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Genetic, anthropometric, and lifestyle factors associated with IGF-1 and IGFBP-3 levels in Hispanic and non-Hispanic white women

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Abstract

Circulating concentrations of IGF-I and IGFBP-3 are associated with risk of pre-menopausal breast cancer. Racial differences in levels of these factors have been reported, and determinants of IGF-I and IGFBP-3 levels within racial and ethnic groups are unclear. In this study we examine genetic, anthropometric, diet, and lifestyle factors that may predict serum levels of IGF-I and IGFBP-3 among Hispanic and non-Hispanic white women. A sample of healthy controls participating in the SHINE (Southwest Hormone, Insulin, Nutrition, and Exercise Study) case-control breast cancer in Arizona, Colorado, New Mexico, and Utah were included in these analyses. Subjects included 210 Hispanic and 284 non-Hispanic white women. Hispanic women had significantly lower levels of IGFBP-3 (mean = 3764.3 mcg/ml) after adjusting for age, body size, physical activity, menopausal status, and dietary factors than non-Hispanic white women (mean = 4058.0 mcg/ml; $p < 0.01$). The CC genotype of the −202 A > C polymorphism of the *IGFBP*3 gene was associated with lower IGFBP-3 levels in both ethnic groups. The frequency of the *IGFBP3* C allele differed between Hispanic (0.65) and non-Hispanic white women (0.53), but serum levels of IGFBP-3 were lower for Hispanic women than non-Hispanic after accounting for *IGFBP*3 genotype. Body size indicators, vigorous physical activity, and dietary factors appeared to influence serum levels of IGF-1 and the ratio of IGF-1 to IGFBP-3 in pre-menopausal women more than in post-menopausal women. On the other hand, using aspirin/NSAIDs appeared to increase IGFBP-3 levels significantly among pre-menopausal Hispanic women. Results from this study suggest that differences in IGFBP-3 levels exist in Hispanic and non-Hispanic white women. These differences could be due to the combined effects of genetic and behavioral factors which could account for ethnic differences in the risk of breast cancer and other chronic diseases.

Introduction

In the southwestern U.S. states of Arizona, New Mexico, Colorado, and Utah, both risk factors for breast cancer and breast cancer incidence and mortality rates vary markedly between non-Hispanic white women and Hispanic women. From studies conducted among predominantly non-Hispanic white women, we have learned that obesity, patterns of weight gain, and lack of physical activity may contribute significantly to breast cancer risk [1–9]. U.S. Hispanic populations have a higher prevalence of overweight and obesity than non-Hispanic whites [10], yet Hispanic women have lower breast cancer incidence rates. There are differences in patterns of expression of breast tumor markers associated with Hispanic ethnicity [11, 12], though, and Hispanic women with breast cancer have higher mortality risk after a breast cancer diagnosis [13–15]. These differences suggest that metabolic pathways between obesity and breast cancer development may differ between Hispanic and non-Hispanic women.

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Circulating insulin-like growth factor 1 (IGF-I) and its predominant binding protein (IGFBP-3) act as regulatory molecules affecting cell proliferation and apoptosis, and are themselves regulated primarily by growth hormone [16]. Serum concentrations of these proteins are predictive of risk of certain cancers [17], with higher concentrations associated with increased risk of premenopausal breast cancer [10, 13, 14]. Associations between body size and cancer risk are hypothesized to be mediated by IGF. IGF-I and IGFBP-3 levels have been reported to vary according to age, body mass index (BMI) [16] and race [18, 19]. However, few data are available describing IGF-I and IGFBP-3 levels and their regulation among Hispanic women. A previous study evaluating IGF-I and IGFBP-3 in several ethnic groups, including Latinos, showed that Latinos had lower IGF-I and IGFBP-3 levels than did Caucasian populations [19], but did not evaluate predictors of IGF-I and IGFBP-3 within ethnic groups.

A dinucleotide (CA) repeat polymorphism in the IGF-I promoter region has been described [20]; the most common allele is 19 repeats. The 19/19 genotype was reported to be associated with lower concentrations of circulating IGF-I [21]. Other studies, however, have reported no association or a reversed association between IGF-I genotype and serum IGF-I levels [19, 22, 23]. Thus there are conflicting data regarding whether the polymorphism has a functional effect on IGF-I expression. An IGFBP−3 single nucleotide polymorphism in the promoter region of the IGFBP-3 gene, A−202C, was reported to be associated with higher promoter activity *in vitro* [24] and presence of the A allele has consistently been found to be associated with higher serum IGFBP-3 levels [24–26] in studies in Caucasian populations.

The purpose of this manuscript is to investigate differences in serum levels IGF-I and IGFBP-3 in Hispanic and non-Hispanic white women and to determine how concentrations of these molecules may be affected by anthropometric, diet, lifestyle, and genetic factors in each group. Our intent is to examine ethnic differences that might relate to differences in breast cancer risk by ethnicity. Data come from healthy controls participating in the Southwest Hormone, Insulin, Nutrition, and Exercise Study (SHINE) of breast cancer. The SHINE Study is a large case-control of breast cancer of women living in the Southwestern United States, Arizona, Colorado, New Mexico, and Utah. The purpose of the study is to evaluate differences in breast cancer risk factors between Hispanic and non-Hispanic white women. These analyses represent a subset of the approximate 5000 cases and controls participating in the larger study.

Methods

Participants were women between 25 and 79 years of age living in Arizona, Colorado, New Mexico, or Utah. In Arizona and Colorado, participants under 65 were randomly selected from a commercial mailing list; in New Mexico and Utah, they were randomly selected from driver's license lists. In all states, women 65 years and older were randomly selected from social security lists. In all states, controls were frequency matched by five-year age-groups to breast cancer cases. Twenty percent of controls were randomly selected to have a fasting blood draw, unless a medical reason existed that prevented fasting.

Questionnaire data

Diet and lifestyle data were collected by trained and certified interviewers using a computerized questionnaire. The diet history questionnaire has been described previously [27] with modification to expand the list of unique foods consumed in the U.S. Southwest. Version 30 of the NCC database was used to estimate nutrients based on reported foods. A detailed physical activity questionnaire captured activity performed at various levels of intensity, including activities performed at leisure, work, and around the home. The physical activity questionnaire was adapted from the Cross-Cultural Activity Participation Study (CAPS) questionnaire that has been used to estimate activity of minority women [28]. The CAPS questionnaire was modified to obtain more information on reported intensity and to include activities that were common in the study population. Height and weight were measured at the time of interview, and a weight change history was asked to obtain information on frequency of adult weight change, defined as the number of times the subject had weight changes of 15 pounds or more, apart from pregnancy. Additionally, study participants were asked to self-report weight at age 15, 30, and 50. Other questionnaire data included race and ethnicity, reproductive history, use of hormone replacement therapy, history of aspirin and non-steroidal anti-inflammatory drug use, and cigarette smoking history.

Anthropometric data

A portable digital scale was used to measure current body weight. Weight was measured with the participants wearing light clothing to the nearest 0.50 lb. Height was measured to the nearest 0.5 inch using a stadiometer. Body mass index was calculated using the formula of weight in kilograms (kg)/ height in meters $(m)^2$. Hip and

waist circumference measurements were taken using a flexible tape with the participant standing and recorded to the nearest 0.25 inch. Waist was measured at the smallest point between the 10th rib and the iliac crest over bare skin or minimal clothing. Hip circumference was measured at the maximum circumference of the buttocks. Weight, height, and waist and hip circumferences were taken twice; a third measurement was taken if the difference in the first two differed by more than one lb or 0.5 inch respectively.

Serum measurements

Participants were enrolled in either the fasting or non-fasting protocol. Controls eligible for the fasting protocol included women who had never taken chemotherapy, were not currently taking tamoxifen, were not currently pregnant, and were not diagnosed with diabetes. Venipuncture was performed in study participants' homes to obtain blood for germline DNA and blood tests requiring serum. In the laboratory, serum that was centrifuged for 15 minutes after collection in the home, stored in a cooler, and was divided into smaller aliquots for shipment to the Maine Center for Osteoporosis Research for IGF-1 and IGFBP-3 testing.

Serum IGF-1 levels were determined using the IGF-1 (IGFBP-blocked) RIA assay (American Laboratory Products Company (ALPCO) Windham, NH). The calculated sensitivity of the assay is 0.02 ng/ml; cross reactivity with IGF-II is small ($<0.05\%$). Using this radioimmunoassay technique, IGF-1 was dissociated from the binding proteins (IGFBPs) by dilution in an acidic buffer. An antibody solution containing excess IGF-II is added to neutralize the samples. The excess IGF-II then occupies the IGF-binding sites and free IGF-1 is measured through addition of a ^{125}I tracer. Separation of the bound and free tracer is carried out by the addition of a second antibody. Using software provided by Packard Instruments, a standard curve is constructed and the concentrations of the unknowns and controls are read from this curve. IGFBP-3 levels were determined using the "Active" IGFBP-3 IRMA kit (Diagnostic Systems Laboratories, Inc., Webster, Texas). The calculated sensitivity of the kit is 0.5 ng/ml. The kit employs a two-site immunoradiometric principle to directly measure non-glycosylated IGFBP-3. In this non-competitive assay, the analyte is "sandwiched" between two antibodies. One antibody is immobilized on a solid carrier (the tube) while the second is labeled with the [^{125}I] tracer. The unbound reagents were removed by decanting and washing the tubes. The activity of the "sandwiched" complex was measured to determine the IGFBP-3 concentration. Concentrations of IGFBP-3 were determined by comparing the resultant cpm of the unknowns and controls to the corrected for the dilution factor. Intra-assay precision was determined for three serum samples containing low, mid-range, and high concentrations from each group of samples run. Replicates of each sample were measured to obtain the mean and standard deviation for the group sample and to calculate the coefficient of variation. The range in the coefficient of variation was 0.06–6.5% for IGF-1 and 0.17–7.5% for IGFBP-3; the majority of all samples had less than two percent variation.

Genotyping

Genotyping was done on germline DNA. Three duplicate known variants were incorporated into each tray. Any deviation from the known genotype resulted in the entire tray being repeated.

The *IGF1* CA repeat was amplified using PCR primers IGF1-F 5′-GCT AGC CAG CTG GTG TTA TT and IGF1-R 5′-ACC ACT CTG GGA GAA GGG TA [29]. PCR conditions consisted of a two-minute denaturation at 94 °C followed by 30 cycles of 94 °C 10 s, 57 °C 10 s, and 72 °C 15 s. The *IGF1* products were electrophoresed on 6% denaturing polyacrylamide gels at 70 watts for three hours. The gels were dried and exposed to X-ray film. Alleles were assigned by size of fragment in base pairs and classified as "192" or not "192." "192" is the PCR product size of the most common allele which contains 19 CA repeats.

The -202 A > C polymorphism in the *IGFBP3* gene was amplified using primers F 5′-CCA CGA GGT ACA CAC GAA TG and R3 5′-TGA GCA GCC GGG GCC GAG and Alw21I digestion [24]. 0.5 units of Amplitaq gold and 5% DMSO were used to increase efficiency of amplification. PCR conditions were nine minute initial denaturation at 95 °C followed by 40 cycles at 95 °C 10 s, and 66 °C 20 s. The resulting PCR product was digested with 4 units of Alw21I at 37 °C overnight. Digested products were separated on a 2% Nusieve gel stained with ethidium-bromide and visualized with ultraviolet light. Alleles were scored as either A or C allele (presence or absence of the restriction site, respectively).

Statistical analyses

Analyses were based on 210 Hispanic and 284 non-Hispanic white women. All analyses were stratified by ethnicity to yield ethnic specific results. Models were adjusted for age and other factors as indicated in the tables. Total physical activity level (PAL) was based on all moderate and intense activities; vigorous activities

were restricted to activities with MET values of six or greater and included activities such as jogging, swimming, and running.

The focus of the analyses was to: (1) describe differences in anthropometrics, physical activity, diet, and other breast cancer risk factors between Hispanic and non-Hispanic white women; (2) to determine if those diet and lifestyle factors were associated with IGF-1 and IGFBP-3 levels in Hispanic and non-Hispanic white women; (3) to determine if genetic, diet, and lifestyle factors were associated with IGF-1 and IGFBP-3 levels in Hispanic and non-Hispanic white women; and (4) to determine if these associations are influenced by menopausal status in Hispanic and non-Hispanic white women. Chi-squared tests and *t*-tests were done to examine differences in proportions and mean levels of diet, lifestyle factors, and genetic factors. Spearman correlation coefficients were used to further estimate associations between diet, lifestyle and IGF-1 and IGFBP-3 levels in Hispanic and non-Hispanic white women. Least squares regression was performed on data that were transformed to normalize the variables in order to evaluate mean levels of IGF-1 and IGFBP-3 and their ratio to compare differences in Hispanic and non-Hispanic white women. To determine the set of variables that best predicted IGF-1, IGFBP-3, and the ratio of IGF-1 to IGFBP-3 levels, we used backward stepwise linear regression including all variables described in Table 1. We report the model R^2 that measures the amount of the variability in the data is that accounted for by the set of diet and lifestyle variables in the model. SAS (SAS institute, Cary, NC) statistical package was used to complete all analysis.

Results

There were no significant differences by ethnicity (Hispanic versus non-Hispanic white women) for menopausal status, recent aspirin use, and current cigarette

Table 1. Description of study population

	Hispanic N = 210 N (%)	Non-Hispanic White N = 284 N (%)	*p*-value
Center: Arizona	21 (10.0)	40 (14.1)	< 0.01
Colorado	102 (48.6)	76 (26.8)	
New Mexico	11 (5.2)	45 (15.8)	
Utah	76 (36.2)	123 (43.3)	
Post-menopausal	132 (65.7)	188 (67.4)	0.69
Current aspirin/NSAID users	57 (27.1)	80 (28.4)	0.76
Current cigarette smokers	24 (11.5)	32 (11.3)	0.94
Genotype: *IGF1* 19/19	80 (48.5)	100 (44.1)	0.35
19/other	61 (37.0)	100 (44.1)	
other	24 (14.5)	27 (11.9)	
IGFBP3 AA	75 (44.4)	67 (28.8)	< 0.01
AC	72 (42.6)	118 (50.6)	
CC	22 (13.0)	48 (20.6)	
	Hispanic Mean (SD)	Non-Hispanic Mean (SD)	*p*-value[‡]
Age (yrs)	54.5 (12.4)	55.2 (12.5)	0.55
BMI (kg/m^2)	30.0 (6.2)	28.4 (7.1)	< 0.01
Waist (inches)	35.7 (5.7)	34.2 (6.2)	< 0.01
Hip (inches)	42.9 (5.2)	43.1 (5.6)	0.61
Waist to hip ratio	0.83 (0.07)	0.79 (0.07)	< 0.01
Birth weight (kg)[†]	3.1 (0.7)	3.2 (0.7)	< 0.01
Time weight change	1.7 (4.3)	3.0 (5.9)	< 0.01
Total PAL (MET/minutes)	3284 (3462)	2859 (2586)	0.14
Vigorous PAL (MET minutes)	388 (785)	484 (1022)	0.23
Energy intake (kcal)	2535 (1376)	2435 (6836)	0.81
Dietary fiber g/1000 kcal	12.5 (3.9)	11.6 (4.4)	0.02
Sucrose g/1000 kcal	21.9 (8.3)	22.7 (9.5)	0.33
Sucrose/fiber ratio/1000 kcal	1.0 (0.9)	1.3 (1.0)	< 0.01
Carbohydrate g/1000 kcal	129 (20)	129 (24)	0.99
Protein g/1000 kcal	38.2 (6.5)	39.6 (7.9)	0.03
Cholesterol mg/1000 kcal	120 (41)	117 (48)	0.48

[†] Respondents reported birth weight.

[‡] The *p*-values are derived from Student's *t*-test using transformed variables. The means and standard deviations are from the untransformed variables.

smoking (Table 1). Mean BMI level was higher among Hispanic women, although non-Hispanic white women reported significantly higher birth weight and more occasions of weight changed by 15 or more pounds. Mean total activity levels were similar for Hispanic and non-Hispanic white women, although non-Hispanic white women reported more vigorous activity. Significant differences in mean dietary intake were observed for dietary fiber, sucrose to fiber ratio (SFR), and protein. Hispanic women reported consuming more protein as well as more dietary fiber. SFR was lower in Hispanic women than in non-Hispanic white women. The *IGFBP3* AA genotype was significantly more common among non-Hispanic white women. The C allele was more frequent among Hispanic than non-Hispanic white women (65% vs. 53% $p < 0.01$ (data not shown in Table). *IGFBP3* was in Hardy Weinberg Equilibrium in both Hispanic and non-Hispanic white women, however *IGF1* was only in Hardy Weinberg Equilibrium for non-Hispanic white women.

Assessment of associations between diet and lifestyle factors and levels of IGF-1, IGFBP-3, and IGF-1/GFBP-3 ratio levels in pre- (Table 2) and post-menopausal women (Table 3) showed stronger associations for all factors among pre-menopausal women than among post-menopausal women. Further, there were some differences in level of associations for Hispanic and non-Hispanic white women. BMI, weight change, and waist and hip circumference were consistently inversely correlated with levels of IGF-1 and the IGF-1 to IGFBP-3 ratio for both pre- and post-menopausal women. BMI and number of times weight changed were also inversely associated with IGFBP-3 levels among Hispanic women but not non-Hispanic white women. Birth weight was not associated with IGF-1, IGFBP-3, or the ratio of IGF-1 to IGFBP-3. Vigorous physical activity was associated with levels of IGF-1 and the IGF-1/IGFB-3 ratio for pre-menopausal women and Hispanic post-menopausal women. For most dietary variables, associations were stronger for pre-menopausal women, although were often not significant because of the smaller sample size. Among post-menopausal Hispanic women, dietary cholesterol was significantly inversely related to IGF-1 levels. Currently using aspirin/NSAIDs was significantly associated with higher IGFBP-3 levels among Hispanic post-menopausal women.

Assessing the variability in IGF-1, IGFBP-3, and the ratio of IGF-1/IGFBP-3 in a backward selection model that included age and the diet and lifestyle factors showed that these factors accounted for much more of the variability in serum levels among pre-menopausal women than post-menopausal women (data not shown in table). The R^2 values for Hispanic pre-menopausal women were 0.37, 0.20, and 0.25 and for 0.51, 0.16, and

Table 2. Spearman Correlations between IGF-1, IGFBP-3, IGF-1/IGFBP-3 ratio, with body size, physical activity level (PAL), diet, cigarette smoking, and aspirin/NSAIDs in pre-menopausal Hispanic and non-Hispanic white women

	Hispanic women			Non-Hispanic white women		
	IGF-1 N = 69	IGFBP-3 N = 68	IGF-1/IGFBP-3 Ratio N = 68	IGF-1 N = 91	IGFBP-3 N = 86	IGF-1/IGFBP-3 Ratio N = 86
Body size						
BMI	−0.26*	−0.20	−0.22	−0.18	−0.05	−0.19
Weight change	−0.25*	−0.24*	−0.14	−0.23*	0.01	−0.22*
Waist	−0.33*	−0.17	−0.34*	−0.33*	−0.09	−0.32*
Hip	−0.19	−0.17	−0.16	−0.29*	−0.04	−0.33*
Waist to hip	−0.36*	−0.05	−0.42*	−0.28*	−0.11	−0.21
Birth weight†	0.02	< −0.01	< 0.01	−0.10	0.02	−0.16
PAL						
Total	−0.01	−0.02	0.03	0.23*	0.12	0.19
Vigorous	0.28*	0.17	0.22	0.25*	0.07	0.25*
Diet						
Energy	−0.25	−0.22	−0.14	0.10	−0.02	0.06
Dietary fiber	−0.05	−0.13	0.06	0.22*	0.02	0.18
Sucrose	−0.11	−0.14	−0.04	0.17	−0.06	0.13
SFR	−0.07	0.01	−0.10	0.05	−0.01	< −0.01
Carbohydrate	−0.21	−0.19	−0.10	0.19	−0.08	0.14
Protein	−0.19	−0.18	−0.10	0.08	0.02	0.03
Cholesterol	−0.23	−0.19	−0.13	< 0.01	0.04	−0.02
Smoking	0.11	−0.02	0.16	0.18	0.12	0.15
Asprin/NSAIDs use (current, ever, never)	0.16	0.10	0.12	0.06	0.16	−0.04

* Statistically significant at the 0.05 levels using Spearman correlation analysis; adjusted values for age.

† Categorical birth weight. Includes women who did not know exact birth weight but knew if < 2.5 kg or > 4.5 kg.

Table 3. Spearman Correlations between IGF-1, IGFBP-3, IGF-1/IGFBP-3 ratio, with body size, physical activity level (PAL), diet, cigarette smoking, and aspirin/NSAIDs in post-menopausal Hispanic and non-Hispanic white women

	Hispanic			Non-Hispanic white		
	IGF-1 N = 132	IGFBP-3 N = 127	IGF-1/IGFBP-3 N = 127	IGF-1 N = 188	IGFBP-3 N = 176	IGF-1/IGFBP-3 N = 176
Body size						
BMI	−0.36*	−0.13	−0.36*	−0.11	0.04	-0.22*
Weight change	−0.22*	−0.15	−0.18*	−0.19*	−0.03	−0.17*
Waist	−0.32*	−0.07	−0.36*	−0.04	0.08	−0.15
Hip	−0.35*	−0.08	−0.40*	−0.11	0.08	−0.25*
Waist to hip	−0.06	0.07	−0.15	0.01	< 0.01	< 0.01
Birth weight†	−0.15	0.04	−0.17	0.08	−0.03	0.06
PAL						
Total	−0.12	−0.02	−0.06	0.02	−0.15*	0.14
Vigorous	0.12	< −0.01	0.22*	0.05	−0.02	0.08
Diet						
Energy	−0.09	−0.06	−0.09	−0.15	−0.13	−0.05
Dietary fiber	−0.07	−0.05	−0.08	−0.11	−0.11	−0.03
Sucrose	−0.05	−0.06	−0.02	−0.13	−0.05	−0.15*
SFR	0.02	−0.07	0.06	−0.04	0.05	−0.13
Carbohydrate	−0.09	−0.07	−0.09	−0.15*	−0.09	−0.09
Protein	−0.10	−0.06	−0.10	−0.06	−0.07	−0.01
Cholesterol	−0.22*	−0.16	−0.13	−0.08	−0.09	−0.01
Smoking	−0.10	−0.07	−0.07	−0.01	0.08	−0.11
Asprin/NSAIDs use (current, ever, never)	−0.04	−0.23*	0.11	0.04	0.01	0.05

* Statistically significant at the 0.05 level using Spearman correlation analysis; adjusted for age.
† Categorical birth weight. Includes women who did not know exact birth weight but knew if < 2.5 kg or > 4.5 kg.

0.39 for non-Hispanic white women for IGF-1, IGFBP-3, and their ratio respectively. Comparable R^2 values for post-menopausal women were 0.20, 0.17, and 0.21 for Hispanic women and 0.08, 0.04, and 0.13 for non-Hispanic white women. Age was a major predictor of all serum values in all groups evaluated.

Evaluation of the 19 CA repeat in *IGF1* gene, the −202 A > C polymorphism in the *IGFBP3* gene, with age-adjusted serum levels of IGF-1, IGFBP-3, and the ratio of IGF-1 to IGFBP-3 showed that among both Hispanic and non-Hispanic white women the AA *IGFBP3* genotype was associated with the highest levels of IGFBP-3, the AC genotype, intermediate levels, and the CC genotype the lowest levels of IGFBP-3 (Table 4). Results were similar for pre- and post- menopausal women. Although results were not significant, there are suggestions that the IGF-1 serum levels go in the opposite direction with IGF-1 CA among Hispanic and non-Hispanic white women. After adjusting for age, body size, physical activity, and dietary variables described in Table 1, IGFBP-3 levels were significantly lower in Hispanic than non-Hispanic white women ($p \leq 0.01$) (Table 5). These trends were similar for pre- and post-menopausal women. This difference remained significant after adjusting for *IGFPB*3 genotype. Evaluation of IGF-1 by age for Hispanic and non-Hispanic white women (Figure 1) showed decreasing IGF-1 levels until 50–59 years age group, at which point they leveled off. IGFBP-3 levels appeared to be less influenced by age, especially among non-Hispanic white women, with only slight differences between those diagnosed between 25–39 years and those diagnosed between 70 and 79 years. Although IGF-1 and IGFBP-3 levels were higher among non-Hispanic white women; the ratio of the two showed fewer differences by age, with Hispanic women between 50 and 59 and 70 and 79 actually have the same or slightly higher IGF-1/IGFBP-3 ratio.

Discussion

IGF-1 and IGFBP-3 have been evaluated with cancer because of their mitogenic properties as well as because of the co-regulatory effects on estrogen and insulin-like growth factor signaling [30, 31], two pathways that may be important for several types of cancers [32–35]. Studies evaluating associations with serum levels of IGF-1 and IGFBP-3 and breast cancer specifically report mixed results, with positive associations more frequently being reported for pre-menopausal women [36–43]. To date, few studies have evaluated differences

Table 4. Mean level of serum IGF-1, IGFBP-3, IGF-1/IGFBP-3 ratio by *IGF1* and *IGFBP3* genotype in Hispanic and non-Hispanic white women[a]

Genotype	Hispanic			Non-Hispanic white		
	IGF-1 N = 169	IGFBP-3 N = 166	IGF-1/IGFBP-3 Ratio N = 166	IGF-1 N = 233	IGFBP-3 N = 221	IGF-1/IGFBP-3 Ratio N = 221
IGF1						
19/19	107.4	3684.1	0.0290	123.6	4032.3	0.0304
19/no19	116.4	3805.1	0.0303	117.8	4070.5	0.0291
No 19	119.9	3711.2	0.0330	116.2	3934.4	0.0298
p-value	0.37	0.73	0.12	0.54	0.76	0.51
IGFBP3						
AA	124.2	3950.4	0.0313	122.3	4433.7	0.0274
AC	109.8	3899.9	0.0283	121.8	4073.2	0.0302
CC	110.6	3504.6	0.0310	115.8	3728.9	0.0306
p-value	0.44	0.01*	0.10	0.60	< 0.01*	0.05

[a] Adjusted for age and menopausal status.

in IGF-1 and IGFBP-3 by race/ethnic groups, groups known to have widely disparate breast cancer incidence rates. Our evaluation of differences in IGF-1 and IGFBP-3 in Hispanic and non-Hispanic white women living in the Southwestern United States contributes to our knowledge regarding not only differences that exist in these populations, but also genetic, diet, and lifestyle factors that contribute to those differences

In the current study, we observed significantly lower levels of IGFBP-3 among Hispanic women compared with non-Hispanic white women after adjusting for age. These lower levels were remained after adjusting for genotype. Anthropometric indicators were more strongly associated with IGF-1 and IGFBP-3 levels in Hispanic women than in non-Hispanic white women.

Studies conducted in African–American women also have shown differences in serum IGF-1 and IGFBP-3 levels compared with non-Hispanic white women [18]. Investigators of a multi-ethnic cohort found that Latino-American women had significantly lower age-adjusted IGF-1 and IGFBP-3 levels than other ethnic groups studied [19, 44]. Collectively, these findings indicate that ethnic differences in IGF-1 and IGFBP-3 levels. It remains to be determined if these ethnic differences influence rates of breast cancer incidence or survival in diverse populations. In the present study, mean IGF-1 levels were lower among Hispanic than non-Hispanic white women, but we did not observed significant differences between IGF-1 levels after adjusting for multiple predictors of IGF-1. The levels of IGFBP-3 were also significantly lower among Hispanic women, even after taking into account all other predictors. It may be important to note that the levels of IGFPB-3 among both Hispanic and non-Hispanic white women in this study among women living in the SW were considerably higher than those reported in the Multiethnic Cohort Study [19]. Given the relatively low rates of breast cancer in Hispanic women living in the Southwest, data showing significantly lower levels of IGFBP-3 for Hispanic women than for non-Hispanic white women suggest that IGFBP-3 levels may explain some of the differences observed in breast cancer incidence rates. How this might

Table 5. Differences in mean levels of IGF-1, IGFBP-3, and IGF-1/IGFBP-3 ratio between Hispanic and non-Hispanic white women, SHINE Study

	Hispanic N	Non-Hispanic N	Age & Menopause-adjusted			Full model			Full model with *IGFBP3* genotype		
			Hispanic Mean	Non-Hispanic Mean	*p*-value	Hispanic Mean	Non-Hispanic Mean	*p*-value	Hispanic Mean	Non-Hispanic Mean	*p*-value
IGF-1 mcg/ml	210	284	111.4	118.7	0.07	114.6	118.3	0.39	116.3	118.8	0.59
IGFBP-3 mcg/ml	204	266	3781.7	4076.6	< 0.01*	3764.3	4058.0	< 0.01*	3805.2	4071.9	< 0.01*
IGF-1/IGFPB-3 ratio	204	266	0.0294	0.0293	0.89	0.0305	0.0294	0.15	0.0305	0.0292	0.14

* Significant difference between mean values.

† Full model adjusted for age, BMI, number of times weight changed, vigorous physical activity, energy intake, dietary carbohydrates, dietary cholesterol, and menopause status

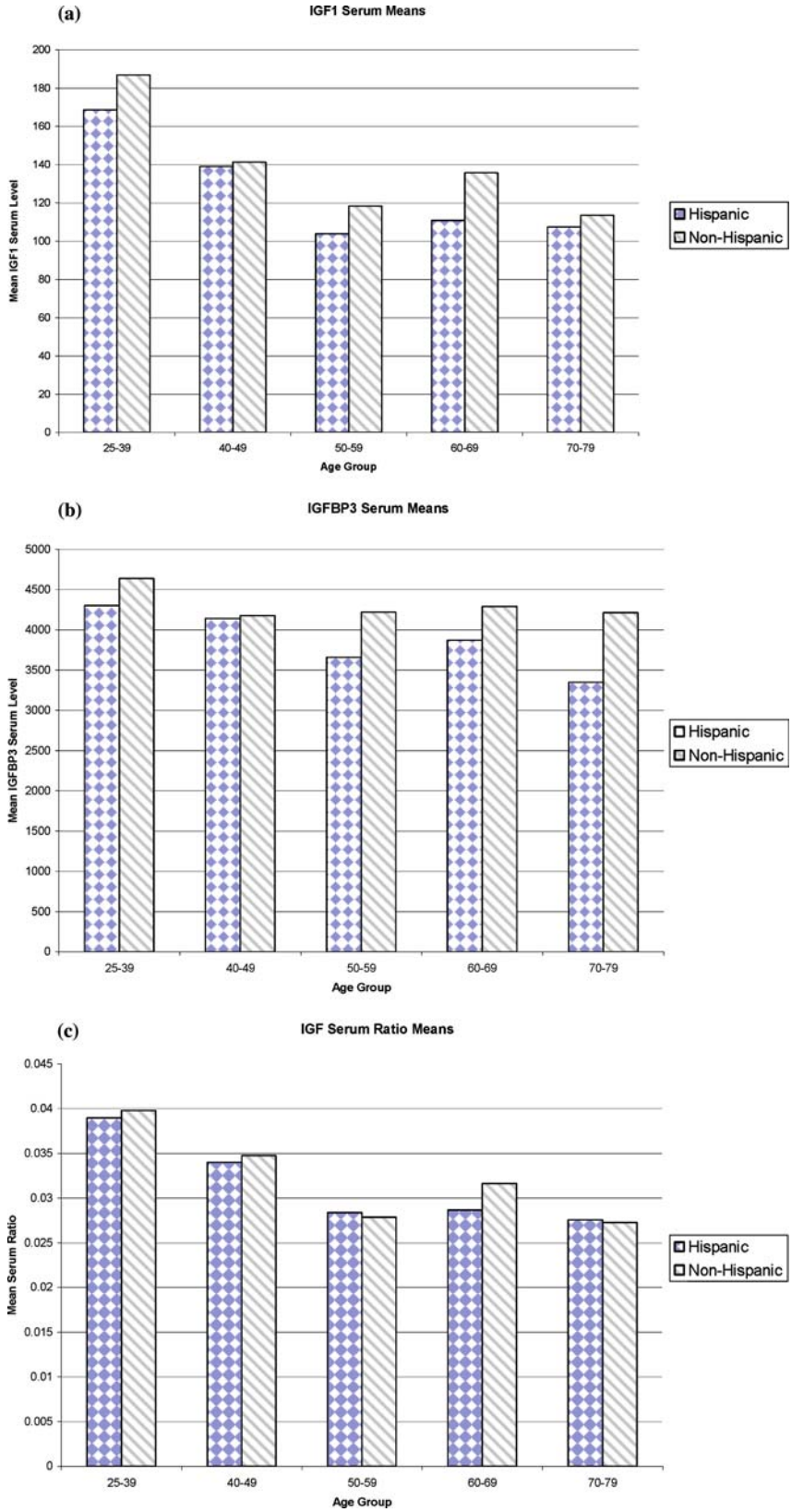

Fig. 1. Associations between IGF-1, IGFBP-3, IGF-1–IGFBP-3 ratio and age for Hispanic and non-Hispanic white women.

relate to more adverse prognosis after breast cancer among Hispanic women is less clear.

Given the potential role for IGF-1, IGFBP-3, and insulin-related factors in the etiology of cancer, it is important to identify factors that are associated with these levels in various ethnic groups. Others, evaluating correlates of IGF-1 and IGFBP-3, have shown body weight and waist and hip circumference measurements to be strongly associated with IGF-1 and insulin levels in older women [45–47]. A study examining both birth weight and current weight showed inverse associations between birth weight and IGF-1 levels, while current weight was directly associated with IGF-1 levels [46]. In this population we did not observe any association between birth weight and levels of IGF-I or IGFBP-3. Others have reported associations between smoking and alcohol and IGF-1 levels in Japanese men [47], although we did not detect associations with alcohol in this population. We did not detect associations between reported levels of physical activity or intensity of activity and IGFBP-3 levels; this corroborating the findings by Voskuil and colleagues [48]. Although we were somewhat limited to examine differences in physical activity because few women reported frequent involvement in vigorous activity, we did find a positive association between vigorous activity and IGF-1 for both Hispanic and non-Hispanic white women.

Previous studies have shown lower IGF-1 serum levels among vegan women compared to women who ate meat [49]. Increased *IGF1* levels also have been observed for men reporting high intake of total protein [50]. Our results show little evidence for an association between *IGF1* and protein intake either ethnic group. In this study, total energy intake was inversely associated with IGF-1 in Hispanic women only. Studies results have been mixed for the reporting of genetic factors that may influence IGF-1 and IGFBP-3 levels. The Multiethnic Cohort did not find that the *IGF1* CA repeat was associated with *IGF1* levels in postmenopausal women. A study conducted primarily in African American women taking oral contraceptives showed a direct association between plasma levels of IGF-1 and the [51] *IGF1* repeat polymorphism [18]. The same CA repeat in the *IGF1* gene was evaluated among women in the Nurses' Health Study and showed that women without a 19 CA allele had lower IGF-1 levels. We did not observe a significant association between IGF-1 levels and the 19 CA repeat in the *IGF1* gene. However, we did observe significant associations between the -202 A > C polymorphism of the *IGFBP3* gene and circulating IGFBP-3 levels. We found, as others also have reported, that those with the CC genotype have lower IGFBP-3 levels [25]. We have shown this association to be present among both Hispanic and non-Hispanic white women.

The effects of IGF-1 and IGFBP-3 on breast cancer risk have been hypothesized as being stronger for pre-menopausal breast cancer than post-menopausal breast cancer [39, 52, 53]. Thus, we evaluated associations between IGF-1 and IGFBP-3 and diet and lifestyle factors by menopausal status. In general, we observed that most diet and lifestyle factors contribute more to variability in IGF-1, IGFBP-3, and their ratio among pre-menopausal women, especially among Hispanic women. However, IGF-1/IGFBP-3 levels were more strongly associated with anthropometric indicators among post-menopausal women than among pre-menopausal women. Given these observations, there are implications for mechanisms to reduce breast cancer risk associated with IGF-1 and IGFBP-3 levels.

The study has several potential limitations. First, we have only evaluated two polymorphisms for *IGF1* and *IGFBP3*. It is possible that other polymorphisms may have additional functional relevance that is not shared by these polymorphisms. Likewise, given the *IGF1* polymorphism is a length variant, it is possible that repeats other than the 19CA repeat may be important. The sample included in these analyses is a subset of a larger sample of controls; however correlations between genotype and serum levels may not vary by sample selection. A larger limitation is the small sample size that has prohibited us from doing further subgroup analysis that may reveal factors that influence serum levels of IGF-1 and IGFBP-3.

In summary, these findings suggest that behavioral as well as genetic factors may contribute to IGF-1 and IGFBP-3 levels and that these factors may vary by ethnicity. Hispanic women had significantly lower IGFBP-3 levels than non-Hispanic white women. The differences in these levels may be partially attributed to genetic factors, but differences in IGFBP-3 levels remained after IGFBP-3 C-202A polymorphism was taken into account. Recent publications have reported the presence of additional polymorphisms in IGF-1 [54] and IGFBP-3 [26]; functional consequences of these variants are not well understood. Future research should further explore genetic influences on expression of IGF-1 and IGFBP-3. Diet and lifestyle factors also appear to influence levels of IGF-1 and IGFBP-3, especially among pre-menopausal women. Additional research on the potential contribution of IGFBP-3 to cancer risk and survival after diagnosis and how it may differ in diverse populations is needed.

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