Human sex hormone-binding globulin does not provide metabolic protection against diet-induced obesity and dysglycemia in mice

Yael Sofer¹, Nava Nevo², Michal Vechoropoulos¹, Gabi Shefer¹, Etty Osher¹, Nathan Landis¹, Karen Tordjman¹, Geoffrey L Hammond³ and Naftali Stern¹

¹Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv-Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
²Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel
³Departments of Cellular & Physiological Sciences and Obstetrics & Gynaecology, University of British Columbia, Vancouver, British Columbia, Canada

Correspondence should be addressed to N Stern: naftalis@tlvmc.gov.il

Abstract

Background: Sex hormone-binding globulin (SHBG) is the main transporter of sex hormones in most vertebrates. Low SHBG levels have been linked to increased risk for diabetes and metabolic syndrome. Polymorphisms of the SHBG gene linked to low SHBG protein levels also strongly predicted increased risk of type 2 diabetes, thus raising the possibility that SHBG may play a role in the pathogenesis of insulin resistance and diabetes.

Aim: To examine whether expression of human SHBG in mice may ameliorate the development of diabetes and metabolic syndrome in response to a high-fat diet (HFD).

Methods: Transgene mice expressing a human SHBG transgene (SHBG+) (N = 10/11; males/females) and their wild type littermates (N = 12/8; males/females) were fed HFD for 4.5 months.

Results: HFD induced comparable obesity in control and SHBG+ mice. Male transgenes had higher muscle mass after 2–3.5 months HFD (0.43 ± 0.028 (n = 4) vs 0.38 ± 0.053 g (n = 7), P = 0.05). Fasting blood glucose, as well as insulin or HOMA-IR, was not different in transgenic vs wild-type males after 4–5 months HFD. Female transgenes had higher fasting glucose (152 ± 29 (n = 7) vs 115 ± 27 mg/dL, P = 0.01 (n = 8)), but mean insulin and HOMA-IR were not different. Likewise, insulin tolerance test and intra-peritoneal glucose tolerance test (GTT) were not different. Finally, SHBG+ mice were not different from controls in terms of liver enzymes, serum triglyceride levels and blood pressure.

Conclusion: In mice with diet-induced obesity, human SHBG did not protect against development of obesity or dysglycemia.

Introduction

SHBG is a homodimeric plasma glycoprotein produced by the liver that acts as the main transporter of active estrogens and androgens in almost all vertebrates (1). The protein has high affinity and selectivity to these sex steroids, and it has been traditionally postulated that the role of SHBG is limited to the transport of sex steroids into the target cells (2, 3). However, it has also been reported that SHBG interacts with a trans-membrane receptor that activates cellular cascades after binding sex steroids (4, 5).

The relation between low SHBG levels and diabetes now appears well established, both in men and women (6, 7, 8) and remains significant even after adjustment...
for BMI (9, 10, 11) and waist circumference (12). A meta-analysis of more than ten prospective studies has demonstrated that women with SHBG levels higher than 60 nmol/L had 80% lower risk of type 2 diabetes (7). Men with SHBG higher than 28.3 nmol/L had a 52% smaller risk of developing diabetes (7). High SHBG levels are apparently also associated with lower incidence of insulin resistance in men (10). Furthermore, cross-sectional studies in non-diabetic men and women have shown an inverse relationship between SHBG levels and HbA1c (11, 13). Hypoandrogenism in men and hyperandrogenism in women (e.g., the polycystic ovary syndrome) have been linked to insulin resistance and the metabolic syndrome (13, 14). In one report, SHBG levels predicted the development of gestational diabetes as early as 11–13 weeks of gestation (15). Of note, several studies have shown that the relationship between SHBG levels and diabetes is independent of sex hormones, in both female and male subjects (16, 17, 18). In a few studies, SHBG has also been linked to the metabolic syndrome in both adolescents (19, 20) and adults (21). Other metabolic components such as liver fat have also been strongly and inversely correlated with SHBG levels (22). Favorable lifestyle changes caused a parallel regression of fatty liver and increase in plasma levels of SHBG (23). A few studies have suggested that elevated insulin levels suppress SHBG production (24, 25), and insulin-sensitizing drugs, such as metformin and thiazolidinediones, have been shown to increase SHBG levels in men and women (26, 27). Other studies have shown that dietary monosaccharides can decrease SHBG expression (28).

There is evidence that SHBG levels may be influenced by specific polymorphisms in the SHBG gene per se and also by an array of loci in genes involved in biologic networks such as liver function, lipid metabolism, glucose metabolism, androgen and estrogen receptor function and epigenetic effects (29). Recent studies have also shown that specific genetic polymorphisms of SHBG were not only predictive of SHBG protein levels, but also of the apparently consequent risk of type 2 diabetes in men and women (12, 30). Also, one study suggested an association between a polymorphism in the SHBG promoter and polycystic ovary syndrome (31). Collectively, these findings are consistent with the hypothesis that the SHBG protein may not only comprise a ‘reverse’ biomarker of insulin resistance and diabetes, but could curb their evolution (32). To directly address this question, we examined whether or not mice expressing human SHBG have attenuated tendency to develop diabetes and other characteristics of the metabolic syndrome.

Materials and methods

High-fat diet-fed mice

Animal care and experiments were approved by the Institutional Animal Care and Use Committee of the Tel Aviv University. Twenty C57Bl/6J WT and twenty transgene littermates (non-congenic) expressing a 4 kb human SHBG transgene under the control of its own promoter sequence (33) were separated by sex at age 3 weeks and at age 6 weeks were placed on a high-fat diet (HFD, Research Diets, Inc, New Brunswick, NJ, USA). The diet was composed of 58% fat from lard, 25.6% carbohydrate and 16.4% protein (total caloric value, 23.4 kJ/g). Weight of mice was checked weekly. Identification of transgenic mice was done using PCR for human SHBG, as described previously (32). DNA was extracted from mice tails using REDExtract-N-Amp Tissue PCR Kit. Thereafter, the DNA was subjected to PCR amplification (1 cycle of 95°C for 2 min, 40 cycles of 94°C for 30 s, 64°C for 30 s and 72°C for 30 s) using specific forward (5’-GATCCCGAGGGTGATAGC-3’) and reverse (5’-GGGTAAAGGAAACAGGGGCAC-3’) primers designed to amplify a 153-bp region in the human SHBG promoter (34).

Further validation of the model was done by measuring serum SHBG (Siemens 06603393 Immulite SHBG Kit). After 20 weeks of the HFD, blood samples were collected for the measurement of fasting glucose, insulin, lipids, liver enzymes and testosterone levels. A glucose tolerance test (GTT) was done after an overnight fast with an intra-peritoneal injection of glucose (2 mg/kg). Glucose was measured at the following time points: 0, 15, 30, 60, 90 and 120 min after the injection of glucose. Similarly, an insulin tolerance test (ITT) was carried out with an intra-peritoneal injection of insulin (Humalog, 0.75 U/kg; Eli Lilly) and subsequent measurement of glucose at same time points. Blood pressure was measured in awake mice by a non-invasive approach using a three-channel computerized tail-cuff method (35, 36). The recording system consisted of an animal restrainer, which had a sensor block containing a photoelectric sensor built inside an occlusion cuff, an inflation bulb, a sphygmomanometer, an amplifier (model 3M229 BP, attached to the 31BP software package, IITC, Inc. Woodland Hills, CA, USA) and a chart recorder.

Technical procedures

Assays

Serum insulin was determined using a radioimmunoaassay

Insulin-CT (MP Biomedicals, Orangeburg, NY) with...
SHBG provides no metabolic protection in mice

Y Sofer et al.

Published by Bioscientifica Ltd

Table 1

| Duration HFD | 2 months | 3.5 months | 5 months |
|-------------|----------|------------|----------|
|              | Muscle mass (median) (g/cm²) | %FAT mean (median) | Muscle mass (median) (g/cm²) | %FAT mean (median) | Muscle mass (median) (g/cm²) | %FAT mean (median) |
| WT          | 0.39 ± 0.05 (0.38) | 21.89 ± 10 (16.23) | 0.43 ± 0.1 (0.38) | 24.76 ± 3.4 (27.04) | 0.48 ± 0.02 (0.49) | 25.94 ± 2.55 (27.13) |
| n = 7       | n = 7     | n = 3       | n = 3       | n = 3       | n = 3       |
| SHBG+       | 0.43 ± 0.03 (0.43) | 27.58 ± 9.48 (32.36) | 0.68 ± 0.19 (0.67) | 34.42 ± 11.3 (32.21) | 0.39 ± 0.09 (0.37) | 21.12 ± 7.7 (23.79) |
| n = 4       | n = 4     | n = 6       | n = 6       | n = 8       | n = 8       |
| P           | NS        | NS          | 0.032       | 0.065       | 0.02        | 0.097       |

Results

Validation of the model

Because mice and rats do not postnatally express Shbg in the liver, which is the site of production of circulating SHBG in other mammals such as humans, circulation testosterone in these rodents are generally low and tend to exhibit large fluctuations, presumably owing to the lack of the buffering effect afforded by SHBG (32). Expectedly, on the background of the previously described large variability in circulating testosterone in wild-type mice, serum total testosterone levels were much higher in female SHBG transgenic mice as compared to wild-type mice. Indeed, testosterone levels were undetectable in 5 of 6 wild-type females, with the exception of one female mouse showing a value of 0.82 ng/mL. In contrast, in the 10 female SHBG transgenmic mice, the mean testosterone level was 1.47 ± 1.57 ng/mL. Male SHBG transgenes had non-significantly higher testosterone levels than the wild-type mice (5.77 ± 6.09 ng/mL; (n = 12) vs 2.31 ± 4.31 ng/mL (n = 11)), owing to the large spread of testosterone levels in both strains. There was a 100% concordance between the presence of a human SHBG gene as detected by PCR and the presence of measurable serum SHBG levels (data not shown). Concordantly, SHBG levels were undetectable in wild-type mice.

Effect of SHBG expression on weight gain and body composition on HFD

There was no significant difference in the initial weight of SHBG-expressing mice as compared to wild-type littermates. Further, weight gain on HFD and weight were similar throughout the experiment (data not shown). In male mice, median peak weight (4 months) was 48.3 ± 2.87 g and 50.2 ± 5.72 g for WT and SHBG+, respectively, and the corresponding attained mean weights for female mice were 45.5 ± 7.4 g and 40.0 ± 8.7 g (P= ns, for both comparisons).

Due to the known association between total testosterone and muscle (38), we compared muscle and fat mass of the gastrocnemius muscle, using dual-energy X-ray absorptiometry (DEXA). Male transgenes had significantly higher muscle mass after 3.5-month HFD, 0.68 ± 0.19 (median 0.67) g/cm², as compared to 0.43 ± 0.1 (median 0.38) g/cm², respectively (P=0.032), but this difference was reversed after 5 months (Table 1). Bone mineral density and % fat were not different between transgenoses and controls. In HFD female mice, % fat and muscle mass was not related to the SHBG status: WT mean muscle mass 0.3125 ± 0.07 g/cm² (median 0.295) (n = 4) as compared with SHBG+ mean muscle mass 0.413 ± 0.1 g/cm² (median 0.37) (n = 3). WT mean % fat is 33.12 ± 4.08
Effect of SHBG on glucose metabolism

Fasting blood glucose was not different in WT and SHBG+ mice ~6 months on HFD (143±36 vs 166±33 mg/dL, respectively; P=0.18), and likewise, no statistically significant differences were seen in insulin (51±30 vs 59±29 IU/L, P=0.25 or HOMA-IR 18.94±14.61 vs 25.67±15.58, P=0.13) in male wild-type mice compared to transgenic mice. Four to five months into the HFD, female transgenes had significantly higher fasting glucose than their WT controls (152.30±27.12 mg/dL vs 114.50±28.73 mg/dL, P=0.01), but there were no differences in mean insulin or HOMA-IR (Table 2). Similarly, there was no discernible difference between transgenic wild-type mice in the liver enzymes (GOT and GPT) and serum triglycerides and HDL. GTT and ITT were similar in WT and transgene male and female mice (data not shown).

Discussion

The mechanisms through which SHBG, a binding protein for sex hormones with high affinity to active androgens and estrogens, is linked to protection from diabetes or insulin resistance (39) remain enigmatic. Prospective and cross-sectional studies have already established that such putative protection was not mediated through the prevention of weight gain or the presence/degree of obesity (10, 11, 12, 13). Interestingly, SHBG-related protection from diabetes was not seen in both women and men (12) and is independent of sex hormone levels per se. Still, it is apparently linked to insulin resistance, as several studies noted a strong reverse correlation between SHBG levels and metabolic syndrome and insulin resistance (40).

In the present study, SHBG+ mice were not protected from high fat diet-induced obesity. Further, diabetes, as evidenced by high glucose levels, was evident in mice fed on high-fat chow regardless of the expression of SHBG, in both intact male and female mice.

This outcome has some similarities and major differences compared to the human data. First, the presence of SHBG did not prevent weight gain on HFD in our experiments. In humans, higher SHBG levels appear to confer protection from diabetes after adjustment for the level of obesity (12). Phrased alternatively, in humans, SHBG is linked to lesser risk for diabetes even in the obese state (12, 31). Second, in contrast to what might have been expected based on the human data, high circulating SHBG levels did not protect mice fed on a HFD from diabetes. Unlike humans, SHBG does not circulate in adult rodents (39). Despite early interest in a putative cellular receptor for SHBG (41), evidence for its physiological role is lacking. Therefore, the possibility that SHBG’s putative protective metabolic effect can be exerted by some direct interaction with cellular target sites/receptors to enhance insulin sensitivity remains entirely speculative.

In a recent report (42), crossbreeding of the same SHBG+ transgenic mouse used in our study with the db–db transgenic mouse lacking the leptin receptor, attenuated the massive weight gain seen in the latter mouse model, but did not affect the metabolic anomalies seen in this setting, i.e., high glucose and increased serum lipid levels. The latter is in general agreement with our results, in that neither baseline nor post-HFD glucose and triglycerides differed between SHBG+ and wild-type mice in the obese state.

Whether the linkage of SHBG to dysglycemia and diabetes in humans is species specific or mediated by some mechanism that is not operative in the HFD mouse model cannot be determined based on our experiments. Our data are limited to a particular animal model under a particular experimental set of metabolic conditions (HFD). Hence, we are unable to extrapolate our results to the much more complex clinical setting in humans, where genetics, nutrition, environment, life style and aging all interact in the pathogenesis of diabetes. Nevertheless, our results do not support the concept that SHBG expression alone modifies the abnormalities in glucose metabolism, which are linked to HFD and the associated obese state.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
The work was supported by the Sagol Foundation for the Metabolic Syndrome Research Center at TASMC and by Dr Sofer’s TASMC research grant for excellence in research.

References
1 Hammond GL. Diverse roles for sex hormone-binding globulin in reproduction. Biology of Reproduction 2011 85 431–441. (https://doi.org/10.1095/biolreprod.111.092593)
2 Hammond GL, Wu TS & Simard M. Evolving utility of sex hormone-binding globulin measurements in clinical medicine. Current Opinion in Endocrinology, Diabetes and Obesity 2012 19 183–189. (https://doi.org/10.1097/MED.0b013e3283537320)
3 Anderson DC. Sex hormone-binding globulin. Endocrinology 1974 3 69–96. (https://doi.org/10.1210/jcem-157-4-4473-4486)
4 Rosner W, Hryb DJ, Kahn SM, Nakha AM & Romans NA. Interactions of sex hormone-binding globulin with target cells. Molecular and Cellular Endocrinology 2010 316 79–85. (https://doi.org/10.1016/j.mce.2009.08.009)
5 De Toni L, Guidolin D, De Filippis V, Strapazzon G, De Toni L, Guidolin D, De Filippis V & Tescari S, Strapazzon G. Osteocalcin and sex hormone binding globulin compete on a specific binding site of GP63A. Endocrinology 2016 157 4473–4486. (https://doi.org/10.1210/en.2016-1312)
6 Haffner SM. Sex hormone-binding protein, hyperinsulinemia, insulin resistance and non-insulin-dependent diabetes. Hormone Research 1996 45 233–237. (https://doi.org/10.1159/000184794)
7 Ding EL, Song Y, Malik V, Liu S, Sitzer PK, Murali JT, Hammond GL, Nisker JA, Raymond WJ & Kuhn RW. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. Jama 2006 295 1288–1299. (https://doi.org/10.1001/jama.295.11.1288)
8 Wang Q, Kangs AJ, Soininen P, Tiainen M, Tiainen M, Tynkkynen T, Puukka K, Ding EL, Song Y, Malik VS, Liu S, Singh HJ, Singh HJ, Puukka K, De Filippis V, Nanda S, Savvidou M, Syngelaki A, Akolekar R & Nicolaides KH. Sex hormone-binding globulin levels and high estradiol levels are independent predictors of type 2 diabetes in men. European Journal of Endocrinology 2010 162 747–754. (https://doi.org/10.1530/EJE-09-0943)
9 Kalyani RR, Franco M, Dobs AS, Ouyang P, Vaidya D, Bertoni A, Capistrano SM & Golden SH. The association between sex hormones, adiposity, and insulin resistance with incident diabetes in postmenopausal women. Journal of Clinical Endocrinology and Metabolism 2009 94 4127–4135. (https://doi.org/10.1210/jc.2009-0910)
10 Agirbasli M, Agaoglu NB, Orak N, Caglioz O, Ocek T, Poci N, Salaj A & Maya S. Sex hormones and metabolic syndrome in children and adolescents. Metabolism 2009 58 1256–1262. (https://doi.org/10.1016/j.metabol.2009.01.024)
11 Glueck CJ, Morrison JA, Daniels S, Wang P & Stroop D. Sex hormone-binding globulin, ooligomenorrhea, polycystic ovary syndrome, and childhood insulin at age 14 years predict metabolic syndrome and class III obesity at age 24 years. Journal of Pediatrics 2011 159 308–313. (https://doi.org/10.1016/j.jpeds.2011.01.018)
12 Li C, Ford ES, Li B, Giles WH & Liu S. Association of testosterone and sex hormone-binding globulin with metabolic syndrome and insulin resistance in men. Diabetes Care 2010 33 1618–1624. (https://doi.org/10.2337/dc09-1788)
13 Jaruqongvanich V, Sanguankee A, Siangwattan T & Uapa S. Testosterone, sex hormone-binding globulin and nonalcoholic fatty liver disease: a systematic review and meta-analysis. Annals of Hepatology 2017 16 382–394. (https://doi.org/10.4103/ajh.ajh_166_17)
14 Peter A, Kantartzis K, Machann J, Schick F, Staiger H, Machiao C, Schleicher E, Fritsche A, Haring HU & Stefan N. Relationships of circulating sex hormone-binding globulin with metabolic traits in humans. Diabetes 2010 59 3167–3173. (https://doi.org/10.2337/db10-0179)
15 Pons J, Matei LA, Jones RE & Friedl KE. Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. Current Journal of Endocrinology and Metabolism 1998 67 460–464. (https://doi.org/10.1210/jcem-67-3-460)
16 Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, Clove JW & Blackard WG. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism 1991 72 823–89. (https://doi.org/10.1210/jcem-72-1-83)
17 Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gambineri A, Biscotti D, Vicennati V, Calvi S, Cogna GE, Filocori M & MOSERI-LABATE AM. Effect of long-term treatment with mifemulin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic
ovary syndrome. Journal of Clinical Endocrinology and Metabolism 2000 85 2767–2774. (https://doi.org/10.1210/jcem.85.8.6738)
27 Dunai A, Scott D, Finegood D, Quintana B & Whitcomb R. The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism 1996 81 3299–3306. (https://doi.org/10.1210/jcem.81.9.8784087)
28 Selva DM, Hogeveen KN, Innis SM & Hammond GL. Monosaccharide-induced lipogenesis regulates the human hepatic sex hormone-binding globulin gene. Journal of Clinical Investigation 2007 117 3979–3987. (https://doi.org/10.1172/JCI32249)
29 Covelli AD, Haring R, Wellons M, Vaidya D, Lehtimäki T, Keildson S, Lunetta KL, He C, Fornage M, Lagou V, et al. A genome-wide association meta-analysis of circulating sex hormone-binding globulin reveals multiple Loci implicated in sex steroid hormone regulation. PLoS Genetics 2012 8 e1002805. (https://doi.org/10.1371/journal.pgen.1002805)
30 Perry JR, Weedon MN, Langenberg C, Jackson AU, Lysenko V, Sparso T, Thorleifsson G, Grallert H, Ferrucci L, Maggio M, et al. Genetic evidence that raised sex hormone binding globulin (SHBG) levels reduce the risk of type 2 diabetes. Human Molecular Genetics 2010 19 535–544. (https://doi.org/10.1093/hmg/ddp522)
31 Xita N, Tsatsoulis A, Chatzikyriakidou A & Georgiou I. Association of the (TAAAA)n repeat polymorphism in the sex hormone-binding globulin (SHBG) gene with polycystic ovary syndrome and relation to SHBG serum levels. Journal of Clinical Endocrinology and Metabolism 2003 88 5976–5980. (https://doi.org/10.1210/jc.2003-030197)
32 Le TN, Nestler JE, Strauss JF 3rd & Wickham EP 3rd. Sex hormone-binding globulin and type 2 diabetes mellitus. Trends in Endocrinology and Metabolism 2012 23 32–40. (https://doi.org/10.1016/j.tem.2011.09.005)
33 Janne M, Deol HK, Power SG, Yee SP & Hammond GL. Human sex hormone-binding globulin gene expression in transgenic mice. Molecular Endocrinology 1998 12 123–136. (https://doi.org/10.1210/mend.12.1.0050)
34 Janne M & Hammond GL. Hepatocyte nuclear factor-4 controls transcription from a TATA-less human sex hormone-binding globulin gene promoter. Journal of Biological Chemistry 1998 273 34105–34114. (https://doi.org/10.1074/jbc.273.51.34105)
35 Yaden S, Palmer RT, Elko EE & Lal H. Comparative activity of antihypertensive drugs as determined by indirect measurement of blood pressure. Drug Development Research 1985 5 129–136. (https://doi.org/10.1002/ddr.430050205)
36 Whitesell SE, Hof JB, Yoliner AP & D’Aleyc LG. Comparison of simultaneous measurement of mouse systolic arterial blood pressure by radiotelemetry and tail-cuff methods. American Journal of Physiology: Heart and Circulatory Physiology 2004 286 2408–2415. (https://doi.org/10.1152/ajpheart.01089.2003)
37 Stern N, Sowers JR, Taylor IL & Golub M. Dopaminergic modulation of meal-stimulated and circadian secretion of pancreatic polypeptide in man. Journal of Clinical Endocrinology and Metabolism 1983 56 300–304. (https://doi.org/10.1210/jcem-56-2-300)
38 Herbst KL & Bhasin S. Testosterone action on skeletal muscle. Current Opinion in Clinical Nutrition and Metabolic Care 2004 7 271–277. (https://doi.org/10.1097/00075197-200405000-00006)
39 Gunsalus GL, Musto NA & Bardin W. Immunoassay of androgen binding protein in blood: a new approach for study of the seminiferous tubule. Science 1978 205 65–66. (https://doi.org/10.1126/science.635573)
40 Thaler MA, Seifert-Klauss V & Luppa PB. The biomarker sex hormone-binding globulin – from established applications to emerging trends in clinical medicine. Best Practice and Research Clinical Endocrinology and Metabolism 2015 29 749–60. (https://doi.org/10.1016/j.beem.2015.06.005)
41 Fortunati N, Raineri M, Cignetti A, Hammond GL & Frairia R. Control of the membrane sex hormone-binding globulin-receptor (SHBG-R) in MCF-7 cells: effect of locally produced SHBG. Steroids 1998 63 282–284. (https://doi.org/10.1016/S0039-128X(98)00021-X)
42 Saéz-López C, Rivera-Giménez M, Hernández C, Simó R & Selva DM. SHBG-C57BL/ksJ-db/db: a new mouse model to study SHBG expression and regulation during obesity development. Endocrinology 2015 156 4571–4581. (https://doi.org/10.1210/end.2015-1677)

Received in final form 19 October 2017
Accepted 15 November 2017
Accepted Preprint published online 15 November 2017