SHBG-C57BL/ksJ-db/db: a new mouse model to study the link between SHBG regulation and obesity development

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Introduction

See hormone-binding globulin (SHBG) is produced by the human liver under control of hormones and nutritional factors. The human liver secretes SHBG to the blood where it binds sex steroids with high affinity regulating their bioavailability. Low serum SHBG levels in overweight individuals are a biomarker for the metabolic syndrome and are predictive of type 2 diabetes and cardiovascular disease risk.

There are no in vivo models to study SHBG expression and regulation during obesity development. The main reason for this is that the obesity-prone rodent models cannot be used to study this issue since rodents unlike humans do not express the SHBG gene in their livers, instead they express the SHBG in the Sertoli cells of the testis.

To circumvent this issue, we have taken advantage of having the human SHBG transgenic mice and have developed a unique mouse model that overexpresses the human SHBG and develop obesity, by crossing the human SHBG transgenic mice with the C57Bl/6J-db/db mouse.

The aim of the study has been the characterization of this new SHBG-C57Bl/ksJ-db/db mouse model, which has allowed us to determine the molecular mechanisms and transcription factors involved in the downregulation of SHBG during obesity development. These mechanisms of SHBG downregulation involve changes in hepatic HNF-4α and PPARβ/δ target gene levels.

Methods

Animals

The human SHBG transgenic mice were backcrossed onto C57Bl/6J-db/db background in order to obtain mice expressing human SHBG and developing obesity and NAFLD. Male mice were maintained under standard conditions with food (Global Diet 2008, Ibarra Laboratories, Barcelona, Spain) and water provided ad libitum and a 12 h light/dark cycle. Experimental procedures were approved by the Institutional Animal Care Committees of the Vall d’Hebron Research Institute and the Universitat Autònoma de Barcelona (151-CEA).

For gene expression

One set of lean SHBG-C57Bl/ksJ-db/db and db/db mice (n=6 each) were followed up for 12 weeks on the diet. Blood samples were obtained after overnight fasting by tail snip at the beginning and the end of the diet period. Blood samples were collected by enucleation via venous puncture of male SHBG-C57Bl/ksJ-db/db and db/db mice (n=6 each) sacrificed at 6 weeks of age and blood and tissues were collected and weighed for RNA and protein isolation.

Statistical analysis

Human SHBG levels and total and free testosterone levels from plasma mice were measured using an ELISA (Ismatek Diagnostic GmbH).

Results

Total RNA was extracted from mouse liver and adipose tissue and human liver samples using TRIzol reagent (Invitrogen, MA). Reverse transcription (RT) was performed at 42°C for 50 min and the cDNA obtained was amplified with specific primers and probes provided by Invitrogen. The intensity of the RT product was amplified in a 25 μl-reaction containing SYBR Green/ROX (1:1) mixture with appropriate magnesium chloride primer pairs corresponding to human HNF-4α, mouse PPARβ/δ, human TNP, mouse (38), human TRA (13), mouse and human SHBG, human HNF-4α, human PPARβ/δ and human SHBG. Results were analyzed using the 7500 Fast program.

Results in diet analysis

Male mice were fasted in the dark (2A) and performed insulin resistance test (Insulin treated) (B). DEX treatment (C) was performed for 28 days and the weight of the mice were measured every 4 days by an electronic balance and the body weight was measured by an electronic balance. The weight was measured every 4 days by an electronic balance and the body weight was measured by an electronic balance.

Conclusions

The SHBG production is downregulated in obese SHBG-C57Bl/ksJ-db/db mice in comparison with lean SHBG-C57Bl/ksJ-db/db mice. The SHBG production is downregulated via a leptin feedback in the levels of the transcription factor HNF-4α and upregulation of PPARβ/δ.

In human liver biopsies, SHBG mRNA levels were positively correlated with HNF-4α and negatively correlated with PPARβ/δ.

Cytokine production is increased in obese SHBG-C57Bl/ksJ-db/db mice compared to lean SHBG-C57Bl/ksJ-db/db mice, which may influence the low hepatic levels of HNF-4α.

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