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Cross-sectional study of factors influencing sex hormone–binding globulin concentrations in normally cycling premenopausal women

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Objective: To assess the relationship between SHBG and 18 other hormonal and metabolic parameters in well characterized, normally cycling premenopausal women.
Design: Cross-sectional study.
Setting: University general clinical research center.
Subject(s): A total of 319 young healthy women with ovulatory menstrual cycles.
Intervention(s): None.
Main Outcome Measure(s): Midfollicular serum SHBG concentrations.
Result(s): In our final linear regression model, SHBG was negatively associated with bioavailable T and positively associated with adiponectin, associations that were independent from other parameters. SHBG was also positively associated with estrone sulfate, but only when taking into account confounding variables. Unexpectedly, there was no straightforward relationship between SHBG and insulin resistance according to homeostasis-model assessment.
Conclusion(s): Our results highlight the link between androgen action, as reflected by bioavailable T, and circulating SHBG concentrations in all premenopausal women and speak to the importance of the relationship between SHBG and adiponectin, which is at least in part independent from androgen action.
Clinical Trial Registration Number: NCT00393172. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
Key Words: Sex hormone–binding globulin, premenopause, testosterone, adiponectin, insulin resistance

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Sex hormone–binding globulin (SHBG) is a homodimeric plasma glycoprotein produced by hepatocytes and serves as the principal transport protein for T and E_2 (1, 2). According to the free hormone hypothesis, the biologic activity of sex steroids is affected by their free or unbound concentrations (3), providing one means by which SHBG can regulate sex steroid action. Other means that involve a cell membrane receptor for SHBG have also been proposed (4), including receptor-mediated endocytosis of steroids (5). The role of SHBG in regulating sex steroid action has long been of interest (6); in particular, low SHBG concentrations in women have been linked to conditions of androgen excess (2). As well, more recent work has emphasized the potential value of SHBG measurements in assessing risks for several other conditions, including the metabolic

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syndrome, type 2 diabetes, cardiovascular disease, and breast cancer (2, 7, 8).

Many studies have assessed the relationships between SHBG and assorted hormonal and metabolic parameters in different populations. Nonetheless, the precise mechanisms involved are poorly understood, complicated by many of the parameters having unidirectional or bidirectional cause and effect relationships with one another, and relationships explained by confounding factors. For example, estrogens increase hepatic SHBG production at least in part through a direct effect involving estrogen receptor α (9), but any resulting increase in SHBG might be expected to decrease bioavailable E_2. An example of confounding is that the effect of oral contraceptives to decrease bioavailable T, often attributed to increased SHBG, could simply be due to decreased ovarian production of androgens because of negative feedback on gonadotropins. Still another example of the complexity of the relationships is that type 2 diabetes risk for women is associated with high T concentrations, which is opposite of the case for men (10).

In any event, because few of the many studies of SHBG and physiologically related parameters have focused on well characterized normally cycling women, we performed a cross-sectional study of such women. We specifically used only baseline data from subjects who completed the Women in Steady Exercise Research (WISER) study, the primary purpose of which was to assess the effect of an exercise intervention on parameters associated with breast cancer risk (11, 12). As previously reported, the exercise intervention had no significant effect on SHBG or sex hormone concentrations despite significant increases in aerobic fitness and lean body mass and a significant decrease in percentage of body fat (13, 14), possibly because overall, the body composition changes that did occur were modest in degree.

MATERIALS AND METHODS

The WISER study was a randomized, controlled, parallel-arm study that investigated the effects of a 16-week aerobic exercise intervention on breast cancer biomarkers in young, healthy, sedentary, eumenorrheic women. The study was approved by the Human Subjects Review Committee at the University of Minnesota (IRB ID no. 0505M69867). Written informed consent was obtained from each participant before participation.

Details of the study design and methods have been described previously (11). Briefly, nonsmoking women aged 18–30 years with a body mass index (BMI) of 18–40 kg/m^2 (inclusive), having self-reported menstrual cycle length of 24–35 days, exercising fewer than two times a week, and residing in the Minneapolis–St. Paul metropolitan area were randomized into exercise and control groups. Exclusion criteria included use of any hormonal contraception in the past 3 months or depot-medroxyprogesterone acetate in the past 12 months, gynecologic disorders, metabolic or endocrine-related diseases, nonmelanoma cancer in the past 5 years, alcohol consumption of more than seven servings per week, current or recent (past 6 months) pregnancy, and body weight changes >10% over the past year. The primary study was completed by 319 of the 391 women who started the study by undergoing baseline measurements during the luteal phase of the menstrual cycle and subsequent follicular phase, and the findings presented here are based only on the baseline measurements of those 319 subjects (both exercisers and control subjects) who completed the study.

All biologic, anthropometric, and body composition measurements were taken at the General Clinical Research Center at the University of Minnesota. Height was measured with the use of a stadiometer to the nearest 0.1 cm without shoes (Scale Tronix). Body mass was measured to the nearest 0.1 kg with the use of an electronic scale (Scale Tronix). Body mass index was calculated by dividing weight in kg by height in meters squared (kg/m^2). Body fat percentage and android-gynoid fat ratio were assessed by dual-energy x-ray absorptiometry (DXA) with the use of a Lunar Prodigy apparatus (Lunar Radiation). A submaximal treadmill test was used to provide a measure of aerobic fitness, metabolic equivalents (METs) at 85% maximal heart rate, with workload converted into METS by means of a standard conversion formula (15).

Biologic Sample Analyses

Blood samples for serum and plasma concentrations were drawn between 6:45 and 11:00 a.m. after an overnight fast and the specimens stored frozen at −70°C. Blood draws took place during specific days of the menstrual cycle. Occurrence and timing of ovulation were assessed with the use of a commercial 9-day Assure LH ovulation kit (Conception Technologies). Day of ovulation was considered to be the day following a positive LH surge result. A midluteal-phase blood drawing was scheduled 6–9 days after ovulation for analysis of P, followed by a single midfollicular-phase blood drawing between cycle days 7 and 10 of the following cycle for all other serum or plasma concentrations. During this same cycle urine was collected for three consecutive 24-hour periods on cycle days 7–9, with the three refrigerated 24-hour collections pooled for aliquots, which were then stored at −20°C until analysis.

Serum concentrations of T, E_2, estrone sulfate, P, and SHBG were measured with the use of commercially available RIA kits for sex steroids and an ELISA for SHBG as previously described (13). Bioavailable fractions of T and E_2 were calculated with the use of the equations of Vermeulen et al. (16) and association constants estimated by Mazer (17). Plasma concentrations of insulin were measured by means of chemiluminescent immunoassay and combined with glucose concentrations to determine the homeostasis-model assessment (HOMA) index, a measure of insulin resistance that correlates well with insulin sensitivity as determined by the criterion-standard euglycemic clamp technique (18). Plasma concentrations of insulin-like growth factor 1 (IGF-1), IGF-binding protein 3 (IGFBP-3), and adiponectin were determined with the use of ELISA, those of leptin and C-reactive protein with the use of multiplex bead array assays, and those of F_2-isoprostanes with the use of a gas chromatography–mass spectrometry method, all as previously described (11, 19, 20), with IGF-1–IGFBP-3 ratios calculated on a mass basis. The urinary estrogen metabolites 2-hydroxyestradiol