Sex Hormone-Binding Globulin Protects Against Non-Alcoholic Fatty Liver Disease

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Introduction and Aim

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of death worldwide due to excess alcohol intake. The increased prevalence of diabetes and obesity is considered to be the most common cause for NAFLD. Several studies have shown that obese subjects, type 2 diabetic patients and individuals with NAFLD have low SHBG levels, a protein produced by the liver that carries sex steroids and regulates their bioavailability at the tissue level.

Apart from the increase in lipogenesis that drives fat accumulation in the liver, there are evidences supporting a central role of TNF-a in the development of NAFLD. We have previously demonstrated that an increase in hepatic lipogenesis and TNF-a downregulated SHBG production. Therefore, lipid accumulation and lower grade inflammation present in NAFLD-related disease by reducing the low circulating SHBG levels in this latter raises the intriguing question of whether low SHBG could contribute to the progression of NAFLD, rather than simply being a consequence and a surrogate biomarker.

Aim of this study is to address the importance of SHBG expression in NAFLD in vivo, we developed a unique mouse model by crossing the human SHBG transgenic mice with the C57Bl/6j-db/db mice. The characterization of this SHBG-db/db mice allowed us to demonstrate for the first time that SHBG overexpression reduces hepatic steatosis by limiting lipogenesis. In addition, in a diet induced model of NAFLD using the human SHBG transgenic mice and their WT littermates, we demonstrated that SHBG overexpression protected against NAFLD development induced by a high fructose diet (HFD) feeding.

Methods

1 Subjects and samples: we recruited 36 obese subjects (body mass index [BMI] 42.7 ± 16.7 kg/m2, waist circumference [WC] 109.0 ± 16.0 cm, systolic blood pressure (SBP) 140.0 ± 15.5 mmHg, diastolic blood pressure (DBP) 96.0 ± 14.5 mmHg, low-density lipoprotein cholesterol (LDL-C) 138.0 ± 43.5 mg/dL, triglycerides (TG) 221.0 ± 107.0 mg/dL, high-density lipoprotein cholesterol (HDL-C) 41.0 ± 7.0 mg/dL, and Fasting Glucose [FG] 116.0 ± 22.0 mg/dL) suffering from metabolic diseases were included. Liver biopsies were obtained using a needle biopsy and the biopsy sample was used for Western blot and qPCR analysis and the study was approved by the ethics committee from the university that consented the study. All of the samples were taken during a 24-hour fast to ensure a basal state and the sample was divided for the efficiency of nuclear extracts.

2 In vitro experiments: male and female mice of the four genotypes were used in this study. All mice (4–6 weeks old) were fasted overnight until 4–6 hours before the experiment was performed and the mice were divided into two groups: one for the control group and the other for the HFD group. The mice were fasted for 4–6 hours before the experiment was performed. The mouse was fasted for 4–6 hours before the experiment was performed. The mouse was fasted for 4–6 hours before the experiment was performed.

3 Animal experiments: to determine the role of SHBG in the development of NAFLD, we used a mouse model of diet-induced obesity. Mice were divided into two groups: control and HFD. The control group was maintained on a normal diet, while the HFD group was fed a high-fat diet. After 12 weeks of diet, the mice were killed and liver samples were collected for analysis. The results were analyzed using a t-test.

Results

1 SHBG overexpression reduces liver weight and fat accumulation in SHBG-db/db mice.

2 Western blot analysis: liver samples were harvested from mice and analyzed for the expression of SHBG, TNF-a, and related proteins. Results were analyzed using the Western blot program. For immunochemical analysis, liver samples from mice were homogenized in MEF buffer with Complete (Roche). Protein extracts were used for western blotting with antibodies for TNF-a, ACY, and ACP. Specific antibodies against TNF-a were identified using the corresponding MAb-based reagent. Anti-TNF-a and anti-ACY were used with 1:1000 and 1:1000 dilutions, respectively.

3 Microarray hybridization and analysis: the goal of the study was to compare hepatic gene expression patterns between db/db and SHBG-db/db mice. Microarray data were carried out using the Agilent microarray platform and the GeneSpring software. GeneSpring (v 12.6, Agilent) analysis was performed at the High Through put Unit of Our Research Institute as described elsewhere (www.omics.org). Data obtained from the microarrays were analyzed by the GeneSpring and Statistica Software package. All the biological analysis was done using the statistical software R and the GeneSpring software. GeneSpring software version was used to establish the association between SHBG levels and the other parameters. For graphs, a linear regression test was applied. Significance was accepted at the level of p < 0.05. Statistical analysis were performed with the SPSS statistical package (SPSS, Inc., Chicago, IL).

Conclusions

1 Human SHBG overexpression protects against liver fat accumulation in a genetically-induced and a diet-induced mouse models of NAFLD.

2 Human SHBG overexpression reduces hepatic fat accumulation by downregulating key lipogenic enzymes (ACC, FAS and ACY).

3 Using HepG2 cells we show a cell autonomous effect of SHBG expression levels on lipogenesis.

4 SHBG mRNA levels correlate significantly with TG and ACC mRNA levels in human liver biopsies.

5 Overall, our results suggest that SHBG protects against NAFLD development. Further research should address whether SHBG could be a new therapeutic target for preventing or arresting NAFLD.

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