model 2		
	$\beta$	<i>p</i> -value	R-squared	$\beta$	<i>p</i> -value	R-squared
TT	0.451	0.046	0.002	0.195	0.003	0.193
FT	−0.180	0.003	0.032	−0.004	0.952	0.155
BioT	−0.195	0.001	0.038	0.009	0.886	0.155
SHBG	0.339	<0.001	0.115	0.186	0.005	0.184

Model 1: no adjustment; model 2: adjusted for age, smoking, alcohol consumption and physical activity.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; TT, total testosterone; FT, free testosterone; BioT, bioavailable testosterone; SHBG, sex hormone-binding globulin.

( $p = 0.058$ ). After adjusting for age, smoking, alcohol consumption and physical activity, the OR for hepatic steatosis in the fourth quartile of TT (0.41 [95% CI: 0.17–0.95]) was significantly lower than in the lowest quartile.

Compared with the lowest quartile, the ORs for hepatic steatosis in the third and fourth quartiles (0.27 [95% CI: 0.12–0.61] and 0.21 [95% CI: 0.03–0.54], respectively) of SHBG were significantly lower. After adjusting for age, smoking, alcohol consumption and physical activity, the inverse associations remained significant. However, there was no association between quartiles of FT, BioT and hepatic steatosis. These results showed that higher TT and SHBG levels were associated with reduced risk of hepatic steatosis, independent of age, smoking, alcohol consumption and physical activity.

#### ***Associations of TT, FT, BioT and SHBG with AST/ALT ratios***

Table 4 shows the relationships between TT, FT, BioT, SHBG and the AST/ALT ratios. TT and SHBG were positively associated with AST/ALT ratios with and without adjusting for age, smoking, alcohol consumption and physical activity. FT and BioT were inversely associated with AST/ALT ratios without adjustments.

## **Discussion**

The association of hepatic steatosis and SHBG levels has been studied previously. In the Multiethnic Study of Atherosclerosis, conducted on 2,835 postmenopausal women and 2,899 men, both men and women with hepatic steatosis had lower SHBG levels, and higher SHBG tertiles were associated with lower risk of hepatic steatosis [27]. A study of middle-aged diabetic patients showed that men with hepatic steatosis have lower

SHBG levels, and the odds ratio (OR) for NAFLD is significantly lower with increased quartiles of SHBG after adjustment for covariates in both genders [9]. Similarly, in type 2 diabetes patients, lower serum SHBG levels are strongly correlated with a higher incidence of NAFLD [28]. Several epidemiological studies also show that hepatic steatosis is inversely associated with lower SHBG concentrations [8, 10, 29]. Furthermore, in a study of 118 subjects with lifestyle (diet and exercise) intervention, an increase in SHBG levels is associated with a decrease in hepatic steatosis [30]. Our finding supports these previous studies. Men with hepatic steatosis have lower SHBG levels (Table 1), and SHBG is associated with a lower risk of hepatic steatosis (Table 2). Compared with the lowest quartile, the ORs for hepatic steatosis in the third and fourth quartiles of SHBG are significantly lower with or without adjustment for age, smoking, alcohol consumption and physical activity (Table 3). Our results together with previous studies confirm that higher SHBG levels decrease the risk of hepatic steatosis.

An association between hepatic steatosis and TT levels has also been studied. In middle-aged Korean men and middle-aged diabetic patients, the TT level is independently and inversely correlated with risk of NAFLD [9, 18]. Similarly, men with fatty liver have been reported to have lower levels of TT [27, 31]. In contrast, in a study in China, no association could be found between TT and NAFLD [29]. In our study, we find that men with hepatic steatosis have lower TT levels (Table 1). TT is a protective factor for hepatic steatosis (Table 2). After adjusting for age, smoking, alcohol consumption and physical activity, the OR for hepatic steatosis in the fourth quartile of TT is significantly lower than in the lowest quartile (Table 3). Our results suggest that higher TT levels reduce the risk of hepatic steatosis.



NAFLD is characterized by asymptomatic, mild elevations of serum liver enzymes: ALT and AST [20]. Consistent with the previous study reporting that serum levels of AST and ALT are fairly similar in healthy people [32], our study shows that the AST/ALT ratios in men without hepatic steatosis are 1.04 (Table 1). The AST/ALT ratios in men with hepatic steatosis are 0.83 (Table 1), which is less than 1:1 [21, 22]. Furthermore, our study shows that the AST/ALT ratios in men with hepatic steatosis decrease because of the elevated ALT levels (Table 1). We first find that TT and SHBG are positively associated with AST/ALT ratios, independent of age, smoking, alcohol consumption and physical activity (Table 4). Additionally, higher levels of TT and SHBG show the lower risks of hepatic steatosis (Table 2 and 3), and men with hepatic steatosis represent the lower levels of TT, SHBG and higher levels of ALT (Table 1). Those results imply that the decreased levels of TT and SHBG in men with hepatic steatosis may be associated with the increased levels of ALT. Furthermore, the AST/ALT ratios are varied in different liver

diseases. The AST/ALT ratios in alcoholic steatohepatitis are at least 2:1 [33]. When fibrosis and cirrhosis is present, AST/ALT ratios are slightly above 1.0 [34, 35]. Therefore, the relationships between TT, SHBG and AST/ALT ratios in other liver diseases need to be further investigated.

In conclusion, our study shows that TT and SHBG are inversely associated with the risk of hepatic steatosis, and positively associated with AST/ALT ratios, independent of age, smoking, alcohol consumption and physical activity. Furthermore, the decrease of the AST/ALT ratios in men with hepatic steatosis is because of the elevated ALT levels.

## Acknowledgements

This work was supported by Scientific Research Foundation of Shantou University Medical College (50010203) and Innovative Experimental Project of University Students, Guangdong Province, China (201510560025).

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