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Comments on ‘Low serum sex hormone binding globulin is associated with nonalcoholic fatty liver disease in type 2 diabetic patients*’

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Nonalcoholic fatty liver disease (NAFLD) is the liver manifestation of metabolic syndrome, visceral obesity and insulin resistance, all of which are associated with increased risk of type 2 diabetes mellitus and atherosclerosis.¹ NAFLD is characterized by an increase in hepatic triglyceride content with or without inflammation and fibrosis. NAFLD is one of the most common causes of elevated aminotransferases worldwide including Asia² and is a public health problem due to high prevalence and risk of developing nonalcoholic steatohepatitis, cirrhosis and liver failure.³ The development of NAFLD has been shown to be related to insulin resistance in most studies.¹ Many studies have shown an inverse relationship between obesity, insulin resistance, metabolic syndrome and type 2 diabetes and serum testosterone and sex-hormone-binding protein (SHBG) concentrations.^{4,5} The manuscript by Hua *et al.* in this issue of the journal attempts to clarify the relative roles of serum SHBG and testosterone in the development of NAFLD in men and women with type 2 diabetes in a case–control study.⁶

Sex-hormone-binding globulin (SHBG), in addition to its role as a circulating protein that transports androgens and oestrogens to their target tissues, may have independent cell signalling actions by binding to a membrane receptor.⁷ SHBG affects the delivery of the sex steroid to the body and the metabolic clearance of these steroids. Testosterone and estradiol circulate in three forms: SHBG bound; albumin bound; and free hormone. It is widely believed that the SHBG-bound component is less available to tissues than the albumin-bound and free steroid fractions (bioavailable components). SHBG levels and the binding affinities of androgens and oestrogens to SHBG influence the concentrations of total testosterone/estradiol in the blood and the percentage of testosterone/estradiol that is in a biologically available form. Free hormone concentrations measured by equilibrium dialysis provide a SHBG-independent assessment of the free-circulating biological hormone,

whereas calculated free hormone levels depend on the concentrations of both serum total hormone and the SHBG concentrations.⁸ Heterogeneity in the characteristics of SHBG in the population may affect the concentrations of total, bioavailable and free testosterone/estradiol as well as influence possible direct effects of SHBG on metabolic disorders. These possibilities were emphasized by recent genome-wide association studies (GWAS) of large cohorts of men identifying two single-nucleotide polymorphisms (SNPs rs12150660 and rs6258) at the SHBG gene that were independently associated with serum testosterone concentration. Men with 3 risk alleles of these variants had 6.5-fold higher likelihood of having low-serum testosterone than subjects with no risk allele. Both SNPs also affected serum SHBG concentrations but even after adjustment for SHBG concentrations, the association between these two SNPs and serum testosterone remained significant. SNP rs6258 also affected the binding of testosterone to SHBG and the free testosterone fraction.⁹ These genetic modifications add complexity to the relationship between SHBG and serum total and free testosterone.

One paradox in understanding the relationship of circulating androgen levels to metabolic disorders and NAFLD is that low total and free testosterone are also associated with the metabolic syndrome and type 2 DM in men,¹⁰ whereas high total and free androgens (e.g. polycystic ovarian disease) are associated with metabolic syndrome and insulin resistance in women.^{4,11} Epidemiologic studies in both men and women show that low SHBG concentrations are bi-directionally associated with and predictive of development of obesity, metabolic syndrome^{4,12} and type 2 DM.¹⁰ Insulin resistance plays a key role in the association between low SHBG and type 2 diabetes.^{11,12} These studies together suggest that SHBG plays an important role linking insulin resistance, metabolic dysfunction and NAFLD.

Hua *et al.* in this issue of the journal conducted a case control study in men and women with type 2 DM patients with and without NAFLD that was diagnosed by liver ultrasound or fatty liver index. Not unexpectedly the group with NAFLD had significantly higher waist circumference, serum triglyceride, insulin and c-peptide levels, lower total testosterone in men and marginally higher free testosterone in women. Both men and women with NAFLD had significantly lower SHBG levels compared with those without hepatic steatosis. After adjustment for confounders (age, smoking, alcohol use, duration of diabetes, BMI, and fasting c-peptide), serum SHBG levels remained inversely associated with NAFLD (fatty liver index). In contrast, after adjustment for multiple risk factors and SHBG levels, the relationship between serum total testosterone for men and free testosterone in women was both attenuated, suggesting that low-serum SHBG is more strongly associated with NAFLD than testosterone. The association of low-serum SHBG concentration with the severity of NAFLD in type 2 DM patients has been reported in another case-control study in Asian men and women. In that study, the odds ratio of predicting high-grade NAFLD decreased significantly with increasing SHBG concentrations even after adjustment for risk factors of metabolic dysfunction, testosterone and estradiol.¹³ Interpretation of these data requires confidence in the assays used to measure testosterone and SHBG in serum. Hua *et al* indicated serum total testosterone was not measured by the 'gold standard' liquid chromatography and tandem mass spectrometry assays.⁶ Thus, low total and calculated free

testosterone levels in women may be both imprecise and inaccurate as calculated free testosterone is dependent on the accuracy of the total testosterone and SHBG levels.¹⁴ The finding of lack of association of free testosterone with NAFLD in the study reported by Hua *et al* may be related to the inability to accurately measure free testosterone in women. The studies of Hua *et al*.⁶ and others¹³ emphasized the strong association between low SHBG levels and the occurrence of NAFLD in patients with type 2 DM. These studies while of great interest do not prove causality of low SHBG in inducing NAFLD in men and women with type 2 diabetes. Insulin resistance is a major factor associated with lowering SHBG levels and in most instances the development of NAFLD in type 2 diabetes.¹

Recent GWAS on SNPs of the SHBG gene showed that carriers of the SNP rs6257 have a 10% lower serum level of SHBG whereas carriers of SNP rs6259 have 10% higher SHBG levels compared with homozygous genotype. For each standard deviation increase in SHBG levels, the predicted odds ratio for developing type 2 DM is 0.29 for men and 0.28 for women independent of body mass.¹⁵ Another GWAS study showed that presence of SNP rs1799941 was associated with increased SHBG levels and lower risk of type 2 DM.¹⁶ These genetic studies suggest that SNPs in the SHBG gene modify serum SHBG concentrations and the risk of type 2 diabetes. Genetic polymorphisms of the SHBG gene may also play a causal role in the development of NAFLD in type 2 diabetes. With the small sample size, Hua *et al*. did not perform genetics analyses of the SNPs of the SHBG gene.⁶

Can low SHBG concentrations be a consequence of hepatic steatosis? SHBG is produced by the liver. *In vitro* studies reported that insulin and glucose decrease SHBG production by hepatocytes.¹⁷ It is known that SHBG synthesis is regulated by androgens and oestrogens and inversely associated with insulin resistance and type 2 diabetes. GWAS study confirmed that human serum SHBG concentration is regulated by 12 main genes that control hepatic function, lipid and carbohydrate metabolism, androgen and oestrogen receptor, and hormone-dependent cancers.¹⁸ All these factors in addition to low SHBG may contribute to development NAFLD in type 2 DM.

To directly study whether low SHBG plays a key causal role in the development of NAFLD in type 2 DM independent of sex hormone concentration in men and women is difficult because of complex relationships between measured serum SHBG and testosterone and estradiol and the inability to perform intervention studies modifying SHBG concentrations without affecting serum sex hormone levels. In rodents, where SHBG is not excreted by the liver to the circulation, lack of the action of androgens in the male is associated with the development of NAFLD with or without presence of insulin resistance.^{19,20} This study by Hua *et al*.⁶ challenges investigators to dissect the role of sex hormones and its binding protein in the development of metabolic dysfunction and NAFLD in men and women.

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