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Maternal Testosterone Levels are Associated with C-Peptide Levels in the Mexican American Subset of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study Cohort

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Abstract

Altered sex hormone levels are thought to play an important role in adult-onset diseases including obesity, cardiovascular disease, and diabetes. They contribute to these complex diseases through changes in their availability, which is influenced, in part, by binding proteins. Insulin resistance, which is characteristic of these diseases, along with increased insulin secretion, is a physiologic change that occurs normally during pregnancy. To determine the relationship between insulin resistance and sex hormone levels, we examined the associations of sex hormone-binding globulin (SHBG) and testosterone with measures of glycemia and insulinemia in a healthy pregnant population. We measured fasting serum SHBG and testosterone levels in 215 Hispanic mothers of Mexican ancestry from the HAPO Study cohort and tested for associations between SHBG and testosterone levels and maternal plasma glucose and C-peptide. After adjusting for confounding variables, serum total testosterone (TT) was positively associated with fasting C-peptide (0.18 μg/l higher for TT higher by 1 SD, $p = 0.001$) and 1-h C-peptide (0.79 μg/l higher for TT higher by 1 SD, $p < 0.001$). Free testosterone (FT) was also positively associated with fasting C-peptide (0.19 μg/l higher for FT higher by 1 SD, $p < 0.001$), and 1-h C-peptide (0.83 μg/l higher for FT higher by 1 SD, $p < 0.001$). Although these findings are from a single cohort, this study provides evidence for an association between testosterone and C-peptide during pregnancy in a nondiabetic Hispanic obstetric population.

Introduction

Altered levels of sex hormones have been linked to adult-onset diseases like type 2 diabetes, polycystic ovary syndrome (PCOS), cardiovascular disease, obesity, and cancer [1, 2]. Hormones contribute to many diseases either through abnormal circulating levels of

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individual hormones, perturbations in the ratios between them, or the amount of biologically active hormone available through alterations in binding proteins like sex hormone-binding globulin (SHBG) [2].

Insulin resistance and increased gluconeogenesis are physiologic metabolic changes that occur during pregnancy to meet the demands of the growing fetus [3]. Increased peripheral insulin resistance during pregnancy is compensated for by an increase in insulin secretion to maintain glucose homeostasis [4]. Since both sex hormones and SHBG have been shown to be independently associated with metabolic diseases when there is a disruption of glucose homeostasis [1], they may be important in maintaining glucose homeostasis during pregnancy.

Total testosterone and SHBG rise throughout normal pregnancy [5]. The increase in SHBG and the placental aromatization of androgens to estrogens protect the mother and fetus from excess androgen exposure [5]. Women with lower levels of SHBG in the first trimester of pregnancy are at increased risk of developing gestational diabetes (GDM) as pregnancy progresses [6]. Increased androgens during pregnancy are often seen together with hyperinsulinemia in GDM and in pregnant women with PCOS [7]. Therefore, imbalances in both testosterone and SHBG during pregnancy may lead to metabolic consequences to the mother.

In this study, we used a subset of the large, rigorously phenotyped Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study cohort to address the hypothesis that SHBG and testosterone levels are associated with measures of glucose and C-peptide in a nondiabetic pregnant population. SHBG and testosterone levels were measured in Hispanic mothers of Mexican ancestry from Bell-flower, CA, USA and tested for associations between fasting serum SHBG and testosterone levels and maternal metabolic measures.

Materials and Methods

Participants

The HAPO Study investigated the association of maternal glycemia with adverse pregnancy outcomes. HAPO Study protocols were approved by the field center's institutional review board and written informed consent was obtained from all participants [8].

We studied 215 Hispanic participants of Mexican ancestry from Bellflower, CA, USA who self-identified as white Hispanic. Principal component analyses of the ancestry of these participants demonstrated similarities with the HapMap Phase III Mexican reference population from Los Angeles, CA, USA (MXL) (data not shown). All pregnant women were eligible to participate, except where specific exclusion criteria were present [8, 9].

Study protocols

Detailed HAPO Study methods have been published previously [8]. Gestational age was determined from the date of the last menstrual period or from an ultrasound performed between 6 and 24 weeks gestation, as described [8].

Oral glucose-tolerance test (OGTT)

Mothers provided information on ethnicity, smoking, and other parameters, and then underwent a 75 g OGTT and measurement of hemoglobin A1c (HbA1c) from whole blood at approximately 28 weeks gestation, as published previously [8]. Blood samples were obtained for plasma glucose levels at 0, 1, and 2 h, and serum C-peptide levels at 0 and 1 h. Caregivers and HAPO Study staff were “blinded” to the OGTT results except when exceeding specific thresholds.

Glucose analysis and unblinding

Fasting and 2-h OGTT samples were analyzed at the field center laboratory. Values were unblinded if fasting plasma glucose exceeded 5.8 mmol/l, if 2-h OGTT plasma glucose exceeded 11.1 mmol/l, or if any plasma glucose value was < 2.5 mmol/l. These cutoff values were predefined during recruitment of the HAPO participants [8], and are slightly less stringent than the new diagnostic thresholds determined by the International Association of Diabetes and Pregnancy Study Groups (IADPSG), which are based on the analysis of the HAPO study data [10]. Aliquots of all OGTT samples were also analyzed at the HAPO Central Laboratory for comparison. Unblinded individuals were excluded from the analyses since unblinded subjects may undergo medical interventions that can impact outcome measures of the study.

SHBG and testosterone assays

SHBG and testosterone assays were performed at the Steroid Hormone Assay Laboratory of Boston University School of Medicine (Boston, MA) on fasting serum collected during the OGTT. SHBG levels were measured by 2-site-directed immunofluorometry with a sensitivity of 0.5 nmol/l. The intra- and interassay coefficients of variation were 7.9 % and 8.4 %, respectively [11]. Total testosterone (TT) levels were measured by liquid chromatography-tandem mass spectrometry [12]. The limit of detection was 0.017 nmol/l (0.5 ng/dl), and intra- and interassay coefficients of variation were 8.9 % and 6.9 %, respectively [13]. Free testosterone (FT) was calculated from TT and SHBG levels using a method developed by Mazer [14].

Statistical analysis

Multivariate linear regression analyses were performed to evaluate the associations of serum SHBG and testosterone (TT and FT) levels with quantitative measures using the Statistical Package for the Social Sciences (SPSS) (v17.0, SPSS Inc, Chicago, IL, USA). Measures tested were glucose (fasting, 1-h and 2-h), C-peptide (fasting and 1-h), and HbA1c levels. C-Peptide, which is secreted in equimolar amounts with insulin, was used as a marker of insulin secretion.

Three models were analyzed for each measure. Model I was an unadjusted model. Model II was a minimally adjusted model that included adjustment for variables thought to be major confounders of glycemia during pregnancy: principal components of ancestry, maternal age, parity, gestational age at OGTT, and newborn gender. Model III was the fully adjusted model, adjusting for all covariates included in models I and II as well as maternal mean arterial pressure (MAP), height, and BMI at OGTT. When the measures tested were C-

peptide levels; we used fasting, 1-h, and 2-h plasma glucose values as covariates. When the measures tested were plasma glucose measures; we used fasting and 1-h C-peptide levels as covariates. When HbA1c was tested, we used fasting, 1-h, and 2-h plasma glucose values and fasting and 1-h C-peptide levels as covariates. Collinearity of all covariates in the fully adjusted model was excluded by appropriate tests. MAP was calculated as diastolic pressure + 0.33 (systolic pressure-diastolic pressure).

All covariates, except parity and newborn gender, were recorded at the time of the OGTT. Parity and newborn gender were determined from abstraction of the medical record following delivery. Principal components of ancestry were determined using SNPs on the Illumina IM Duo platform (data not shown). The first 2 principal components were used as covariates in our analyses. p-Values < 0.05 were considered significant.

Results

Descriptive characteristics for the HAPO Study have been previously reported [8]. After adjusting for confounding variables, serum total testosterone (TT) was positively associated with fasting C-peptide (0.18 μg/l higher for TT higher by 1 SD, $p = 0.001$) and 1-h C-peptide (0.79 μg/l higher for TT higher by 1 SD, $p < 0.001$) (Table 1). Free testosterone (FT) was also positively associated with fasting C-peptide (0.19 μg/l higher for FT higher by 1 SD, $p < 0.001$), and 1-h C-peptide (0.83 μg/l higher for FT higher by 1 SD, $p < 0.001$) (Table 1). SHBG levels were not significantly associated with any of the measures of glucose or C-peptide tested. All analyses were also performed with log-transformed SHBG and testosterone values and the results yielded similar p-values (data not shown).

Discussion and Conclusions

The purpose of our study was to investigate the associations of testosterone and SHBG with plasma glucose and C-peptide in nondiabetic pregnant women from the HAPO Study cohort. To our knowledge, this is the first study to examine the associations of SHBG and testosterone with maternal metabolic measures during pregnancy in a reproductively healthy Hispanic population. Our results demonstrate that pregnancy can be used as a model to further understand the relationship between androgen production and hyperinsulinemia.

Both TT and FT were positively associated with fasting and 1-h C-peptide. Similar positive associations have been found in other cohorts of healthy pregnant women [15, 16], as well as diabetic pre- and postmenopausal women [17, 18], indicating that in conditions with altered glucose metabolism and insulin resistance, like pregnancy and diabetes, there may be an interaction between glucose metabolism and sex hormone production. This interaction may be due to insulin resistance as a result of changes in sex hormone levels, or to alterations in sex hormone and SHBG production due to an already present hyperglycemic or hyperinsulinemic state. Evidence for the former is demonstrated by the increased frequency of GDM in hyperandrogenic conditions such as PCOS and congenital adrenal hyperplasia, regardless of the presence of pre-pregnancy insulin resistance [19, 20], indicating that hyperandrogenic states during pregnancy contribute to maternal glycemia.

We did not detect significant independent associations between SHBG levels and C-peptide, despite recent studies indicating that SHBG may have a direct role in diabetes. One reason may be that during pregnancy SHBG levels are influenced by a number of factors, effectively obscuring any possible association between SHBG and C-peptide. However, this lack of association has been observed by others as well, who have found associations between insulin sensitivity, but not measures of insulin secretion, including C-peptide [1].

Since these findings are from a single population, further studies are required in other cohorts to replicate the present findings and to examine the direction of causation in the relationship between testosterone and C-peptide during gestation. In conclusion, we have found that both total and free testosterone were positively associated with fasting and 1-h C-peptide levels in a healthy Hispanic obstetric population, thus providing evidence for a role for testosterone in glucose homeostasis during pregnancy.

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Table 1

Associations of SHBG, TT, and FT levels with metabolic measures in Hispanic mothers of Mexican ancestry.

Measure	Model I				Model III			
	Beta	L95	U95	p-Value	Beta	L95	U95	p-Value
SHBG								
Fasting pg (mg/dl)	−0.01	−0.01	0.00	0.004	0.00	−0.01	0.00	0.609
1 h pg (mg/dl)	−0.02	−0.05	0.00	0.047	−0.01	−0.04	0.01	0.392
2h pg (mg/dl)	−0.02	−0.03	0.00	0.105	−0.01	−0.03	0.01	0.462
Fasting C-peptide (μg/l)	0.00	0.00	0.00	0.001	0.00	0.00	0.00	0.080
1h C-peptide (μg/l)	0.00	−0.01	0.00	0.013	0.00	0.00	0.00	0.153
HbA1c (mmol/mol)	0.00	0.00	0.00	0.601	0.00	0.00	0.00	0.503
TT								
Fasting pg (mg/dl)	−0.01	−0.03	0.01	0.257	−0.03	−0.05	0.00	0.020
1 h pg (mg/dl)	0.02	−0.07	0.11	0.651	−0.02	−0.12	0.07	0.600
2h pg (mg/dl)	−0.01	−0.08	0.06	0.782	0.00	−0.08	0.07	0.922
Fasting C-peptide (μg/l)	0.01	0.00	0.01	<0.001	0.00	0.00	0.01	0.001*
1 h C-peptide (μg/l)	0.02	0.01	0.03	<0.001	0.02	0.01	0.03	<0.001*
HbA1c (mmol/mol)	0.00	0.00	0.00	0.017	0.00	0.00	0.00	0.528
FT								
Fasting pg (mg/dl)	0.68	−0.56	1.93	0.281	−0.71	−1.92	0.49	0.244
1 h pg (mg/dl)	4.17	−0.88	9.22	0.105	0.38	−4.87	5.62	0.888
2h pg (mg/dl)	2.09	−1.79	5.96	0.290	1.12	−3.00	5.24	0.592
Fasting C-peptide (μg/l)	0.40	0.25	0.56	<0.001	0.25	0.13	0.37	<0.001*
1 h C-peptide (μg/l)	1.40	0.87	1.93	<0.001	1.07	0.58	1.57	<0.001*
HbA1c (mmol/mol)	0.00	0.00	0.00	0.004	0.00	0.00	0.00	0.457

pg = Plasma glucose; HbA1 c = Hemoglobin A1 c variant

L95/U95 = lower and upper limits of the 95 % confidence interval

Both the unadjusted model (model I) and the fully adjusted model (model III) are shown for comparison

†The regression coefficient (Beta) and p-values are from linear regressions of measures against SHBG, TT, and FT levels. Beta is the regression coefficient for the measure per 1 SD change in SHBG, TT, FT

*Associations significant at $p < 0.05$ in the fully adjusted model