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An association between a common variant (G972R) in the *IRS-1* gene and sex hormone levels in postmenopausal breast cancer survivors

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Abstract

Insulin receptor substrate-1 (*IRS-1*) is a key downstream signaling molecule common to both the insulin and IGF signaling pathways that can interact with the estrogen pathway to regulate breast cell growth. We investigated whether a putative functional variant for *IRS-1* (G972R) influences circulating levels of sex hormones, sex hormone binding globulin (SHBG), C-peptide, and insulin-like growth factor 1 (IGF-1) levels among postmenopausal African-American and non-Hispanic white breast cancer patients enrolled in the Health, Eating, Activity, and Lifestyle (HEAL) Study. Circulating levels of sex hormones and growth factors can influence breast cancer recurrence and survival. Serum estrone, estradiol, testosterone, SHBG, IGF-1 and C-peptide were measured in 468 patients at 30+ months post diagnosis. Non-protein bound hormone levels (free estradiol, free testosterone) were calculated. In African-American patients, the *IRS-1* variant was associated with increased serum levels of estrone (p=0.02), free estradiol (p=0.04), total testosterone (p=0.04), free testosterone (p=0.006) and decreased levels of sex hormone-binding globulin (p=0.02). No association was present for white patients. Our findings provide suggestive evidence that *IRS-1* G972R variant may be associated with circulating levels of sex hormones and SHBG in African American breast cancer survivors.

Keywords

African-American; breast cancer; *IRS-1*; polymorphism; sex hormones

Introduction

Of the established determinants of breast cancer occurrence, estrogen is one of the most important predictors of disease and prognosis. The majority of accepted risk factors for breast cancer such as early age at menarche, late age at menopause, late age at first full-term pregnancy, and use of hormone replacement therapy (HT) influence a woman's lifetime exposure to estrogens, progesterone and other sex hormones [1]. Cumulative estrogen exposure after the diagnosis of breast cancer may also influence breast cancer prognosis. High plasma estrogen levels have been associated with a shortened disease-free interval in postmenopausal breast cancer patients with recurrent disease [2]. Tamoxifen, an antiestrogen in the breast, blocks estrogen binding to the estrogen receptor (ER) and is now a standard part of clinical breast cancer care. It has been shown to be effective in reducing both disease progression and recurrence in ER positive tumors [3,4]. Aromatase inhibitors, which block estrogen production, have been shown to improve disease free survival when used as the initial adjuvant treatment [5] or after 2-3 years of tamoxifen therapy [6].

Like estrogens, the insulin and insulin-like growth factor (IGF) pathways have been associated both with breast cancer development and prognosis [7-10]. The effect of estrogen appears to be modified by its interaction or cross-talk with the insulin and IGF pathways [11,12] and these pathways act synergistically with estrogen to enhance breast cell proliferation [9] in both normal [13] and cancerous cells [14]. Estradiol (E₂) modifies the effect of the IGF-1 pathway by upregulating the expression of several components of the pathway including the insulin-like growth factor 1 receptor (IGF1R), insulin receptor substrate 1 (IRS-1), and IGF binding proteins (IGFBPs) [11,15-17]. Conversely, the insulin and IGF-1 pathways have been shown to induce ER phosphorylation and therefore receptor activity through a phosphatidylinositol 3-kinase (PI3-kinase) or extracellular signal-regulated (ERK-mediated) mechanism [12,18-20]. It also has been shown through *in vitro* assays, that co-administration of estrogen and growth factors to cells has synergistic effects on proliferation compared to either treatment alone [11,12]; however, the exact mechanism of this synergy has not been resolved.

A key downstream signaling molecule common to both the insulin and IGF-1 signaling pathways that is up-regulated by E₂, IRS-1, is a protein that is likely to influence the synergistic relationship between sex hormones, insulin, and IGF-1. IRS-1 is the first substrate after the activation of IGF-1 or insulin receptors; upon phosphorylation, IRS-1 activates downstream signaling pathways involved in cell cycle progression, including PI3-kinase and ERK [21,22]. IRS-1 has an essential mediating role in apoptosis, cell differentiation, and cell transformation through its activity in these pathways [23,24]. A common variant in the *IRS-1* gene results in an amino acid change at codon 972 (G972R) that has been associated with impaired insulin signaling [25], obesity [26], body fat distribution [27], type II diabetes [28], hyperlipidemia, and coronary artery disease [29-31]. *In vitro* assays suggest the missense variant changes the ability of the molecule to bind the p85 subunit of PI3-kinase, but does not alter IRS-1 protein expression levels [32]. The critical role of *IRS-1* in sex hormone and growth factor pathways, as well as the association of the G972R variant with measures of impaired insulin signaling and disease, led us to hypothesize that variation in this gene also may influence circulating sex hormone, IGF-1, and C-peptide levels.

We measured the association between the *IRS-1* G972R variant, sex hormone levels, IGF-1, and C-peptide (a surrogate measure for insulin) in the Health, Eating, Activity, and Lifestyle (HEAL) Study, a population-based prospective cohort study of women with breast cancer. The study includes post-menopausal breast cancer patients from California, Washington,

and New Mexico [33] and was designed to evaluate the roles of hormones, genetics, diet, and physical activity on breast cancer prognosis and survival. In the present report, we tested the hypothesis that the G972R variant of *IRS-1* is associated with circulating sex hormone and IGF-1 levels among stage 0-IIIa African-American and non-Hispanic white breast cancer patients approximately 30 months after diagnosis. Because the frequency of the *IRS-1* variant differs across populations [24,34], we examined this effect by race/ethnicity. To our knowledge, the role of the G972R variant in the *IRS-1* gene has not previously been investigated with regards to steroid hormone or IGF-1 levels.

Material and methods

Population

The HEAL study has been described previously [33]. In brief, we recruited patients with newly diagnosed stage 0-IIIa breast cancer who were identified at one of the three participating study centers affiliated with the Surveillance, Epidemiology and End Results (SEER) registries including Los Angeles County (California), Seattle (Washington), and New Mexico. Eligible patients were residents of Los Angeles County (California), King, Pierce, or Snohomish counties (Washington), or Bernalillo, Santa Fe, Sandoval, Velencia, or Taos counties (New Mexico) at the time of diagnosis. Baseline interviews were conducted, on average, 6 months after diagnosis and follow-up interviews were completed 24 months after the baseline interview (at approximately 30 months post-diagnosis). To be eligible for this analysis, participants were required to have completed the baseline and 24-month follow-up evaluations; provided a blood sample at the 24-month follow up interview; been post-menopausal at the time of blood draw; and be African-American or non-Hispanic white (Hispanic women were not included due to inadequate numbers with the *IRS-1* variant). All participants from Los Angeles, CA were African-American, while non-Hispanic white participants were from Seattle, WA or New Mexico. Participants who died, had severe illness or declined to participate at the time of follow-up interview were excluded from the present analysis. Patients who met either one of the following criteria were defined as post-menopausal: natural menopause with no periods for at least 1 year and/or surgical menopause with bilateral oophorectomy. Subjects who began taking HT prior to or within one year after the last menstrual period, who were premenopausal at 30 months post diagnosis (time of blood draw), or who had unknown menopausal status were excluded from the analyses. Of the 1,223 patients who completed both the baseline and follow-up interviews for the HEAL Study (202 from Washington, 654 from New Mexico, and 367 from California), 490 patients were eligible to be included in this analysis (101 from Washington, 235 from New Mexico, and 154 from USC). Of the ineligible subjects, 179 patients were excluded because they were premenopausal; 334 had unknown status of menopause; 146 did not have a blood sample for hormone or genetic measure; and 74 were in racial/ethnic categories with too few numbers for individual analyses.

Measurements

In-person interviews (California, New Mexico) or self-administered forms (Washington) were used to collect information on demographics, reproductive and menstrual history (age at menarche, regularity of periods when menstruating, age at menopause, type of menopause), hysterectomy and oophorectomy status, history of oral contraceptive and hormone replacement therapy use, medical history including history of endocrine problems and other medical problems, history of tobacco, caffeine, and alcohol use, maximal adult height and height at age 18 and 65, previous weight (ages 18, 35, 50, and 65). Both baseline and/or 24-month updated information from these questionnaires was used in the analyses. Anthropometric measurements including weight, height, skinfold thickness (tricep, subscapular, thigh, calf), and circumference (waist, hip, midarm, midthigh, calf) were made

and serum samples were collected at the 24-month follow-up. Cancer stage at diagnosis was determined by medical record review and summary data available from the National Cancer Institute's SEER program at each center. Treatment information, including chemotherapy and tamoxifen history, was obtained from baseline and follow-up interviews, examination of medication bottles, medical record review, and summary abstracts from the SEER registries.

Blood Collection and Hormone Measurements

Fasting blood samples (35 ml) were obtained from each participant at the 24-month follow-up interview. Blood was processed within 3 hours of collection; serum and buffy coat were stored in 1.8-ml aliquot tubes at -70 to -80°C.

In Washington and New Mexico, assays for sex-hormone binding globulin (SHBG), insulin-like growth factor-1 (IGF-1), C-Peptide of insulin, total testosterone (T), and insulin-like growth factor binding protein-3 (IGFBP3) were completed at the University of New Mexico endocrinology laboratory. Samples were shipped to Quest Diagnostics at the Nichols Institute (San Juan Capistrano, CA) for analysis of estrone (E₁) and estradiol (E₂). All assays for California samples were done in the Endocrine Research Laboratory at USC, with the exception of T, which was completed at the University of New Mexico endocrinology lab. All samples were randomly assigned to assay batches and were randomly ordered within each batch. Laboratory personnel performing the assays were blinded to patient identity and personal characteristics.

¹²⁵I radio-immunoassay (RIA) methods were utilized to measure serum hormone and growth factor levels [33]. Serum extraction and chromatographic purification were performed before radio-immunoassays for E₁ and E₂ were conducted. Assay sensitivities and interassay precision are less than 10 pg/mL and 10%, respectively for E₁ and less than 2 pg/mL and 8% for E₂. Free (non-SHBG bound) E₂ was estimated based on serum SHBG and total E₂ levels [35]. A Total Testosterone ¹²⁵I RIA Kit, supplied from Diagnostic Products Corporation (DPC) was utilized with sensitivity as 4 ng/dL and an inter-assay precision of 5.9-11%. SHBG levels were determined by SHBG ¹²⁵I RIA kit (Wein Laboratories, Succasunna, NJ) with a sensitivity of 6nmol/L. Free (non-SHBG bound) T was estimated from serum SHBG and total T levels [35]. The C-peptide of Insulin was measured using the ¹²⁵I RIA kit from INCSTAR Corp. (sensitivity of 0.1ng/mL). IGF-1 levels were determined by ¹²⁵I RIA kits supplied from Nichols Institute Diagnostics with sensitivity of 0.1ng/mL. IGFBP3 levels were determined from serum using a ¹²⁵I IGFBP3 RIA kit, supplied by Nichols Institute Diagnostics. The sensitivity of the assay is 0.0625 ug/mL with an inter-assay precision of 5.3-6.3%.

Intra-assay variability as measured by the coefficient of variation (CV) after assay replication was assessed in a reduced randomly selected sample for all hormones in California (n=24) and Washington (n=10 to 24, depending on the measure). The CV was estimated by dividing the standard deviation of the difference of replicated measures by the mean of the two measures. The intra-assay CVs for California replicate samples were 9.3%, 26.2%, 15.4%, 15.8%, 6.2%, 17.6%, and 10.5%, respectively, for SHBG, E₁, E₂, T, IGF-1, IGFBP3 and C-peptide. In Washington, intra-assay CV's were calculated using a random effects model. The intra-assay and total CVs were 3.8% and 5.9%, respectively; for SHBG, 12.0% and 14.4%; for T, 29.1% and 13.3%; for E₁, 28.8% and 13.3% for E₂. These CVs are similar to those observed in other studies using similar methods for serum concentration of sex hormones [36,37].

Determination of IRS-1 G972R polymorphism

Genomic DNA was extracted from peripheral blood leukocytes of the post-menopausal patients selected for this study. The IRS-1 G972R variant for all samples was determined by allelic discrimination in a fluorogenic Taqman assay at Albany Molecular Research in Bothell, Washington, with the ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA), which has been described [38]. The sequences of the primers and probes used in the Taqman assay were: primers, GGGTCGAGATGGGCAGACT and GGGACAACCTCATCTGCATGGT; and probes, CTGCACCTCCCGGGGCTG (FAM probe) and CTGCACCTCCCAGGGCTGCTAG (VIC probe). In each 25 μ l PCR solution, there were 900 nM primers, 100 nM probes, 40 ng DNA template, and 12.5 μ l Taqman Universal PCR master mix. ABI Temperature cycling of PCR was: 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 62°C for 1 minute. The fluorogenic G (wild-type) and A (variant) allele specific probes were complementary to their corresponding strands and labeled with FAM or VIC reporter dye at the 5' end, and the TAMRA quencher at the 3' end. After PCR, the fluorescence in each tube was measured by ABI PRISM 7700 Sequence Detection System version 1.6.5. Taqman genotyping results were confirmed by sequencing each allele of several samples.

Statistical Analysis

Patients were classified as obese if their body mass index was greater than or equal to 30 kg/m², based on the World Health Organization (WHO) definition of obesity [39].

Statistical analyses were performed on logarithmically transformed values for hormone and growth factor levels, and geometric mean values with 95% confidence intervals (CI) are presented. We determined whether sex hormone, IGF-1, and C-peptide levels were related to IRS-1 genotype within the two racial/ethnic groups using analysis of covariance methods. The final analysis of covariance models included age at 24-month interview (continuous), obesity (obese versus not obese), and tamoxifen use history (current users versus not current or never users). No confounding effect by other 24-month interview variables including years since menopause (continuous); smoking (current versus former/never); cancer stage (*in situ*, localized, regional); bilateral oophorectomy (yes/no); diabetes (yes/no); and fasting status (yes/no) was observed for any hormone or protein measurement in multivariable analysis of covariance models. Stratified analysis by tamoxifen history (current user versus not current user) and obesity (obese versus not obese) separately were completed to evaluate potential effect modification. Because the California sample included patients who were, on average, younger at diagnosis than patients from the other two geographic sites, a sub-analysis restricted to age groups sampled at all three study sites was conducted. Two-sided p-values comparing G972R wildtype (GG) to the variant (GA) genotype are presented. Calculations were performed using the PROC GLM procedure in SAS Version 8.0 (SAS Institute, Cary, NC).

For patients who had a hormone concentration below the detection limits of the assay, the midpoint value between zero and the lowest detectable value was assigned (35, 18, and 8 women were assigned a value for E1, T and C-peptide, respectively).

Results

The analysis included 335 non-Hispanic white and 155 African-American female breast cancer survivors. African-American patients had a slightly lower mean age at the 24-month interview and fewer years since menopause than non-Hispanic white patients. Further, African-American patients were more likely to be current smokers, obese, and to have diabetes than non-Hispanic white patients (Table 1). Among the participants, 430 were

scored as wildtype for the *IRS-1* gene (GG) and 60 as the variant (GA); no homozygous carriers (AA) were identified in our sample. Allele frequencies were in Hardy-Weinberg equilibrium in each racial/ethnic group. The frequency of the variant allele was 0.06 for non-Hispanic whites and 0.07 for African Americans; these frequencies were similar to the value of 0.07 (range 0.051-0.11) previously described for a general population sample [40].

Characteristics by *IRS-1* genotype for African-American and non-Hispanic white cases are shown in Table 1. We found no significant difference in age, smoking status, tamoxifen use, obesity, or stage of disease with *IRS-1* genotype.

The concentrations of E₁, T and free T (Table 2) were significantly higher for African-American variant G972R carriers compared to those with the wildtype allele, and SHBG levels were significantly lower [E₁: 30% (p=0.02), T: 40% (p=0.03), free T: 59% (p=0.005), and SHBG: -21% (p=0.02)]. Other hormone and growth factor levels did not differ statistically between the two genotypes in African Americans. No statistically significant differences by G972R genotype were observed for non-Hispanic white patients. Results were similar for strata of current tamoxifen versus women not currently using tamoxifen and obese versus non-obese women. When restricting analyses to patients in the age-ranges common to all three study sites, we observed statistically significant differences within the two racial/ethnic group strata (data not shown).

Discussion

In the present study, the *IRS-1* G972R variant was associated with circulating sex hormone levels in African-American patients. African-American carriers of the variant (A) allele had higher serum levels of E₁, T, and free T, and decreased levels of SHBG than carriers of the wildtype allele. No statistically significant differences in serum hormone levels were observed for non-Hispanic white patients by genotype.

In general, African-American women have higher breast cancer mortality rates than whites [41,42]. The reasons for this increased mortality are likely to be multifaceted including factors related to socioeconomic status, access to health care, course of treatment, or characteristics of the tumor such as tumor size, grade, or stage [41,43-46]. Given that circulating estrogen levels are associated both with breast cancer onset [47,48] and prognosis [2], the results of the present study are consistent with a potential role of *IRS-1* in racial/ethnic differences in breast cancer prognosis. However, there is no apparent association of the G972R variant with stage of disease in this data and the women have not been observed long enough to examine survival. The presence of an association between G972R and sex hormones and binding proteins in African-American, but not white patients, may indicate a chance association; however, it may also indicate that the variant is in linkage disequilibrium with a true causal variant. If, however, G972R is a functional variant, the observed differences by race/ethnicity may reflect the influence of genetic background or individual characteristics (e.g. weight) on penetrance.

As a functional variant or a marker of a causal variant in African-American breast cancer patients, G972R may be altering sex hormone and binding protein levels by decreasing insulin-stimulated signaling [25]. It has also been suggested that the *IRS-1* variant may contribute to insulin resistance [49] and elevated insulin levels [50] by impairing the ability of insulin to stimulate glycogen synthesis [51,52] and glucose transport [53]. This could potentially result in hyper-insulinemia [25], a reduction in SHBG [54], and a subsequent increase in circulating levels of E₁ and T. However, these findings have not been supported by all studies [26,28,50,55]. In our data, C-peptide (a surrogate measure of insulin) was not significantly different by genotype within African Americans and whites. Alternatively, it

has been suggested by Ando et al [12] that the *IRS-1* variant might reduce ER binding capacity, which would in turn increase circulating levels of estrogen. Experimental studies are necessary to explore the possible mechanisms through which the *IRS-1* variant may influence sex hormone levels, ER expression and function.

Previous epidemiological studies found that the *IRS-1* G972R variant is associated with insulin resistance [25,28,56], body fat distribution [27], type II diabetes [28], hyperlipidemia, and coronary artery disease [29-31]. Other studies, however, have not confirmed these findings [50,57-63]. The IRS-1 protein is expressed in a variety of solid tumors, including breast cancer, Wilms' tumors, rhabdomyosarcoma, liposarcoma, leiomyoma, leiomyosarcoma, and adrenal cortical carcinoma [64]. A role for IRS-1 in the cross-talk between the estrogen, insulin, and IGF-1 pathways has been shown in experimental studies. In mice, IRS-1 plays a role in mammary gland development and this function is regulated by steroid hormones, especially the combination of estrogen and progesterone [65]. *In vitro* studies [11,66,67] have found that estrogen, especially E₂, can stimulate and increase the expression of IRS-1 protein levels in breast tumor cells resulting in enhanced insulin or IGF-1 signaling. The mechanisms through which the insulin or IGF-1 pathways influence the estrogen pathway are not as well characterized; however, experimental studies have shown that the IRS-1 protein can alter ER expression and function [12]. For example, breast tumor cells with IRS-1 deficiency display up-regulation of ER protein expression and binding capacity, loss of insulin-estradiol synergism and loss of insulin-induced regulation of ER tyrosine phosphorylation [12]. In total, the laboratory evidence suggests that IRS-1 may be an important mediator of the cross-talk or synergistic relationship between insulin/IGF-1 pathways and sex hormones in breast cancer. To our knowledge, human subject data characterizing the relationship between the IRS-1 variant and sex hormone levels have not previously been reported.

While we were able to detect an association between *IRS-1*, sex hormone levels and binding proteins among African-American patients, the generalizability of the findings across racial/ethnic groups is limited by our sample size. The coefficients of variation for some hormones, such as E₂, were large and reflect the difficulty in measuring relatively low sex hormone levels in post-menopausal women. While we designed the analysis to include blood samples taken at 30+ months post-diagnosis, when most women would have finished chemotherapy or radiation therapy, the hormone levels of some breast cancer survivors may have been permanently reduced by past chemotherapy or radiotherapy; we do not expect this to differ by *IRS-1* genotype. Tamoxifen, which is still used by many women at 30+ months post-diagnosis, could potentially influence circulating sex hormone levels. However, the association between hormone levels and genotype in African-American patients persisted, even after controlling for current tamoxifen use and in strata of current tamoxifen users versus non-users.

In summary, our study found a statistically significant association between the *IRS-1* G972R variant and sex hormones in African-American post-menopausal breast cancer survivors that was not observed for white patients; these findings will need replication in additional studies. African-American women diagnosed with breast cancer are considered to have a poorer prognosis than white women diagnosed with a similar stage of disease; variation in the IRS-1 pathway may represent one factor that contributes to these differences. Follow-up of the HEAL Study cohort for disease-free survival and mortality in the next several years will provide an opportunity to assess the role of *IRS-1* gene variation on breast cancer prognosis.

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List of abbreviations

CV	coefficient of variation
E₂	estradiol
E₁	estrone
ER	estrogen receptor
HEAL	Health, Eating, Activity, and Lifestyle Study
HT	hormone replacement therapy
IRS-1	insulin receptor substrate-1
IGF-1	insulin-like growth factor 1
IGF1R	insulin-like growth factor 1 receptor
IGFBPs	IGF binding proteins
SHBG	sex hormone binding globulin
T	testosterone
G972R	amino acid change (glycine to arginine) at codon 972

Characteristics of post-menopausal breast cancer cases (N=490) by race/ethnicity, Health, Eating, Activity, and Lifestyle (HEAL) Study.

Table 1

	Non-Hispanic Whites (N=335)		African-Americans (N = 155)	
	Wildtype (N=295)	Variant (N=40)	Wildtype (N=135)	Variant (N=20)
	N (%)	N (%)	N (%)	P
Age, years, mean ±SD	62.6 ± 9.7	62.5 ± 10.3	55.6 ± 6.6	56.5 ± 7.2 0.61
Current Smoker	28 (9.5)	4 (10.0)	24 (17.8)	1 (5.0) 0.18
Bilateral oophorectomy ^a	65 (21.3)	6 (15.4)	28 (20.9)	1 (5.0) 0.12
Tamoxifen use (current)	145 (49.2)	23 (57.5)	57 (42.2)	7 (35.0) 0.54
Obese (BMI>30 kg/m ²)	66 (22.4)	14 (35.0)	68 (50.8)	7 (35.0) 0.19
Diabetes	25 (8.6)	4 (10.0)	25 (18.5)	4 (20.0) 0.87
Cancer Stage				
In Situ ^b	58 (19.7)	6 (15.0)	29 (21.5)	6 (31.6) 0.40
Localized	184 (62.4)	28 (70.0)	51 (37.8)	6 (31.6) 0.50
Regional	53 (17.9)	6 (15.0)	55 (40.7)	7 (36.8) 0.63

^a 5 non-Hispanic Whites (wildtype) and 1 non-Hispanic White (variant) were missing bilateral oophorectomy

^b 1 AA (variant) missing stage.

Table 2

Adjusted geometric mean serum hormone and protein levels by *IRS-1* G972 variant, Health, Eating, Activity, and Lifestyle (HEAL) Study. (N=490)

Hormone	Wildtype Mean (95% CI)	Variant Mean (95% CI)	p ^d
<i>African-Americans</i>			
Estrone (pg/mL)	30.4 (28.2 – 32.9)	39.5 (32.3 – 48.3)	0.02
Estradiol (pg/mL)	13.8 (12.6 – 15.1)	17.5 (13.8 – 22.3)	0.07
Free Estradiol(pg/ml)	0.36 (0.31 – 0.41)	0.45 (0.31 – 0.60)	0.26
Testosterone(pg/ml)	222.4 (199.3 – 248.2)	311.6 (234.2 – 414.5)	0.03
Free Testosterone(pg/ml)	3.7 (3.3 – 4.2)	5.9 (4.4 – 7.9)	0.005
Serum Hormone Binding Globulin (nmol/L)	54.3 (50.5 – 58.4)	42.8 (35.4 – 51.8)	0.02
C-Peptide (ng/ml)	1.8 (1.6 – 2.1)	1.6 (1.2 – 2.3)	0.57
Insulin-like Growth Factor-1 (ng/ml)	89.8 (83.5 – 96.6)	103.4 (85.6 – 124.8)	0.17
IGF-1/IGFBP3 ^{b,c}	24.5 (22.9 – 26.2)	25.5 (21.5 – 30.4)	0.67
<i>Non-Hispanic Whites</i>			
Estrone (pg/mL)	21.8 (19.9 – 23.9)	19.8 (15.5 – 25.4)	0.47
Estradiol (pg/mL) ^a	15.40 (14.06 – 16.86)	13.17 (10.30 – 16.84)	0.24
Free Estradiol(pg/ml) ^a	0.44 (0.40 – 0.49)	0.34 (0.23 – 0.47)	0.13
Testosterone (pg/ml)	172.6 (159.2 – 187.0)	150.2 (120.6 – 187.0)	0.24
Free Testosterone (pg/ml)	3.0 (2.8 – 3.3)	2.5 (2.0 – 3.2)	0.15
Serum Hormone Binding Globulin (nmol/L)	49.8 (47.3 – 52.6)	53.1 (45.9 – 61.4)	0.42
C-Peptide (ng/ml)	2.2 (2.1 – 2.3)	2.3 (2.0 – 2.5)	0.48
Insulin-like Growth Factor-1 (ng/ml)	123.1 (118.0 – 128.4)	123.8 (110.3 – 139.0)	0.92
IGF-1/IGFBP3 ^b	30.6 (29.6 – 31.6)	31.6 (29.0 – 34.4)	0.49

^a 5 wild type carriers missing Estradiol and Free Estradiol

^b ratio of insulin like-growth factor-1 to insulin-like growth factor binding protein-3

^c 1 wildtype carrier missing IGF-1/IGFBP3

^d Test for wildtype vs variant; Model adjusted for age, obesity (kg/m² >= 30) and current tamoxifen use