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Post-heparin lipolytic enzyme activities, sex hormones and sex hormone-binding globulin (SHBG) in men and women: The HERITAGE Family Study

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Abstract

We tested the hypothesis that androgen, estrogen, and sex hormone-binding globulin (SHBG) levels would be significantly related to post-heparin hepatic lipase (HL) and lipoprotein lipase (LPL) activities in a sample of Caucasian men ($n = 233$) and women ($n = 235$) aged 17–64 years from the HERITAGE Family Study. Body composition (hydrostatic weighing), abdominal adipose tissue distribution (computed tomography), plasma lipid–lipoprotein and hormone levels, and post-heparin lipases activities were measured. HL activity was significantly higher in males, whereas LPL activity was higher in women ($P < 0.005$). In women only, HL activity was positively associated with body fat mass ($r = 0.17$, $P < 0.05$) and intra-abdominal adipose tissue area ($r = 0.18$, $P < 0.05$). Significant associations were also found between fasting insulin and LPL activity ($r = -0.16$, $P < 0.05$ and $r = -0.18$, $P < 0.005$) as well as HL activity ($r = 0.22$, $P < 0.005$, and $r = 0.27$, $P < 0.0001$) in men and women, respectively. A positive association between total testosterone and HL activity was noted in men ($r = 0.13$, $P = 0.05$). In women, plasma SHBG levels were negatively associated with HL activity ($r = -0.48$, $P < 0.0001$), and statistical adjustment for body fat mass, visceral adipose tissue area, and fasting insulin did not attenuate this correlation. In multivariate analyses with models including adiposity variables and measurements of the hormonal profile, insulin, and testosterone levels were both independent positive predictors of HL activity in men. In women, hormone use was a significant positive predictor, and SHBG level a strong negative predictor of HL activity, independent of plasma estradiol and testosterone concentrations. Fasting insulin was the only significant predictor of LPL activity in men (negative association), whereas menstrual status, fasting insulin (negative associations), and plasma SHBG levels (positive association) were all independent predictors of LPL activity in women. These results suggest that the postulated sensitivity of lipolytic enzymes to androgens and estrogens is reflected by a strong negative association between SHBG levels and HL, and a lower magnitude positive association of this hormonal parameter to LPL activity in women. These associations appear to be independent from concomitant variation in total adiposity or body fat distribution.

Keywords: Sex hormone-binding globulin; Hepatic lipase; Lipoprotein lipase; HDL-cholesterol; Sex hormones

1. Introduction

The well-known gender differences in plasma lipid and lipoprotein levels suggest a strong regulation of lipid metabolism by sex steroid hormones, which has been postulated to occur partly through androgen and estrogen effects

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on hepatic lipase (HL), and, to a lesser extent, lipoprotein lipase (LPL) [1–3]. HL exerts both triglyceride lipase and phospholipase A1 activities, and is involved in HDL and LDL remodeling [4], whereas LPL catalyzes the hydrolysis of triglycerides contained in chylomicrons and VLDL particles [5]. Sex steroid hormones have been reported to regulate both enzymes directly in some experimental models, with androgens stimulating HL expression and estradiol inhibiting both LPL and HL expression [4,5]. In numerous experimental conditions, it has been shown that subjects with elevated LPL activity have low triglyceride and high HDL-cholestrol (HDL-C) levels, whereas subjects with high HL activity have low HDL-cholestrol levels [6]. Plasma levels of sex hormone-binding globulin (SHBG) are also regulated by estradiol and testosterone [7], and low levels of this protein have been associated with dyslipidemia [8–11], which also supports a possible link between SHBG and lipolytic enzyme activities.

The associations of sex hormones and SHBG levels to blood lipid alterations may also be mediated indirectly through their association with components of the metabolic syndrome [12,13]. For example, associations between low SHBG levels and elevated total and abdominal adiposity [8,12], as well as hyperinsulinemia and insulin resistance have been reported [14–16]. The inhibiting effect of insulin on SHBG secretion in the liver has been considered a primary factor in the relationship between low SHBG and features of the metabolic syndrome [17,18]. Insulin also exerts a direct regulatory effect on both HL and LPL [4,5], which could also explain the associations observed. Finally, total and visceral adiposity measures were found to be critical correlates of the associations between sex hormones and variables of the metabolic profile in a number of our studies [3,8–10]. Whether a significant association between plasma sex hormones, SHBG, and lipolytic enzymes exists and, if so, whether it is independent from variation in adiposity or insulin levels has not been examined in a large cohort.

In the present study, we tested the hypotheses that there are significant associations between SHBG, sex steroid hormone levels, and post-heparin lipolytic enzyme activities, and that these associations are independent of concomitant variation in adiposity and the metabolic profile.

2. Methods

2.1. Subjects

The HERITAGE Family Study cohort has been described previously [19]. In the present study, analyses were performed on a subsample of 233 Caucasian men, aged 36.1 ± 14.8 years, and 235 Caucasian women, aged 34.0 ± 13.9 years. Results for the black population will be reported separately. To be included in the study, subjects had to be healthy and sedentary, which was defined as no regular physical activity over the previous 3 months. Other exclusion criteria were body mass index greater than 40 kg/m^2 because of the metabolic abnormalities commonly associated with extreme obesity, although exceptions were accepted with sufficient clinical justification on a case by case basis. The upper limits of acceptable blood pressure (BP) levels were a systolic BP of 159 mmHg and a diastolic BP of 99 mmHg for two out of three readings in the sitting position after at least 5 min of rest. Individuals on diuretic or antihypertensive drugs at the initial interview were permitted to enter the study if they were free of hypertensive complications, if their personal physician permitted them to discontinue their medication(s), and if their blood pressure level met the above criteria after at least 3 months off medication. Upon physical examination, subjects with suspicious symptoms or suggestive medical histories had to have their personal physicians provide additional medical information, test results, and hospital records prior to inclusion or exclusion. A past history and/or physical or laboratory finding at medical examination required exclusion from the study [19]. Women were asked to provide information on their menstrual cycle and hormone use (oral contraceptives or postmenopausal hormone replacement therapy). The study protocol was approved by the institutional review board at each clinical center involved: Arizona State University (Indiana University since January 1996); Laval University (Pennington Biomedical Research Center since 1999); Washington University; University of Minnesota; and The University of Texas at Austin (Texas A&M since 1997) [20]. All participants signed an informed consent document.

2.2. Anthropometrics, body composition measurements, and fat distribution

Body weight and height were measured according to standardized procedures [21]. Body density was obtained using hydrostatic weighing [22]. The mean of three valid measurements was used to calculate percent body fat from body density using the equations of Siri [23] for men and Lohman [24] for women. Fat mass was obtained by multiplying body weight by percent body fat. The coefficients of variation for repeated measures ($n = 60$, three times) were 4.3, 1.1, and 4.1% for fat mass, fat-free mass, and percent fat respectively. The coefficient of variation for all centers was $\leq$5% for these measurements [25]. Total and visceral adipose tissue areas were measured by computed tomography as previously described [26–28]. Abdominal subcutaneous adipose tissue area was obtained by calculating the difference between total and visceral adipose tissue areas. A single, standardized calibration unit was developed by using lard carefully sealed within a Plexiglas cylinder. This unit was transported to each clinical center every 6–12 mo to ensure the reliability and consistency of the method between the 4 centers. Image analyses were conducted in the same center, at the same time, and by the same technician to minimize technical error.

2.3. Plasma steroid hormone, SHBG, and insulin concentrations

Duplicate blood samples were obtained at 24 h intervals from an antecubital vein into vacutainer tubes containing no anticoagulant (Savant Instrument Co.) in the morning, after a 12 h fasting period. Fasting serum was prepared according to a standard protocol [29]. After centrifugation of blood at 2000 × *g* for 15 min at 4 °C, two aliquots of 2 ml were placed in cryogenic tubes, frozen, and sent to Laval University, where they were stored at −80 °C, until analysis. C_{19} steroids were extracted with hexane–ethyl acetate (9:1, v/v). In-house RIA was performed. Estradiol and SHBG were assayed directly using commercially available kits (Diagnostics Systems Laboratories Inc.). The free androgen index (FAI) was calculated as the ratio of testosterone/SHBG multiplied by 100 [30]. RIA was used for plasma insulin level measurement after polyethylene glycol separation [31]. All immunoreactive insulin (insulin, proinsulin, and split proinsulin) was measured in this study.

2.4. Lipoprotein and lipid profile

All blood samples were prepared according to a standard protocol and sent to the Lipid Core Laboratory (Lipid Research Center, Laval University Medical Center). Measurements were performed with a Technicon RA-500 (Bayer Corporation Inc., Tarry town, Technicon Instruments) analyzer on plasma and lipoprotein fractions by enzymatic methods [32]. Plasma VLDLs were isolated by ultracentrifugation, whereas the HDL fraction was obtained after precipitation of LDL in the infranatant with heparin and $MnCL_2$ [33]. The infranatant fraction was used before and after the precipitation step for cholesterol and triglyceride level measurements in LDL and HDL. The subfractions of HDL_2 and HDL_3 were obtained after further precipitation of HDL_2 with dextran sulfate [34]. Apolipoprotein B (Apo B) concentrations were measured in total plasma and LDL fraction by the rocket immunoelectrophoretic method of Laurell [35] as previously described [32].

2.5. Post-heparin plasma lipase activities

Blood samples were collected 10 min after an intravenous injection of heparin (60 IU/kg body weight) in the 468 subjects of the study. The injection was performed in the morning after a 12 h overnight fast. All frozen blood samples were sent to the Lipid Core Larboratory and post-heparin LPL and HL activities were measured in plasma using a modification of the method of Nilsson-Ehle and Ekman [36] as previously described [6]. Lipase activities were expressed as nanomoles of oleic acid released per milliliter of plasma per minute (nmol ml^{-1} min^{-1})).

2.6. Statistical analysis

Data are presented as mean ± standard deviation. Homogeneity of group variances between men and women was tested using the Levene test, accepting unequal variances at $P < 0.05$. Variances were unequal for the following variables: fat-free mass, intra-abdominal and subcutaneous adipose tissue areas, cholesterol, HDL-C, triglycerides, apolipoprotein B, fasting insulin, estradiol, testosterone, and SHBG levels and estradiol/testosterone, the free androgen index and cholesterol/HDL-C. The Welch analysis of variance procedure was used to compare means for these variables. Mean differences between men and women for other variables were tested using unpaired *t*-tests. Spearman rank correlation coefficients were computed to quantify relationships of lipolytic enzyme activities with adiposity measures as well as metabolic and hormonal variables. Multivariate regression analyses were used to identify the independent correlates of lipolytic enzyme activities. Variables included in the models were: abdominal adipose tissue area, body fat mass and fat-free mass, SHBG, estradiol, testosterone, and fasting insulin. Menstrual status and hormone use (oral contraceptives or hormone replacement therapy) were also included in models for women. Statistical analyses were performed with the JMP software (SAS Institute, Cary, NC).

3. Results

Physical and metabolic characteristics of men and women of the study are shown in Table 1. Subject's age and body fat mass were identical for men and women. However, men were characterized by higher body fat-free mass. BMI was also significantly different between sexes, with 26.4 ± 4.6 kg/m^2 for men and 24.7 ± 4.8 kg/m^2 for women ($P < 0.005$). Men were characterized by a less favorable lipid profile than women in terms of cardiovascular disease risk. Expected gender differences in plasma hormone and SHBG levels were observed. Women had higher estradiol and SHBG levels, while testosterone levels and FAI were higher in men.

Information on menopausal status and hormone use was available for 233 women (99.1% of the sample). The group included 198 premenopausal women (75 oral contraceptive users and 123 non-users), and 34 postmenopausal women (15 hormone replacement therapy users and 19 non-users). LPL activity was significantly higher in postmenopausal women compared to premenopausal women ($P < 0.0001$) and this effect persisted when excluding hormone replacement therapy and oral contraceptive users ($P < 0.04$). In premenopausal women, oral contraceptive users had significantly lower HL activity compared to non-users ($P < 0.0001$), whereas LPL was not different between these two groups. No difference in LPL or HL activity was found between postmenopausal women using or not using hormone replacement therapy (data not shown).

Table 1
Physical characteristics of men ($n = 233$) and women ($n = 235$) of the study

	Men	Women
Age (years)	36.1 ±14.8	34.0 ± 13.9
BMI (kg/m^2)	26.4 ± 4.6	24.7 ± 4.8**
Fat mass (kg)	19.7 ± 10.6	20.8 ± 10.8
Fat-free mass (kg)	63.4 ± 7.7	45.6 ± 5.2**
Abdominal adipose tissue areas (cm^2)		
Total	327.5 ± 173.7	353.8 ± 179.8
Visceral	106.8 ± 63.8	72.3 ± 51.2**
Subcutaneous	220.7 ± 126.2	281.5 ± 141.7**
Lipid profile		
Cholesterol (mmol/l)	4.5 ± 1.0	4.4 ± 0.9
LDL-C (mmol/l)	3.1 ± 0.9	2.9 ± 0.8*
HDL-C (mmol/l)	0.9 ± 0.2	1.2 ± 0.3**
Triglycerides (mmol/l)	1.5 ± 0.9	1.2 ± 0.6**
Cholesterol/HDL-C	5.1 ± 1.6	4.0 ± 1.1**
Apolipoprotein B (g/l)	0.9 ± 0.2	0.8 ± 0.2**
HL activity (nmol ml^{-1} min^{-1})	243.8 ± 61.3	173.4 ± 62.6**
LPL activity (nmol ml^{-1} min^{-1})	48.9 ± 25.9	63.5 ± 32.1**
Insulin/glucose homeostasis		
Insulin (pmol/l)	4.1 ± 0.6	4.0 ± 0.5
Glucose (mmol/l)	5.2 ± 0.6	5.0 ± 0.7**
Insulin/glucose	13.5 ± 8.8	12.2 ± 5.4*
Hormonal profile		
Estradiol (pg/ml)	67.1 ± 43.8	146.5 ± 198.6**
Testosterone (ng/ml)	14.8 ± 6.0	1.4 ± 0.7**
Estradiol/testosterone ratio	5.3 ± 5.8	160.5 ± 357.0**
Free androgen index	43.1 ± 22.8	2.0 ± 1.8**
SHBG (nmol/l)	39.1 ± 16.3	89.0 ± 51.1**

BMI: body mass index; HL: hepatic lipase; LPL: lipoprotein lipase.
* Significantly different from men, $P < 0.05$.
** Significantly different from men, $P < 0.005$.

Table 2
Spearman rank correlation coefficients between HL activity as well as LPL activity and the metabolic profile in men ($n = 233$) and women ($n = 235$) of the study

	Men		Women	
	HL	LPL	HL	LPL
Age	−0.24**	0.06	−0.07	0.24**
BMI	0.07	−0.08	0.24**	0.03
Fat mass	0.10	−0.04	0.17*	0.06
Fat-free mass	0.04	−0.03	0.09	−0.08
Abdominal AT areas				
Total	0.06	−0.06	0.17*	0.08
Visceral	−0.05	0.03	0.18*	0.07
Subcutaneous	0.11	−0.11	0.16*	0.08
Lipid profile				
Cholesterol	−0.11	0.03	−0.10	0.23**
LDL-C	−0.05	0.06	0.002	0.17*
HDL-C	−0.02	0.31***	−0.40***	0.50***
Triglycerides	−0.05	−0.18**	−0.06	−0.16*
Cholesterol/HDL-C	−0.06	−0.20**	0.23**	−0.23**
Apolipoprotein B	−0.09	−0.03	−0.05	0.08
Insulin/glucose homeostasis				
Insulin	0.22**	−0.16*	0.27***	−0.18**
Glucose	0.01	0.06	0.15*	−0.01
Insulin/glucose	0.23**	−0.17*	0.25***	−0.19**

BMI: body mass index; HL: hepatic lipase; LPL: lipoprotein lipase; AT: adipose tissue.
* $P < 0.05$.
** $P < 0.005$.
*** $P < 0.0001$.

Associations between plasma lipolytic enzyme activities and metabolic variables are shown in Table 2. In men, LPL activity was positively associated with HDL-C ($P < 0.0001$) and negatively with the cholesterol/HDL-C ratio and triglyceride levels ($P < 0.005$). Several significant associations were noted in women. HL activity was positively associated with abdominal adipose tissue area ($P < 0.05$) and insulin/glucose ratio ($P < 0.001$), but negatively with HDL-C ($P < 0.0001$). LPL activity was negatively correlated with variables of the lipid profile, and insulin/glucose variables.

Table 3 shows the correlation coefficients between lipolytic enzyme activities, sex hormone, and SHBG levels. A significant positive association between plasma testosterone

Table 3
Spearman rank correlation coefficients between post-heparin HL activity and LPL activity with hormonal profile in men ($n = 233$), premenopausal women (75 oral contraceptive users and 123 non-users), and postmenopausal women (15 hormone replacement therapy users and 19 non-users) of the study

	Men		Women							
			Premenopausal		Premenopausal-OC		Postmenopausal		Postmenopausal-HRT	
	HL	LPL	HL	LPL	HL	LPL	HL	LPL	HL	LPL
Estradiol	0.12	0.09	−0.04	0.12	0.02	0.09	−0.22	0.14	−0.01	0.04
Testosterone	0.13+	0.01	−0.11	0.09	0.24*	−0.17	0.05	0.01	−0.07	−0.004
Ratio estradiol/testosterone	0.02	0.05	0.01	0.07	−0.08	0.16	−0.28	0.13	0.05	−0.04
Free androgen index	0.19**	0.01	0.03	0.03	0.59***	−0.28*	0.25	−0.35	0.16	−0.09
SHBG	−0.10	0.03	−0.17	0.06	−0.56***	0.20	−0.42	0.56*	−0.51+	0.25

HL: hepatic lipase; LPL: lipoprotein lipase, Premenop.: OC: oral contraceptive use; HRT: hormone replacement therapy use.
+ $P = 0.05$.
* $P < 0.05$.
** $P < 0.005$.
*** $P < 0.0001$.

levels and HL activity was noted in men ($P = 0.05$). Plasma levels of estradiol, testosterone, and SHBG were not significantly correlated with lipolytic enzyme activities in premenopausal women not using oral contraceptives. However, plasma testosterone levels and the free androgen index were positively correlated, whereas SHBG concentration was negatively correlated with HL activity in premenopausal women using oral contraceptives. SHBG level was also positively associated with LPL activity in postmenopausal women not using hormone replacement therapy ($P < 0.05$) and negatively with HL activity in postmenopausal women using hormone replacement therapy ($P = 0.05$). A trend for a negative association between SHBG and HL activity was noted in postmenopausal women not using hormone replacement therapy.

The association between plasma SHBG levels and post-heparin HL activity in the whole sample of women is shown in Fig. 1. A significant positive association was also noted between plasma HDL-C levels and SHBG. These associations were independent from concomitant variations in metabolic and adiposity variables, including fasting insulin, body fat mass, and visceral adipose tissue area.

Multivariate regression analyses were performed with models including adiposity variables and measures of the hormonal profile to identify independent correlates of post-heparin lipolytic enzyme activities (Tables 4 and 5). In men, we found that fasting insulin and testosterone were independent positive predictors of hepatic lipase activity. SHBG level and visceral adipose tissue area were also independent correlates of HL activity in men, although the variance explained was small and the association negative. Fasting insulin was the only significant predictor of LPL

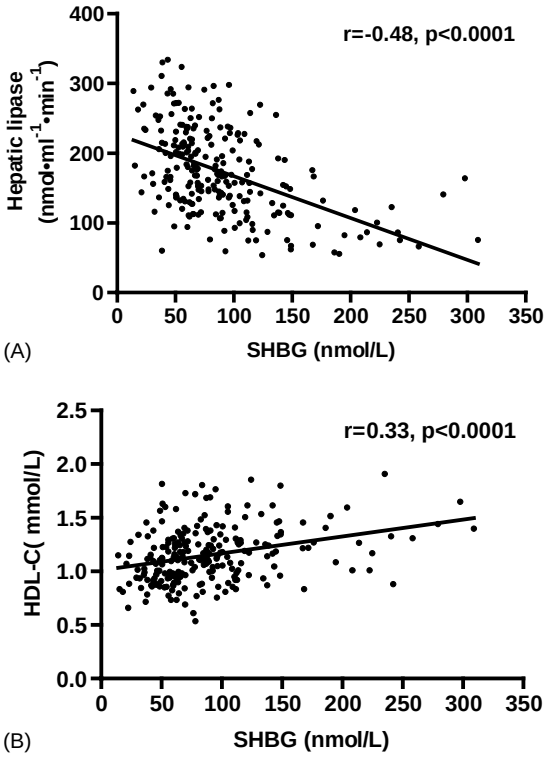

Fig. 1. Correlation between plasma SHBG levels and (A) post-heparin HL activity; and (B) HDL-cholesterol levels in women of the study. Unadjusted Spearman rank correlation coefficients are shown. Fat mass, fasting insulin, and visceral adipose tissue area-adjusted coefficients were $r = -0.39$, $P < 0.001$ for the association between SHBG and HL activity, and $r = 0.24$, $P < 0.0002$ for the association between SHBG and HDL-C.

Table 4
Multivariate regression analyses in men

Dependent variable	Independent variable	Parameter estimate	Partial ($R^2 \times 100$)	Total ($R^2 \times 100$)	$P \leq$
HL	Fasting insulin	24.42	4.9	11.1	0.0008
	Testosterone	2.35	2.3		0.02
	SHBG	−0.40	1.6		0.05
	Visceral AT area	−0.29	2.3		0.02
LPL	Fasting insulin	−6.75	1.8	1.8	0.04

Regression models included abdominal adipose tissue areas, body fat mass and fat-free mass, SHBG, estradiol, testosterone, and fasting insulin. HL: hepatic lipase; LPL: lipoprotein lipase; AT: adipose tissue.

Table 5
Multivariate regression analyses in women

Dependent variable	Independent variable	Parameter estimate	Partial ($R^2 \times 100$)	Total ($R^2 \times 100$)	$P \leq$
HL	SHBG	−0.49	22.8	26.0	0.0001
	Fasting insulin	18.67	2.0		0.01
	Hormone use	7.73	1.2		0.06
LPL	Menstrual status	−10.80	6.5	13.1	0.0001
	Fasting insulin	−15.74	4.7		0.0007
	SHBG	0.10	1.2		0.03

Regression models included abdominal adipose tissue areas, body fat mass and fat-free mass, SHBG, estradiol, testosterone, fasting insulin, menstrual status, and hormone use (oral contraceptives or hormone replacement therapy). HL: hepatic lipase; LPL: lipoprotein lipase.

activity in men (negative association). In women, menstrual status, fasting insulin (negative associations), and plasma SHBG levels (positive association) were all predictors of LPL activity, independent of estradiol and testosterone levels. Fasting insulin and hormone use (oral contraceptives or hormone replacement therapy) were also significant positive predictors of HL activity in women. SHBG was a strong independent negative predictor of post-heparin HL activity in women, explaining 22.8% of its variance.

4. Discussion

The purpose of this study was to investigate the relationships between post-heparin lipolytic enzyme activities, plasma sex hormones, and SHBG levels. We specifically tested the hypothesis that SHBG as well as androgen/estrogen levels are related to post-heparin lipolytic enzyme activities independently of variations in adiposity and fasting insulin. Significant correlations were observed between lipolytic enzyme activities and several metabolic variables, especially in women. As expected, we found a positive correlation between total testosterone and HL activity in men whereas hormone use was a significant positive predictor of HL activity in women. We found a strong inverse and independent association between SHBG levels and HL activity in women. A lower magnitude positive association between SHBG and LPL activity was also found in women. These results suggest that the postulated sensitivity of HL to androgens and estrogens is reflected by a strong significant association with SHBG in women. Few studies have focused on the relationships between lipolytic enzyme activities and endogenous sex hormones in large samples. In fact, to our knowledge, this is the first study to examine this issue in a large cohort of men and women.

Several studies performed by the group of Nikkilä (reviewed in [37]) have examined the regulation of hepatic lipase by sex steroids. These studies demonstrated that androgens increase, and estrogens decrease HL activity, and lead to related changes in HDL particle concentration and composition [37,38]. In the present study, a significant positive association was found between total testosterone levels as well as the free androgen index and HL activity in men. Since the unbound fractions of estrogen and testosterone are thought to be biologically active [11], it is possible that direct measures of free testosterone or free estradiol levels would have been stronger correlates of lipolytic enzyme activities in the present study. On the other hand, Sorva et al. [2] found that changes in endogenous testosterone levels following human chorionic gonadotropin injections 13 boys correlated positively with post-heparin plasma HL activity [2]. The fact that androgen levels were not hormonally manipulated in the present study may also partly explain the fact that total testosterone was a relatively minor contributor to HL activity variance.

In women, the relationships between endogenous sex hormones, SHBG, and the lipoprotein profile have been investigated in many studies. The reduced estrogen levels at menopause are associated with higher LDL-C and lower HDL-C levels [3,39], and elevated free androgen levels are associated with increased total cholesterol, LDL-C and triglyceride levels in premenopausal women [40]. Further, Pugeat et al. [41] reviewed the interrelations between SHBG, plasma lipoprotein, and cardiovascular risk, and concluded that plasma SHBG levels generally correlate positively with HDL-C. These relationships between sex hormones, SHBG, and lipoprotein levels, especially HDL-C, have been postulated to result, at least partly, from the regulation of HL by androgens and estrogens [2,3,12,37,41]. In women of the present study, we found a significant positive correlation between SHBG and HDL-C, which is consistent with this suggestion. Also in agreement with previous studies [9–11], we found that this relationship was independent of total adiposity, body fat distribution, and fasting insulin levels, as SHBG was a strong positive correlate of HL activity in multivariate models.

Hormone use was also a significant positive predictor of HL activity, whereas plasma levels of estrogens were not. Accordingly, the correlation pattern between SHBG and HL appeared to be slightly different among hormone status groups. Although some of these differences may be partly attributable to the size of each subgroup, we suggest that they may also be due to the variability inherent to a single fasting plasma estradiol measurement [42]. In this sense, the variable “hormone use” may represent a more robust assessment of hormonal status. Similarly, SHBG may represent a more stable measure of hormone status, compared to individual sex hormone measurements [43]. We recently suggested that SHBG could act as an integrated marker for not only hormonal, but also metabolic and even nutritional signals, which all modulate its production in the liver [44]. Although the contribution of SHBG to HL activity variance was independent from that of hormone use in multivariate models, it is still possible that SHBG level modulation resulting from both hormonal and metabolic signals explains this relationship.

In the present study, lipolytic enzyme activities were measured in post-heparin plasma. This methodological factor is important to consider in the interpretation of data, especially for LPL activity. As opposed to tissue homogenate LPL activity, post-heparin LPL measures reflect activity from several different tissues including, heart, skeletal muscle, and adipose tissue [45]. A previous report by Després and colleagues [6] found a positive association between abdominal adipose tissue LPL activity and insulin response to oral glucose. However, in the same study, post-heparin plasma LPL activity tended to be negatively associated with the insulin response [6]. Results of the present study are concordant with this study as fasting insulin level was a negative correlate of post-heparin LPL activity in both sexes. This pattern of correlation is

more consistent with that observed with skeletal muscle LPL [5].

Hyperinsulinemia is a central feature of the metabolic syndrome and is frequently observed in association with abdominal obesity and concomitant blood lipid alterations [8,10,14–16]. Accordingly, fasting insulin was a significant positive predictor of HL in men and women of the study. On the other hand, Plymate et al. [17] and Nestler et al. [18] have demonstrated that insulin has a direct inhibiting effect on hepatic SHBG secretion in the liver. In women of the present study, statistical adjustment for fasting insulin or adiposity measures did not affect the negative association between HL and SHBG level. Thus, regulation of HL by free androgens and/or estrogens may be presumably independent from concomitant variations in insulin levels or abdominal adiposity.

In summary, we found that testosterone levels in men and hormone use in women were significant positive predictors of HL activity. SHBG level was the most significant correlate of HL activity in women. These results suggest that the postulated sensitivity of lipolytic enzymes to androgens and estrogens is reflected by a strong negative association between SHBG levels and HL, and a lower magnitude positive association of this hormonal parameter and LPL activity in women. These associations appear to be independent from concomitant variation in adiposity or body fat distribution.

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