BREAST DENSITY AND METABOLIC RISK FACTORS: A CROSS-SECTIONAL ANALYSIS OF A PHASE II STUDY IN PREMENOPAUSAL WOMEN WITH ELEMENTS OF METABOLIC SYNDROME

by

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ABSTRACT

Background

Breast density is an established breast cancer risk factor. Metabolic disturbances and high adiposity also increase breast cancer burden, but their relationships with breast density are not defined. This dissertation research aims to provide more evidence of the associations of metabolic risk factors with breast density measures in a cohort of premenopausal women with elements of metabolic syndrome.

Experimental Design

We performed this dissertation research within the context of a Phase II clinical trial conducted at the University of Arizona Cancer Center in overweight or obese premenopausal women with metabolic dysregulations. The primary aim of the trial was to evaluate if metformin intervention for 12 months can exert changes in breast density. A cross-sectional analysis was performed using the baseline data of the study cohort to evaluate the associations of breast density measurements with metabolic disturbances. The breast density measures, acquired by fat-water MRI, included absolute dense volume, percent density, non-dense volume, and total breast volume. The measures of metabolic disturbances included anthropometric measures, elements of metabolic syndrome, insulin/insulin-like growth factor (IGF) axis, and adipokines.

Results

Our findings indicate that each breast density measurement is non-normally distributed and comprised of distinct subpopulations with normal distributions. We correlated the breast density measurements with anthropometric measures. Those relationships were unaffected by ethnicity but were affected by age and reproductive factors. Total breast volume and non-dense volume were positively related to waist circumference, a measure of central adiposity, after adjustment for potential confounders (i.e., adiposity, ethnicity, age, reproductive factors). These two density measures were also positively correlated with serum leptin levels following adjustment of potential confounders, including waist circumference, but not with other measures of metabolic disturbance. Individuals with elevated fasting glucose had lower absolute dense volume. The insulin and HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) were inversely related to the absolute dense volume after adjustment for potential confounders. In addition, we observed a positive correlation between the absolute dense volume and serum leptin levels. Individuals with elevated fasting glucose had a lower percent density. Individuals with elevated fasting glucose had lower percent density but showed no correlation with insulin and HOMA-IR in analysis adjusted for potential confounders. Furthermore, percent density was positively related to a measure of bioavailable IGF-1 (molar ratio of IGF-1/IGF binding protein 3).

Conclusion

The heterogeneity of breast density measures indicates that there is a wide range of values within our cohort. The observed associations between breast density measures with selected metabolic disturbances suggest the importance of considering these metabolic disturbances when evaluating breast density measures for risk assessment in women with elements of metabolic syndrome. Further investigations are needed to understand the mechanisms responsible for the observed associations.

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CHAPTER I: Introduction

1.1. Breast Density: A Risk Factor for Breast Cancer

The definition of breast density is the amount of parenchyma in relation to the adipose tissue [Destounis 2017]. In a mammogram, the parenchyma appears as light, whereas the fat tissue appears as dark [Lokate 2011, Baglietto 2013]. High breast density, measured either as the percent or absolute density, is related to an increased breast cancer risk [Falcon 2017]. Women with higher mammographic percent density have a 4 to a 6-fold higher risk than those women with fatty breasts [Baglietto 2013].

Several studies suggested that breast density is a modifiable breast cancer risk factor. Findings from the IBIS-I trial showed a reduction in breast density after tamoxifen intervention in a cohort of high-risk women for the development of invasive breast cancer or ductal carcinoma in situ (DCIS) [Cuzick 2015]. Interventions to modify breast density are pharmacological approaches with selective estrogen receptor modulators (SERMs) or by diet [Fabian 2015, Vogel 2010].

Even though these and other studies suggest breast density may be modified, the etiology of breast density is unclear. Breast density has been attributed to non-modifiable factors, like genetic variants and age [Martin 2008]. We briefly describe established factors for breast density, considered as relevant for this dissertation research, in the following section.

1.1.1. Established Factors for Breast Density

There are non-modifiable established factors for mammographic breast density, like age and the menopausal status. Aging exerts an effect on breast density, being at its highest during puberty and decreasing gradually with increasing age, reaching its lowest point after menopause [Pike 1983, Ginsburg 2008]. Hormones can affect the proliferation of epithelial breast cells and may induce changes in breast density [Boyd 2005, Martin 2008].

Among the modifiable factors for breast density are menstrual cycles, parity, and body weight. The menstrual cycle can influence breast density, reaching its highest during the luteal phase (days 15 to 28) [Harvey 2004, Morrow 2010]. Parity can modify breast density [Lokate 2011]. Nulliparous women tend to have higher breast density than parous women [Lokate 2011]. Moreover, parous women have an estimated reduction of 2% in the percent densities per pregnancy [Boyd 2007]. Breast adiposity may also modify mammographic percent density, being lower in women with fatty breasts [Stuedal 2011, Gillmann 2016]. However, the influence of adiposity on absolute density is unclear, as conflicting data exist in the literature [Wanders 2015].

According to these and other studies described above, breast density can be affected by modifiable and non-modifiable factors. It must be noted, though, that the estimations of breast density can vary according to the imaging modalities used for its assessment.

1.2. Imaging Modalities for Assessing Breast Density.

1.2.1. Mammography versus Magnetic Resonance Imaging (MRI) for Breast Density Assessment

There is no gold standard for evaluating breast density. However, the clinic uses mammography as the standard imaging modality for breast cancer screening [Andreea 2011]. In mammography, the parenchyma is depicted as radio-dense or white, whereas the adipose tissue is radio-lucent or black [Falcon 2017]. Mammography, though, presents some limitations when it comes to evaluating dense breasts. The breast tissue overlapping in dense breasts can lead to poor precision, sensitivity, and lack of reproducibility [Chen 2015]. The three-dimensional mammography, or tomosynthesis, offers some advantages over the conventional mammography, such as fewer false positives and greater detection of actual cancers [Haas 2013]. However, tomosynthesis still acquires density from compressed breasts [Batohi 2015]. MRI evaluates density on non-compressed breasts. Therefore, it can overcome issues related to breast compression and may provide a more reliable imaging modality for women with dense or large breasts, reducing the possibility of detection bias [Andreea 2011].

The clinical benefits of breast MRI for breast cancer screening is still a matter of debate [Menezes 2014]. MRI technologies, although highly sensitive for tumor lesions, have a moderate specificity [Berg 2004, Kim 2011]. Some clinical studies indicate that MRI may overestimate lesion size in cases of invasive lobular carcinoma and DCIS [Mann 2008]. Further research might be necessary in order to confirm the utility of breast MRI in detecting invasive breast cancers [McGhan 2010]. In addition, most of the clinical studies conducted with MRI are small, for which the benefits of breast MRI remain unclear [Menezes 2014]. Larger longitudinal studies with more statistical power are required to further confirm the clinical benefits of MRI [Menezes 2014]. For this reason, women at high-risk are advised to take a combined imaging modality approach [Andreea 2011].

1.2.1.1. Evaluation Criteria for Breast Density in Mammography

There are different types of criteria for assessment of breast density. Those are qualitative and quantitative criteria.

<u>Qualitative Criteria for Breast Density Assessment</u>

The Breast Imaging Reporting and Data System (BI-RADS) is the most commonly used criteria as described by the American College of Radiology (ACR). BI-RADS classifies breast density into four categories: almost entirely fat, scattered fibroglandular tissue, heterogeneously dense, and extremely dense breasts [Falcon 2017]. The Wolfe pattern is another qualitative criterion. This one classifies breast density according to the duct patterns in: N1 for normal or entirely fat, P1 for mostly fat, P2 for duct patterns for more than 25% of the breast, DY for dysplasia, and QDT for young women (below 45 years old) who present the DY pattern due to youth [Destounis 2017].

<u>Quantitative Criteria for Breast Density Assessment</u>

Quantitative methods use computer-aided segmentation of the fibroglandular area from digitized mammograms. Quantitative methods are classified into semi-automated and fully-automated methods. Semi- automated methods use reader-based thresholds to define the breast edges on digitized mammograms. An example of the semi-automated method is the Cumulus, which is considered the gold standard due to its high reproducibility [Destounis 2017].

Fully-automated methods are classified as area-methods or as volumetric methods. Area methods include research-only tools, like ImageJ, and automated algorithms that classify breast

density according to BI-RADS, like iReveal and Densitas [Destounis 2017]. On the other hand, volumetric methods estimate the total volume of the breast and the fibroglandular tissue. An example of those is the Volpara, which uses the pixel intensities in the raw mammographic images [Destounis 2017, Chen 2015].

Even though quantitative tools have improved the evaluation for breast densities, there are still caveats on their applications in the clinic. The most common one is the intrinsic tissue overlapping coming from the two-dimensional based analysis [Chen 2015]. For this reason, the clinic recommends other imaging modalities for an accurate assessment of breast density.

1.2.2. Advantages of MRI for Breast Density Assessment

MRI is an imaging modality that may increase the accuracy of breast density measurement. MRI evaluates density on non-compressed breasts. It provides soft-tissue contrast between the adipose tissue and the parenchyma, offering several advantages over mammography [Falcon 2017]. MRI has been shown to be superior in sensitivity, enabling the detection of small lesions in dense breasts [Bluemke 2004, Kuhl 2007, Schrading 2008, Nickson 2009, Zhou 2015, Elsamaloty 2009, Kriege 2004]. MRI has no ionizing radiation, offering the possibility of screening young women who seek breast density assessment and for whom mammography may not be recommended [O'Flynn 2015]. Moreover, MRI provides volumetric parameters of the parenchyma that is superior to that of mammographic breast parameters [Wei 2004, Klyfa 2010]. These and other advantages of MRI may aid in subsequent screening stratification according to the individual's risk.

1.2.2.1. MRI Sequences for Breast Density Assessment

There are different MRI sequences to evaluate breast density. Most MRI sequences evaluate breast density on T1-weighted sequences. In those sequences, the parenchyma is distinguished from fat by the inherent differences in tissue relaxation times [Khazen 2008]. Other MRI sequences evaluate density from fat-suppressed and non-fat-suppressed images [Chang 2011]. In those, the relaxation properties of water and fat are exploited, enabling the acquisition of separate water-only and fat-only images [Chang 2011, Ma 2008]. An example of this is the Dixon sequence, which enables the estimation of the percent of parenchyma by fat-water separation [Ma 2008].

Even though these and other MRI sequences may enable the acquisition of more precise breast density measurements than that of mammograms, their use for breast screening remains controversial. Segmentation methods used to acquire MRI breast parameters can differ significantly [O'Flynn 2015]. Additionally, no consensus has been made about the optimal MRI sequence to be used in the clinic [O'Flynn 2015]. More evidence from large-scale trials is needed to establish the best MRI sequence for its clinical use.

1.2.2.1.1. Fat-Water MRI for Breast Density Assessment

Dixon et al. developed MRI sequences based on fat-water contents [Dixon 1984]. This MRI sequence uses the relative phases of the fat and water spins for data acquisition [Dixon 1984]. The data are then reconstructed into fat and water images, allowing the evaluation of intra- and intercellular compartments of the tissue [Dixon 1984].

Since then, distinct study groups developed several MRI sequences to measure the fat-water content in the breast. An example of those techniques is the radial Gradient-Echo and Spin-Echo (GRASE) [Rosado-Toro 2015]. In this MRI sequence, the fat and water spin allow the acquisition of fat-fraction (Frc) images of the breast. Additionally, this MRI sequence allows correcting for field inhomogeneities and T2-decay. Moreover, the GRASE enables breast imaging in a short time (approximately three minutes) [Rosado-Toro 2015, Li 2007]. Additionally, GRASE quantifies breast density without the need of MRI contrast agents [Li 2007].

The fat-water-MRI derived density measurements from GRASE are consistent with mammographic breast parameters. A study conducted by Trouard et al. showed a significant correlation of the fat fraction 50 (Frc50), acquired by GRASE and defined as the fraction of pixels with less than 50% of fat, with the mammographic percent densities [Trouard 2010]. Thomson et al. found mammographic percent density correlates with the fat fraction 50 (Frc50) and fat fraction 80 (Frc80) (Spearman $\rho = 0.9$ and 0.86 for Frc50 and 80, respectively, $p<0.001$) [Thomson 2015, Thomson 2017]. However, more studies are needed to evaluate fat-water MRI as a method for breast density assessment before its use in the clinic.

1.3. Statement of the Problem

1.3.1. The Conundrum of Adiposity and Breast Density Measurements

Obesity relates to an increased risk for postmenopausal breast cancer [Neuhouser 2015]. The association between obesity and premenopausal breast cancer is not clear. Several studies showed a null or an inverse association of obesity with premenopausal breast cancer risk, suggesting a potential “protective” effect [Vucenik 2012, Tyson 2008]. The most widely accepted mechanism for this inverse association refers to anovulatory menstrual cycles and progesterone deficiency [Eliassen 2006]. However, this mechanism presumably accounts for hormonal-responsive breast cancers, with notable exceptions in the literature. Anderson et al. showed a 70% increase in premenopausal breast cancer risk among obese women in comparison to their lean counterparts [Anderson 2012]. Moreover, high adiposity is related to higher rates of triple-negative breast cancers [Renehan 2015]. Additionally, the detection of breast lumps is more difficult in obese women in comparison to their lean counterparts. The delayed detection of breast lumps may contribute to a late diagnosis at the menopausal stage. Additionally, obesity may contribute to the underreporting of premenopausal breast cancers. Several studies confirm this belief, where obese women had higher incidence rates for postmenopausal breast cancers than their lean counterparts [Renehan 2008, van der Brandt 2000].

It is not clear whether breast density is feasible for breast cancer risk assessment in obese women. Studies have shown an inverse association between percent density and measures of adiposity [Conroy 2011, Tehranifar 2011, Tseng 2010]. If we use the percent density for risk assessment, those studies indicate obese women have a lower breast cancer risk [Conroy 2011, Tehranifar 2011, Tseng 2010]. The relationship between absolute density and high adiposity is not clear [Kim 2015, Heng 2004]. We should notice that breast compression in obese women can obscure breast density measures acquired by mammography.

1.3.2. The Conundrum of Breast Density and Metabolic Disturbances

Metabolic syndrome is a cluster of metabolic disturbances that include hyperinsulinemia, hypertension, abdominal obesity, high fasting glucose, and dyslipidemia [Huang 2009]. Previous studies indicate that metabolic syndrome is considered a risk factor for breast cancer development [Gezgen 2012]. Nonetheless, it is not clear how metabolic syndrome relates to breast density.

Some studies indicated that metabolic risk factors are related to high breast density. Findings from a cross-sectional study indicate that premenopausal Korean women with metabolic syndrome had higher mammographic percent density [Kim 2015]. However, postmenopausal Korean women showed no relationship between metabolic syndrome and breast density [Kim 2015]. This study evaluated the relationship between metabolic risk factors and breast density [Kim 2015]. When comparing the individual elements of metabolic syndrome, all but the large waist circumference (≥ 80 cm) were related to mammographic percent densities in premenopausal women [Kim 2015]. Postmenopausal women with high glucose, but not other components, had higher percent densities [Kim 2015]. A study conducted in premenopausal Mexican women found higher percent densities in the presence of metabolic syndrome [Rice 2013]. Nonetheless, this study showed inconsistent findings across states, as the relationship was observed for Jalisco women but not for Veracruz women [Rice 2013].

Other cross-sectional analyses found no relationship between metabolic disturbances and breast density. A study conducted in the US in a multi-ethnic cohort of premenopausal women found no association between metabolic risk factors and mammographic breast density [Conroy 2011]. Another study performed in a multi-ethnic cohort found no relationships between percent density and metabolic syndrome [Tehranifar 2015]. Additionally, this study reports no associations between the individual components of metabolic syndrome and percent density [Tehranifar 2015]. We need more studies to understand the relationships of metabolic disturbances and breast density.

1.4. Specific Aims

The overall aim of this dissertation was to evaluate the associations between metabolic risk factors and breast density measurements. We sought to evaluate those associations in a cohort of overweight/obese premenopausal women with metabolic disturbances. For this purpose, we used baseline data collected from an ongoing Phase II trial. This clinical trial was double-blinded, randomized, and placebo-controlled.

The hypothesis was as follows: **Breast density was differentially related to metabolic dysfunction in premenopausal women with elements of metabolic syndrome and high adiposity.**

We proposed the following aims to answer the research question:

Aim #1: To evaluate the relationship between breast density measurements, anthropometric measurements, and elements of metabolic syndrome. We hypothesized that breast parameters were affected by measures of adiposity and metabolic risk factors. We evaluated body mass index (BMI), waist circumference, waist-hip ratio, and other elements of metabolic syndrome (i.e., elevated triglycerides (≥150 mg/dl), reduced HDL-C (<50 mg/dl), elevated blood pressure (≥130 mmHg systolic or ≥85 diastolic blood pressure), or elevated fasting glucose (≥100 mg/dl)) at the baseline visit. We used fat-water MRI images from the baseline visit to estimate the breast parameters. We performed statistical analysis to evaluate the associations between anthropometric measurements and breast parameters, and the associations between the elements of metabolic syndrome and breast parameters.

Aim #2: To evaluate the associations between breast density measurements and the insulin/IGF axis. We hypothesized that dysregulations in the circulating metabolites of the insulin/IGF axis were related to breast parameters. We assessed the circulating metabolites of the insulin/IGF axis by ELISA using the serum samples collected at the baseline visit. We conducted statistical analysis to evaluate the associations of the circulating metabolites of the insulin/IGF axis with breast density measurements.

Aim #3: To evaluate the associations between breast density measurements and the adipokines. We hypothesized that the circulating levels of adipokines were related to breast density measurements in the study cohort. We assessed the circulating levels of adipokines by ELISA. We conducted statistical analysis to evaluate the relationship between adipokines with breast parameters.

Impact of this work

Breast density is an important breast cancer risk factor that can be modified by growth factors and hormones. Metabolic syndrome and high adiposity may increase breast cancer risk. However, studies have not elucidated the associations between metabolic risk factors with breast density. This work aims to provide more evidence of those potential relationships in a cohort of premenopausal women with metabolic disturbances.

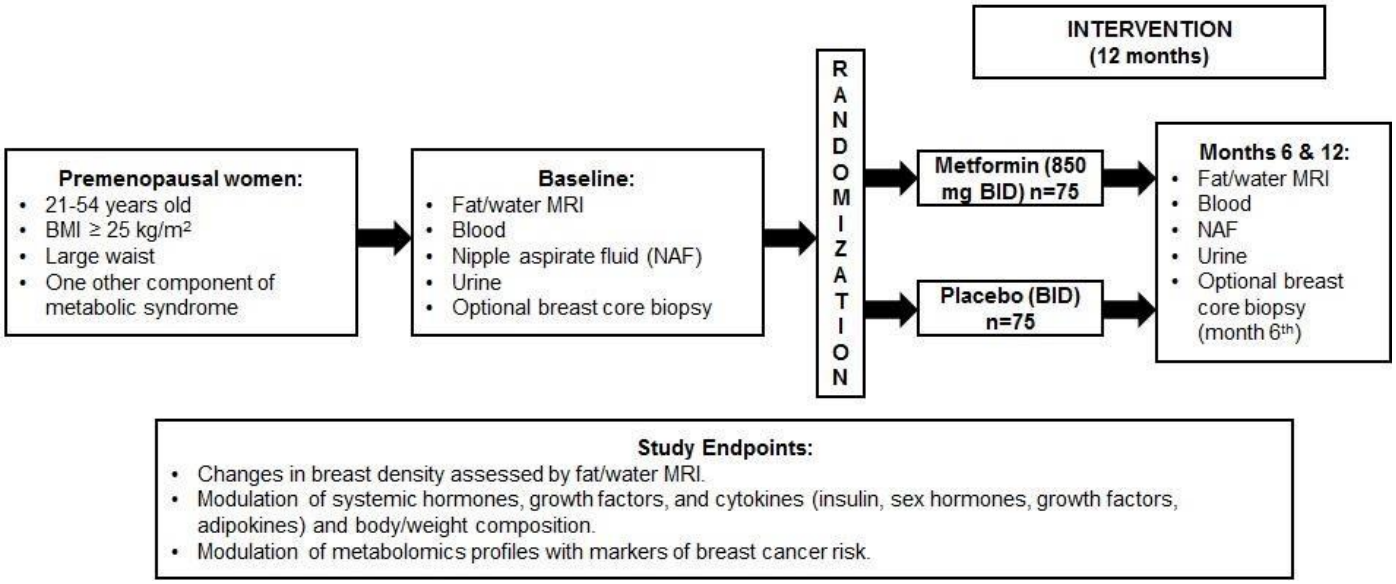
1.5. Clinical Trial: Phase II Study of Metformin for Reduction of Obesity-Associated Breast Cancer Risk.

The University of Arizona Cancer Center was conducting a Phase II, double-blinded, randomized, placebo-controlled trial in overweight/obese premenopausal women with elements of metabolic syndrome to evaluate if metformin can induce changes in breast density after 12 months of intervention.

1.5.1. Clinical Trial Design

The general scheme of this clinical trial was as follows:

Figure 1. Overall Clinical Study Design



1.5.2. Eligibility Criteria

This clinical trial was activated at the University of Arizona on March 7th,2014 and recruited healthy premenopausal women with elements of metabolic syndrome as previously defined by the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III guidelines [Huang 2009]. The key eligibility criteria include:

- Premenopausal women with regular menstrual cycles (defined as 25-32 days in length) for the past six months prior enrollment.
- Ages between 21-54 years old.
- Overweight or obese (BMI ≥25 kg/m^2).
- Central adiposity defined as large waistline circumference (≥35 inches or ≥31 inches for Asians and women with polycystic ovary syndrome).
- Have at least one other component of the metabolic syndrome of the following:

- Elevated triglycerides (≥150 mg/dL) or on drug treatment for it.
- Reduced high-density lipoprotein cholesterol (HDL-C) (< 50 mg/dL) or on drug treatment for it.
- Elevated blood pressure (≥130 mmHg systolic blood pressure or ≥85 mmHg diastolic blood pressure) or on drug treatment for hypertension.
- Elevated fasting glucose (≥100 mg/dL).

- Have normal hepatic or renal function.
- Not on treatment with any drug for diabetes.
- Have no previous history of lactic acidosis.
- Have no history of alcoholism or high alcohol consumption.

We completed the recruitment of study subjects in November, 2017. From 235 subjects assessed for eligibility, 151 subjects met eligibility and underwent baseline evaluation [See Appendix A, Figure A1 for a Consort Diagram].

Participants were asked to keep a menstrual calendar during the study to assist in scheduling the study visits during the mid-luteal phase, when feasible. The information was also used to adjust for the phase of the menstrual cycle as a potential confounder.

1.5.3. Study Evaluation at Baseline Visit

Participants who met all selection criteria underwent the following baseline evaluation during the mid-luteal phase of the menstrual cycle, when feasible:

Anthropometric measurements

Trained staff measured the body weight, waist circumference, and hip circumference of the study subjects. We asked the participants to stand with light clothing on a calibrated scale, with minimal movement with hands by their side. We measured waist and hip circumference in a standing position with a flexible metal tape. We measured the waist circumference at the midpoint between the lowest rib and the top of the iliac crest. We measured the hip circumference at the level of the greatest protrusion of the gluteal muscles.

Study Questionnaires

We asked the participants to complete the Arizona Food Frequency Questionnaire (AFFQ) to measure dietary patterns. We used the Arizona Activity Frequency Questionnaire (AAFQ) to assess physical activity. Participants also completed the Breast Cancer Risk Assessment Questionnaire. We used this questionnaire to collect information on risk factors.

The AFFQ is a modified version of the NCI Health Habits and History Questionnaire (HHHQ) [Block 1986] and includes 175 questions. Those include the frequency of particular food consumption over the prior 12 months, as well as their usual portion size (small, medium, large). The AFFQ includes 800 foods and provides information for 91 nutrients and 34 derived variables (e.g. grams of red meat, grams of cruciferous vegetables). The AFFQ is available in both English and Spanish.

The AAFQ comprises 59 questions and aims to measure the total energy expenditure. The AAFQ provides this information by using metabolic equivalent units (MET) per day and per activity. This questionnaire groups physical activities by categories (leisure, recreational, household, and

other). Moreover, the AAFQ provides the number of physical activities for each category. The AAFQ is available in both English and Spanish [Ainsword 2000, Staten 2001].

The Breast Cancer Risk Assessment Questionnaire comprises 9 questions. The goal is to estimate a woman's personal risk for invasive breast cancer development in the next 5-year period. The Gail model is the basis of this questionnaire. The National Cancer Institute (NCI) and the National Surgical Adjuvant Breast and Bowel Project (NSABP) developed this questionnaire. We used this to collect information on reproductive factors, personal, and family breast cancer risk. We did not use this questionnaire to calculate a risk score.

Collection of baseline samples

A fasting blood sample was collected and sent to Sonora Quest Laboratories for the analysis of a complete blood count with differential (CBC/w diff), comprehensive metabolic panel (CMP), and lipid panel. An additional fasting blood, urine, and nipple aspirate fluid (NAF), if feasible, were also collected. Serum/plasma was separated and aliquots stored at -70°C along with aliquots of urine and NAF samples.

Concomitant Medications

We documented all medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs that were taken by the participants.

Fat-water MRI Assessment of Breast Density

We performed MRI on a Siemens 3T MR system using a 16-channel breast MRI coil system. Fat-water maps were obtained using a multi-point Gradient Echo DIXON imaging method developed by Siemens [Li 2007, Reeder 2007].

Optional Ultrasound-Guided Breast Core Needle Biopsy

For participants who consented to this optional procedure, the medical specialist used a 14-gauge needle under ultrasound guidance to obtain up to 8 tissue cores from areas of high density in one of the breasts.

Following the completion of baseline evaluation, we randomized participants to receive metformin or placebo for 12 months. We asked the participants to take one 850 mg metformin or one placebo tablet daily for the first four weeks, then one 850 mg metformin or one placebo tablet twice daily for the remainder of the study. Participants returned at months 6 and 12 to repeat the evaluation performed at baseline. Participants repeated the optional biopsy only at the month 6.

1.6. Baseline Characteristics of the Study Population

1.6.1. Baseline Characteristics of the Overall Study Cohort

We summarized the baseline characteristics of the cohort in Table 1. The study population had a mean age of 39 years old, with a mean BMI of 37.8 kg/m^2. The cohort presented central adiposity, with a mean of 110.8 cm for waist circumference, and a mean value of 0.9 for the waist-hip ratio.

Most of the study subjects identified themselves as White or Caucasian (83.44%). About 4.6% identified themselves as African-American, 0.6% as Native Hawaiian or Pacific Islanders, 0.6% as Asians, 1.9% as American Indian or Alaska Native, 5.9% as more than two races, and 2.6% of the unknown race. In terms of ethnicity, 36.4% identified themselves as Hispanic or Latino, 62.9% considered themselves as Non-Hispanic or Latino, and 0.6% of unknown ethnicity. This population is representative of the one observed at Southern Arizona [U.S. Census 2016].

Presence of reduced HDL-C (<50 mg/dl or on drug treatment for it) was the most prevalent additional element of metabolic syndrome (64.9%), closely followed by the presence of elevated blood pressure (51.6% with blood pressure ≥130 mmHg systolic or ≥85 mmHg diastolic or on drug treatment for it). In addition, 33.1% of the participants presented with elevated triglycerides (≥150 mg/dl or on drug treatment for it) and 29.1% presented with elevated fasting glucose (≥100 mg/dl).

In addition, we collected the reproductive factors at the baseline visit, where 29.8% of the study subjects were taking hormonal-based contraception, 56.9% were taking non-hormonal contraception, 7.3% were not taking any contraception and 5.9% did not report this information.

With regards to the age at first live birth, this was presented by the age range and by a cut-off of 25 years of age. In terms of age range, 25.1% of the participants had their first child younger than 20 years old, 25.8% of the participants had a child between 20 and 24 years old, 13.9% of the study subjects had a child between 25 and 29 years old, 11.9% of the participants had a child at age 30 or older, 21.1% had no children, and 1.9% did not report this information. When classifying by age cut-off, 50.9% of the participants had a child before 25 years old, 25.8% of the participants had a child at ages 25 or older, 21.1% of the study subjects had no children, and 1.99% did not report this information.

For the age at menarche, 30.4% of the participants had their first period between 7 and 11 years old; 53.6% of the study subjects had their first period between 12 and 13 years old; 13.9% had their first period at 14 years old or older, and 1.9% of the participants did not report the age at menarche.

Most of the study subjects (84.1%) reported no relatives diagnosed with breast cancer, 12.5% of the participants reported one first-degree relative diagnosed with breast cancer, 0.6% reported more than one first-degree relative with breast cancer, and 2.6% had no knowledge of family history of breast cancer.

In terms of the number of breast biopsies performed before the baseline visit of this trial, 86.7% of the study subjects had no prior breast biopsies, 11.2% reported prior breast biopsies, and 1.9% of the participants did not report this information.

Table 1. Baseline Characteristics of the Study Population (N= 151).

Variable	Value
Age (years), mean(SD)	39.5 (8.23)
Body mass index (kg/m^2), mean(SD)	37.8 (6.83)
Waist circumference (cm), mean(SD)	110.8 (12.43)
Waist-hip ratio, mean(SD)	0.9 (0.07)
Race, number (%)	
White or Caucasian	126 (83.44)
Black or African American	7 (4.64)
Native Hawaiian or another Pacific Islander	1 (0.66)
Asian	1 (0.66)
American Indian or Alaska Native	3 (1.99)
More than 2	9 (5.96)
Unknown	4 (2.65)
Ethnicity, number (%)	
Hispanic or Latino	55 (36.42)
Non-Hispanic or Latino	95 (62.91)
Unknown	1 (0.66)
Elevated triglycerides (≥ 150 mg/dl) or on drug treatment for it, number (%)	
No	101 (66.89)
Yes	50 (33.11)
Reduced HDL (< 50 mg/dl) or on drug treatment for it, number (%)	
No	53 (35.10)
Yes	98 (64.90)
Elevated BP (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic) or on drug treatment for it, number (%)	
No	73 (48.34)
Yes	78 (51.66)
Elevated fasting glucose (≥ 100 mg/dl), number (%)	
No	107 (70.86)
Yes	44 (29.14)
Contraception, number (%)	
Hormonal	45 (29.80)
Non-hormonal	86 (56.95)
No contraception	11 (7.38)
Unknown	9 (5.96)
Age at menarche, number (%)	
7-11	46 (30.46)
12-13	81 (53.64)

≥14	21 (13.91)
Unknown	3 (1.99)
Age at first live birth, number (%)	
<20	38 (25.17)
20-24	39 (25.83)
25-29	21 (13.91)
≥30	18 (11.92)
No Births	32 (21.19)
Unknown	3 (1.99)
Category age at first live birth, number (%)	
<25	77 (50.99)
≥25	39 (25.83)
No Births	32 (21.19)
Unknown	3 (1.99)
First degree relatives with breast cancer, number (%)	
0	127 (84.11)
1	19 (12.58)
>1	1 (0.66)
Unknown	4 (2.65)
History of biopsy, number (%)	
No	131 (86.75)
Yes	17 (11.26)
Unknown	3 (1.99)

1.6.2. Baseline Characteristics of the Study Population by Ethnicity

We summarized in Table 2 the baseline characteristics of the study participants by ethnicity. We did not observe statistically significant differences between Hispanics and Non-Hispanics.

Table 2. Baseline Characteristics of the Study Population by Ethnicity.

Variable	Hispanics (n=55)	Non-Hispanics (n=95)	p-value
Age (years), mean(SD)	39.0 (8.23)	39.8 (8.29)	0.575
Body mass index (kg/m^2), mean(SD)	37.0 (5.66)	38.3 (7.42)	0.268
Waist circumference (cm), mean(SD)	109.6 (12.18)	111.5 (12.66)	0.381
Waist-hip ratio, mean(SD)	0.9 (0.07)	0.9 (0.07)	0.797
Elevated triglycerides (≥ 150 mg/dl) or on drug treatment for it, number (%)			0.709
No	36 (65.45)	65 (68.42)	
Yes	19 (34.55)	30 (31.58)	
Reduced HDL (< 50 mg/dl) or on drug treatment for it, number (%)			0.878
No	19 (34.55)	34 (35.79)	
Yes	36 (65.45)	61 (64.21)	
Elevated BP (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic) or on drug treatment for it, number (%)			0.839
No	27 (49.09)	45 (47.37)	
Yes	28 (50.91)	50 (52.63)	
Elevated fasting glucose (≥ 100 mg/dl), number (%)			0.244
No	42 (76.36)	64 (67.37)	
Yes	13 (23.64)	31 (32.63)	
Contraception, number (%)			0.645
Hormonal	19 (34.55)	25 (26.32)	
Non-hormonal	28 (50.91)	58 (61.05)	
No contraception	4 (7.27)	7 (7.37)	
Unknown	4 (7.27)	5 (5.26)	
Age at menarche, number (%)			0.495
7-11	18 (32.73)	28 (29.47)	
12-13	26 (47.27)	55 (57.89)	
≥14	10 (18.18)	10 (10.53)	
Unknown	1 (1.82)	2 (2.11)	
Age at first live birth, number (%)			0.652
<20	17 (30.91)	20 (21.05)	
20-24	16 (29.09)	23 (24.21)	
25-29	6 (10.91)	15 (15.79)	
≥30	5 (9.09)	13 (13.68)	
No Births	10 (18.18)	22 (23.16)	
Unknown	1 (1.82)	2 (2.11)	
First degree relatives with breast cancer, number (%)			0.213
0	33 (60.00)	43 (45.26)	
1	11 (20.00)	28 (29.47)	
>1	10 (18.18)	22 (23.16)	

Unknown	0 (0.00)	0 (0.00)	
History of biopsy, number (%)			0.482
No	50 (90.91)	80 (84.21)	
Yes	4 (7.27)	13 (13.68)	
Unknown	1 (1.82)	2 (2.11)	

1.6.3. Baseline Metabolic Factors of the Study Cohort.

Additionally, we analyzed the serum samples collected at baseline to assess the presence/absence of other metabolic risk factors, such as elevated triglycerides (≥150 mg/dl), reduced high-density lipoprotein (HDL) cholesterol (<50 mg/dl), or elevated fasting glucose (≥100 mg/dl). The average HDL cholesterol levels were lower than the normal values reported for a lean population (> 50 mg/dl), overall (mean 46 mg/dl) and by ethnicity (46 mg/dl Hispanics, 45 mg/dl Non-Hispanics). In addition, the average LDL cholesterol levels were higher than the normal values reported for a lean population (less than 100 mg/dl), overall (111 mg/dl) and in Non-Hispanics (112 mg/dl). Most study subjects presented normal glucose levels. Prediabetics (≥100 mg/dl to 125 mg/dl) and diabetics (≥125 mg/dl) represented the 14.6% and the 2.6%, respectively, in this cohort.

When stratified by ethnicity, 12.7 and 3.9% Hispanics were prediabetics and diabetics, respectively, whereas 15.8 and 2.1% Non-Hispanics were prediabetics and diabetics, respectively. The average total cholesterol (186 mg/dl overall, 183 mg/dl Hispanics, 188 mg/dl Non-Hispanics), and triglycerides (144 mg/dl overall, 141 mg/dl Hispanics, 145 mg/dl Non-Hispanics) were within the range of values reported for a normal population (70-100 mg/dl glucose, < 200 mg/dl total cholesterol, <150 mg/dl triglycerides) [Table 3].

Table 3. Baseline Metabolic Factors of the Study Cohort.

Variable	Overall Cohort	Hispanics	Non-Hispanics	p-value
Fasting Serum Glucose (mg/dl) [a]				
N	151	55	95	0.74
Mean (SD)	96 (15)	96 (13)	97 (16.)	
Median (range)	95 (74 – 212)	93 (75 – 145)	95 (74 – 212)	
95% CI	(94, 99)	(92, 99)	(94,100)	
Fasting Serum Total Cholesterol (mg/dl) [b]				
N	150	55	94	0.32
Mean (SD)	187 (30)	183 (26)	188 (32)	
Median (range)	184 (130 – 275)	178 (137 – 246)	187 (130 – 275)	
95% CI	(182 – 191)	(176 – 190)	(182 – 195)	
Fasting Serum Triglycerides (mg/dl) [c]				
N	150	55	94	0.73
Mean (SD)	145 (69)	142 (55)	146 (77)	
Median (range)	134 (37 – 462)	134 (46 – 338)	133 (37 – 462)	
95% CI	(133 – 156)	(127, 157)	(130, 162)	
Fasting Serum HDL Cholesterol (mg/dl) [d]				
N	150	55	94	0.88
Mean (SD)	46 (11)	46 (10)	46 (11)	
Median (range)	45 (26 – 82)	45 (27 – 82)	45 (26 – 79)	
95% CI	(44, 48)	(43, 49)	(44, 48)	

Fasting Serum LDL Cholesterol (mg/dl) [e]				
N	148	55	92	0.38
Mean (SD)	111 (26)	109 (22)	113 (29)	
Median (range)	107 (46 – 181)	106 (60 – 157)	107 (46 – 181)	
95% CI	(107 – 116)	(103, 115)	(107, 119)	

(a) Fasting serum glucose cutoff values (mg/dl): normal levels < 100 mg/dl, prediabetes from 100 mg/dl to 125 mg/dl, diabetes ≥ 125 mg/dl.
(b) Fasting serum total cholesterol cutoff values (mg/dl): normal levels < 200 mg/dl, borderline high from 200 mg/dl to 239 mg/dl, high ≥ 240 mg/dl.
(c) Fasting serum triglycerides cutoff values (mg/dl): normal levels < 150 mg/dl, borderline high from 150 mg/dl to 199 mg/dl, high from 200 mg/dl to 499 mg/dl, very high ≥ 500 mg/dl.
(d) Fasting serum HDL cholesterol cutoff values (mg/dl): normal levels ≥ 60 mg/dl, borderline low from 40 mg/dl to 59 mg/dl, low < 40 mg/dl.
(e) Fasting serum LDL cholesterol cutoff values (mg/dl): optimal levels < 100 mg/dl, near optimal levels from 100 mg/dl to 129 mg/dl, borderline high from 130 mg/dl to 159 mg/dl, high from 160 mg/dl to 189 mg/dl, very high ≥ 190 mg/dl.

1.7. Summary

Breast density is a well-established breast cancer risk factor [Boyd 2005]. Factors such as age, menopausal status, body weight, and parity, can influence breast density [Boyd 2005, Martin 2008, Lokate 2011]. Breast density can be evaluated by different imaging modalities, like mammography and magnetic resonance imaging (MRI) [Andreea 2011, Chen 2015].

High adiposity and metabolic disturbances are related to breast cancer risk. Nonetheless, the relationships between adiposity, metabolic disturbances, and breast density remain unclear [Wanders 2015, Gezgen 2012 Neuhouser 2015, Vucenik 2012, Tyson 2008]. The cause of inconsistent associations is unknown. However, we may attribute those inconsistencies to measurement bias, which is given by breast compression with mammography. The University of Arizona Cancer Center is conducting a phase II, double-blinded, randomized, placebo-controlled trial in premenopausal women with metabolic risk factors and high adiposity. We used baseline data collected from this phase II clinical trial to determine the association between metabolic disturbances and breast density, with breast density measurements acquired by fat-water MRI on non-compressed breasts.

The baseline demographics of the study cohort are similar to that of southern Arizona, with 36.42% of Hispanics or Latinas and 62.91% of Non-Hispanics or Latinas. The study population is overweight/obese, with the presence of elements of metabolic syndrome such as elevated triglycerides (≥150 mg/dL or on drug treatment for it), reduced HDL- C (<50 mg/dL or on drug treatment for it), elevated blood pressure (≥130 mmHg systolic or ≥85 mmHg diastolic or on drug treatment for it), and elevated fasting glucose (≥ 100 mg/dL). The presence of overall adiposity and metabolic disturbances may indicate a higher risk for breast cancer for our study cohort.

The dissertation research was designed to gain further understanding of the associations between metabolic risk factors and breast density to improve the utilization of breast density for breast cancer risk assessment in overweight/obese women with metabolic disturbances.

CHAPTER II: Breast Density Measurements in the Study Cohort

2.1. Introduction

Breast density is an established breast cancer risk factor. Women with high density have a 4 to 6-fold higher breast cancer risk in comparison to women with low density [Baglietto 2014]. Breast density can be assessed by several imaging modalities, like mammography and MRI. Those technologies allow the acquisition of different breast density measurements, such as the percent density, the absolute density, the non-dense area or volume, and the total breast size [Destounis 2017]. In this dissertation chapter, we review the associations between different breast density measurements with breast cancer risk. In addition, we describe the breast density measurements acquired by fat-water MRI in the study cohort. Additionally, we explored the potential differences in the breast density measurements by ethnicity.

2.1.1. Percent Density and Absolute Density as Breast Cancer Risk Factors

Percent density is the proportion of breast parenchyma in relation to the local breast adiposity. Most studies use percent density for risk assessment. Other studies use the absolute density for risk assessment. The absolute density is the depiction of breast parenchyma.

Table 4 lists examples of studies examining these density measurements with breast cancer risk. Higher mammographic percent densities are consistently related to a higher breast cancer risk [Stone 2009, Maskarinec 2000, Torres-Mejia 2005, Rauh 2012, Vachon 2007]. However, the percent density can be affected by overall adiposity, where large women have low percent densities in comparison to lean women [Stone 2009]. If we use the percent density for risk assessment, we may infer that women with high adiposity have lower breast cancer risk in comparison to their lean counterparts [Stone 2009]. We should consider the confounding effects of adiposity in large women when interpreting the results.

Similarly, higher density areas are related to a higher breast cancer risk in most studies [Maskarinec 2000, Torres-Mejia 2005, Vachon 2007, Lokate 2011, Ursin 2005, Nagata 2005]. However, Rauh et al. showed that women with high absolute dense areas had a lower breast cancer risk (OR= 0.75) [Rauh 2012]. Nevertheless, Rauh et al showed the predictive value of the absolute dense area when used as an adjusting variable for the percent density (OR= 2.12) [Rauh 2012]. For this reason, we may use both the absolute and the percent density for breast cancer risk assessment [Table 4].

Table 4. Case-Control Studies Evaluating the Associations between the Mammographic Percent Density and the Absolute Dense Area with Breast Cancer Risk.

First Author, Year	Total #	Cohort	Predicted Breast Cancer Risk	
			Mammographic Percent Density (PD)	Absolute Dense Area (DA)
Stone, 2009	815	Premenopausal and postmenopausal women from the IBIS-I study	β= 1.24 for moderate PD β= -1.41 for high PD	β= 0.19 for moderate DA β= 0.23 for high DA
Maskarinec, 2000	647	Multi-ethnic pre- and postmenopausal women	OR = 1.8 (95% CI, 1.1 to 3.0) for a comparison between >50% PD vs <10% PD	OR = 1.8 (95% CI, 1.2 to 2.6) for a comparison between the highest and lowest quintiles of DA
Torres-Mejia, 2005	3211	Women ≥ 35 years old from the Guernsey III and Guernsey IV Breast Cancer Research Project	OR = 3.49 (95% CI, 1.69 to 7.18) for a comparison between highest and lowest quartiles	OR = 2.69 (95% CI, 1.40 to 5.16) for a comparison between highest and lowest quartiles
Rauh, 2012	937	Women ≥18 years old from the BBCC1 study	OR = 2.12 (95% CI, 1.25 to 3.62) for a comparison between the highest and lowest quartiles of PD	OR = 0.75 (95% CI, 0.49 to 1.15) for a comparison between the highest and lowest quartiles of DA
Vachon, 2007	1085	Pre- and postmenopausal women	OR = 3.06 (95% CI, 1.88 to 4.98) for comparison between highest and lowest quartiles of PD	OR = 2.45 (95% CI, 1.59 to 3.78) for a comparison between the highest and lowest quartiles of DA
Lokate, 2011	1217	Postmenopausal women from the EPIC-NL study	OR = 1.8 (95% CI, 1.0 to 2.9) for comparison between highest and lowest quintiles of PD	OR = 2.8 (95% CI, 1.7 to 4.8) for a comparison between highest and lowest quintiles of DA
Ursin, 2005	1065	Pre- and postmenopausal women	OR = 5.23 (95% CI, 1.70 to 16.13) for a comparison between >75% PD vs 0% PD	OR = 3.8 (95% CI, 1.43 to 10.08) for a comparison between highest and lowest sextiles of DA
Nagata, 2005	441	Premenopausal Japanese women	OR = 1.36 (95% CI, 0.31 to 6.06) for a comparison between >75% PD vs 0% PD	OR = 2.78 (95% CI, 0.77 to 10.1) for a comparison between highest and lowest quintiles of DA

2.1.2. The Non-Dense Area and Breast Cancer Risk

Limited case-control studies evaluated the relationship between the mammographic non-dense areas and breast cancer risk [Table 5]. Most of them indicated that larger non-dense areas were related to a lower breast cancer risk [Baglietto 2014, Petterson 2011]. This result may suggest local adiposity has a protective effect [Baglietto 2014, Petterson 2011]. In contrast, postmenopausal women with higher local adiposity had an increased breast cancer risk [Lokate 2011]. We should consider the overall adiposity when evaluating the non-dense areas, as it may act as a confounder for breast cancer risk assessment.

Table 5. Case-Control Studies Evaluating the Associations between the Non-Dense Area and the Absolute Dense Area with Breast Cancer Risk.

First Author, Year	Total #	Cohort	Predicted Breast Cancer Risk	
			Non-Dense Area (NDA)	Absolute Dense Area (ADA)
Lokate, 2011	1217	Postmenopausal women from the EPIC-NL study	OR = 2.4 (95% CI, 1.3 to 4.2) for a comparison between the highest and lowest quintiles of NDA	OR = 2.8 (95% CI, 1.7 to 4.8) for a comparison between the highest and lowest quintiles of ADA
Baglietto, 2014	24469	Pre- and postmenopausal women from the MCCS study	RR = 0.48 (95% CI, 0.33 to 0.71) for a comparison between highest and lowest quintiles of NDA	RR = 2.73 (95% CI, 1.95 to 3.83) for a comparison between highest and lowest quintiles of ADA
Petterson, 2011	1462	Premenopausal women from the NHS I and NHS II studies	OR = 0.51 (95% CI, 0.36 to 0.72) for a comparison between highest and lowest tertiles of NDA	OR = 2.01 (95% CI, 1.45 to 2.77) for a comparison between highest and lowest tertiles of ADA

2.1.3. The Total Breast Area and Breast Cancer Risk

There are limited studies that aimed to evaluate the associations between breast size and breast cancer risk [Table 6]. Two studies indicated that large breasts in lean women were related to a higher breast cancer risk [Kusano 2006, Egan 1999]. Japanese postmenopausal women showed a relationship between the total breast size and breast cancer risk [Nagata 2005]. However, premenopausal women did not show this relationship [Nagata 2005]. Others indicated a potential confounding effect of the total breast area, where women with large breasts had a lower risk in comparison to women with smaller breasts [Stuedal 2008]. This result could be due to breast size, as women with large breasts have lower percent and absolute densities in comparison to women with small breasts [Stuedal 2008]. Women with large breasts had a higher risk of postmenopausal breast cancer [Hsieh 1999]. However, this result might be due to the confounding effects of adiposity [Hsieh 1999].

Table 6. Case-Control Studies Evaluating the Association between the Total Breast Area and Breast Cancer Risk.

First Author, Year	Total #	Cohort	Predicted Breast Cancer Risk
Stuedal, 2008	1710	Multi-ethnic pre- and postmenopausal women from the CARE study	Lower breast cancer risk for women with large breasts: Adjusted OR per 10 cm^2 in the absolute dense area were 1.32, 1.14, and 1.02 for the first, second, and third tertiles of the total breast area. Adjusted OR per 10% of the increase in mammographic percent density were 1.22, 1.22, and 1.03 for the first, second, and third tertiles of the total breast area.
Kusano, 2006	89,268	Premenopausal women aged 29- 47 from the NHS II study	Lean women (BMI < 25 kg/m^2) with a bra cup size of "D or larger" had a significantly higher incidence of breast cancer than women with a bra cup size of "A or smaller" (HR=1.80).
Egan, 1999	2556	Postmenopausal women aged 50- 79 from New England and Wisconsin	Lean women (waist circumference < 34 in) with large breasts (cup C and larger) had a higher risk (HR= 1.76) for postmenopausal breast cancer than lean women with smaller breasts (cup size smaller than B, HR= 1.34).
Hsieh, 1991	9333	Pre- and postmenopausal women	Large breasts are associated with increased postmenopausal breast cancer risk ($p = 0.026$) but high adiposity could account for this increased risk.
Nagata, 2005	805	Pre- and postmenopausal Japanese women	Total breast area is significantly associated with breast cancer risk for postmenopausal, but not for premenopausal women, and it is independent of mammographic breast density or absolute dense area. Adjusted OR of postmenopausal women were 1.89, 4.15, and 4.65 for the second, third, and fourth quartiles of the total breast area compared to controls.

Overall, findings from those studies [Table 6] indicated that the variation in the total breast area, and in the distribution of mammographic breast density might impact breast cancer risk differentially. According to the literature, we need to report the total breast area with other breast density measurements for breast cancer risk assessment. Moreover, the total breast area can be affected by adiposity. Hence, it is suggested to consider the confounding effects of adiposity when interpreting the results and for means of comparisons across different studies.

In summary, these and other studies showed the associations between different breast density measurements with breast cancer risk. Therefore, we generated similar breast density measurements using fat-water MRI for our study cohort.

2.1.4. Objectives

In this cross-sectional analysis, we described the breast density measurements acquired by fat water MRI in the study cohort. Our first objective was to evaluate the potential heterogeneity in the breast parameters. The parameters analyzed were the absolute dense volume, the percent density, the non-dense volume, and the total breast volume. We analyzed the breast parameters of the total and the center ± 4 MRI slices. Our second objective was to evaluate the potential correlations between the breast parameters. Furthermore, our third objective was to evaluate the potential differences in the breast density measurements by ethnicity.

2.2. Methodology

2.2.1. Acquisition of Fat Water MRI Images

A total of 107 participants underwent fat-water MRI at the baseline visit as of October 31st, 2017 and were included in the analysis. MRI was performed on a Siemens 3T MR system using a 16-channel breast MRI coil system. Fat-water maps were obtained using a multi-point Gradient Echo DIXON imaging method developed by Siemens [Li 2007, Reeder 2007]. The axial slices had a thickness of 4 mm and both breasts were imaged completely, yielding a total of 48 slices. The total scanning time was approximately 10- 15 min. In order to create the pixel maps, two trained staff drew regions of interest (ROIs) that encompassed the whole breast area per slice. Pixel maps were used to avoid sampling and averaging errors. To avoid spurious signal, enhanced areas (such as muscle or healthy cartilage) and non-enhanced areas (such as the healthy bone) were excluded from the pixel maps. Pixels that contained less than 80% of fat were considered the dense tissue; the sum of these pixels was used as the dense volume Pixels that contained 80% or more fat was considered the non-dense tissue; the sum of these pixels was used as the non-dense volume. Sum of all pixels constituted total breast volume. The percent density was calculated from the dense volume divided by the total volume.

The parameters generated from the center ±4 slices include the total breast volume of center ±4 slices (TBVCS), the percent density from center ±4 slices (PDCS), the non-dense volume from center ±4 slices (NDVCS), and the absolute dense volume from center ±4 slices (ADVCS). Data from all slices were used for the total breast volume (TBV), the percent density (PD), the non-dense volume (NDV), and the absolute dense volume (ADV).

2.2.2. Statistical Analysis

For the identification of potential subgroups for each breast density measurement, we treated the breast parameters as continuous variables without any transformation. Finite mixed models were used to identify subpopulations for each breast density measurement in the study cohort. Correlation analysis was used to evaluate the associations among the breast parameters. Student t-test was used to compare the breast parameters between Hispanics and Non- Hispanics. The analysis was performed using the R package version 3.4.1 [R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org]. *P*-values equal or less than 0.05 were considered statistically significant.

2.3. Results

2.3.1. Distributions of Breast Density Measurements in the Study Cohort

The presence of subpopulations or subgroups in each breast parameter can lead to potential variability in the study cohort, leading to skewed distributions of the data. Variables non-normally distributed can be normalized by distinct models. An example is the exponential model, which uses the square root of the dependent variable y to obtain y' = $\sqrt{y}$. Another example is the logarithmic model, which uses the logarithmic transformation of the independent variable to obtain x' = log(x) [Box 1964]. Nonetheless, data transformations may impair the detection of subgroups within the study cohort. For this reason, we used the approach of finite mixture modeling (FMM) in order to identify distinct, but unobserved, subpopulations within a non-normal distribution. Advantages of the usage of FMM for this purpose are to detect heterogeneity for multimodal, skewed, or asymmetrical data [Benaglia 2009].

The overall study cohort for each breast density parameter followed a non-normal distribution [Figure 2]. Right skewness was observed for the percent density [Fig. 2A], where four normally distributed subpopulations were indicated within the cohort. The distribution of the overall cohort for the absolute dense volume was positively skewed [Fig. 2B], with three underlying normally distributed subpopulations. For the non-dense volume, a left skewness was indicated for the overall population [Fig. 2C], and four normally distributed subgroups. In addition, left-skewness was observed for the distribution of the total breast volume [Fig. 2D] in the overall cohort, where three underlying normally distributed subpopulations were shown.

Overall, we showed that the breast density measurements of the study cohort were not normally distributed but rather were a mix of normally distributed subpopulations. Overlapping across the subgroups was to be expected, as each subpopulation was considered as a Gaussian normal distribution. In addition, the subgroups found for each breast parameter should not be used as a criterion for breast density classification but were rather used to show the heterogeneity of our study cohort for each breast parameter [Figure 2].

Figure 2. Distribution of Breast Density Measurements in the Study Cohort (N=107).

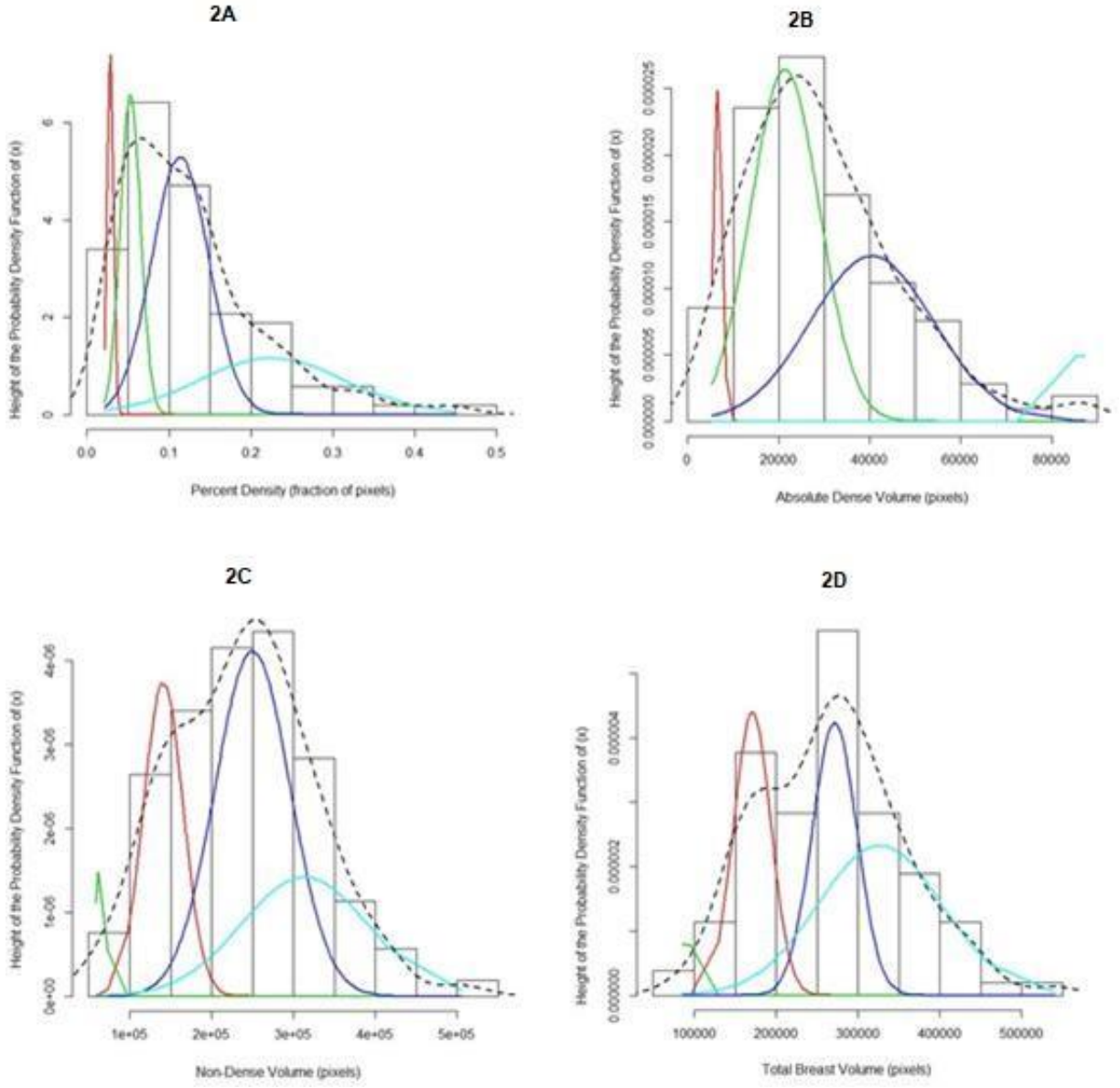


Figure 2A. Distribution of the Percent Density in the Study Cohort. The distribution of the overall cohort is right skewed (dotted black line). Four subpopulations, each one normally distributed, are shown within the study cohort.
Figure 2B. Distribution of the Absolute Dense Volume in the Study Cohort. The distribution of the overall cohort is right skewed (dotted black line). Three subpopulations, each one normally distributed, are indicated within the study cohort.
Figure 2C. Distribution of the Non-Dense Volume in the Study Cohort. The distribution of the overall cohort is left skewed (dotted black line). Four normal subgroups are indicated within the study cohort.
Figure 2D. Distribution of the Total Breast Volume in the Study Cohort. The distribution of the overall cohort is left skewed (dotted black line). Three normal subpopulations are shown within the study cohort.

2.3.2. Correlation Analysis of Breast Density Measurements

We further evaluated the potential correlations between the parameters derived from all breast slices with those derived from the center ± 4 slices. Because breast density measurements were non-normally distributed, they were base 10 log-transformed prior to the correlation analysis.

Table 7 shows that the breast density measurements from the center ± 4 slices were highly and statistically significantly correlated with the breast parameters from all slices (all $p < 0.00001$). Specifically, the percent density of the total slices (PD) showed a significant correlation ($r = 0.96$, $p < 0.00001$) with the percent density of the center ± 4 slices (PDCS). The absolute dense volume of the total slices (ADV) was highly correlated with the absolute dense volume of the center ± 4 slices ($r = 0.95$, $p < 0.00001$). The non-dense volume of the total slices (NDV) was significantly correlated ($r = 0.87$, $p < 0.00001$) with the non-dense volume of the center ± 4 slices (NDVCS). The total breast volume (TBV) was highly correlated ($r = 0.86$, $p < 0.00001$) with the total breast volume of the center ± 4 slices (TBVCS). Because of the high correlation between the breast parameters from all slices with those from the center ± 4 slices, further analyses were performed using only breast density measurements from all slices.

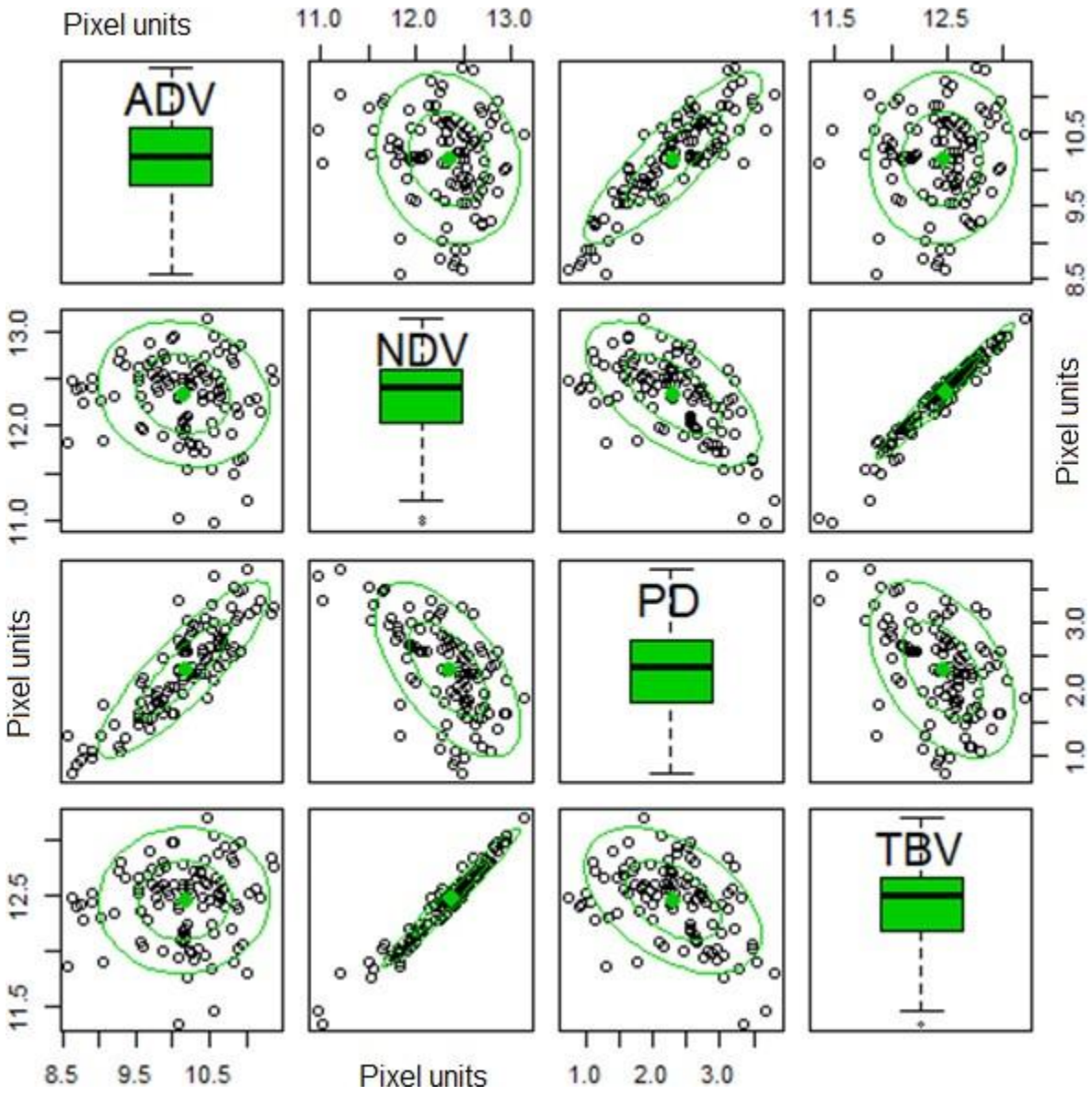
We further examined the correlations between the breast density measurements from all slices. As seen in Figure 3, the percent density (PD) was positively correlated with the absolute dense volume (ADV) but inversely correlated with the non-dense volume (NDV) and total breast volume (TBV). The non-dense volume (NDV) was positively correlated with the total breast volume (TBV). No correlations were observed between the non-dense volume (NDV) and the absolute dense volume (ADV). No correlations were observed for the absolute dense volume (ADV) with the total breast volume (TBV) [Figure 3].

Table 7. Correlation Matrix of Breast Density Measurements

Variable		Breast Density Measurements from Center ± 4 Slices				Breast Density Measurements from Total Slices			
		PDCS	**ADVCS**	**NDVCS**	**TBVCS**	**PD**	**ADV**	**NDV**	**TBV**
Breast Density Measurements from Center ± 4 Slices	**PDCS**	1.00 (na)	0.85 (p<0.00001)	-0.72 (p<0.00001)	-0.49 (p<0.00001)	0.96 (p<0.00001)	0.73 (p<0.00001)	-0.60 (p<0.00001)	-0.46 (p<0.00001)
	ADVCS	0.85 (p<0.00001)	1.00 (na)	-0.34 (p=0.0003)	-0.03 (=0.7232)	0.82 (p<0.00001)	0.95 (p<0.00001)	-0.26 (p=0.008)	-0.07 (p=0.4845)
	NDVCS	-0.72 (p<0.00001)	-0.34 (p=0.0003)	1.00 (na)	0.95 (p<0.00001)	-0.66 (p<0.00001)	-0.21 (p=0.0318)	0.87 (p<0.00001)	0.83 (p<0.00001)
	TBVCS	-0.49 (p<0.00001)	-0.03 (p=0.7232)	0.95 (p<0.00001)	1.00 (na)	-0.44 (p<0.00001)	0.09 (p=0.3506)	0.84 (p<0.00001)	0.86 (p<0.00001)
Breast Density Measurements from Total Slices	**PD**	0.96 (p<0.00001)	0.82 (p<0.00001)	-0.66 (p<0.00001)	-0.44 (p<0.00001)	1.00 (na)	0.87 (p<0.00001)	-0.60 (p<0.00001)	-0.45 (p<0.00001)
	ADV	0.73 (p<0.00001)	0.95 (p<0.00001)	-0.21 (p=0.0318)	0.09 (p=0.3506)	0.87 (p<0.00001)	1.00 (na)	-0.13 (p=0.1885)	0.06 (p=0.5499)
	NDV	-0.60 (p<0.00001)	-0.26 (p=0.0080)	0.87 (p<0.00001)	0.84 (p<0.00001)	-0.60 (p<0.00001)	-0.13 (p=0.1885)	1.00 (na)	0.98 (p<0.00001)
	TBV	-0.46 (p<0.00001)	-0.07 (p=0.4845)	0.83 (p<0.00001)	0.86 (p<0.00001)	-0.45 (p<0.00001)	0.06 (p=0.5499)	0.98 (p<0.00001)	1.00 (na)

Abbreviations: TBVCS, Total Breast Volume of Center ±4 Slices, PDCS, Percent Density of Center ±4 Slices, NDVCS, Non-Dense Volume of Center ±4 Slices, ADVCS, Absolute Dense Volume of Center ±4 Slices, TBV, Total Breast Volume, PD, Percent Density, NDV, Non-Dense Volume, ADV, Absolute Dense Volume. Breast density measurements were base 10 log transformed prior to the correlation analysis. Pearson correlation coefficient estimated by correlation test (R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org).

Figure 3. Scatterplot Matrix of the Breast Density Measurements of the Study Cohort (N= 107).



Abbreviations: ADV, Absolute Dense Volume; NDV, Non-Dense Volume; PD, Percent Density; TBV, Total Breast Volume. Each variable is plotted against each other. Distributions in the shape of a circle indicate no correlations between two variables. Distributions in the shape of an ellipse indicate a non-linear correlation between two variables. Distributions in the shape of a straight line indicate a linear correlation between two variables. According to the distribution of data, the ADV is positively correlated (r= 0.87, p<0.00001) with the PD (green ellipses), but not correlated with the NDV (r= -0.13, p= 0.1885) or with the TBV (r= -0.06, p= 0.5499) (green circles). The NDV is inversely correlated (r= -0.60, p<0.00001) with the PD (green ellipses) and positively correlated (r= 0.98, p<0.00001) with the TBV (linear correlation). The TBV is inversely correlated (r= -0.45, p<0.00001) with the PD (green ellipses). All breast density measurements were base 10 log transformed prior to the correlation analysis.

2.3.3. Means of Breast Density Measurements in the Study Population

2.3.3.1. Means of Breast Density Measurements in the Overall Study Cohort

In order to further characterize the breast density measurements in our cohort, we described the means of each parameter [Table 8]. The average percent density was 12.38% (non-transformed). The average absolute dense volume was 29,707.58 pixels (non-transformed). The average non-dense volume was 237,573.5 pixels (non-transformed). The average total breast volume was 267,281.1 pixels (non-transformed).

Table 8. Means (SD) of breast density measurements (non-transformed and log base 10 transformed) in the study cohort (N = 107).

Breast Density Measurement	Mean (SD)
Percent Density (%)	
Non-transformed	12.38 (8.44)
Log- transformed	2.29 (0.69)
Absolute Dense Volume (pixels)	
Non-transformed	29,707.58 (16840.11)
Log- transformed	10.12 (0.62)
Non-Dense Volume (pixels)	
Non-transformed	237,573.5 (85905.87)
Log- transformed	12.30 (0.41)
Total Breast Volume (pixels)	
Non-transformed	267,281.1 (86079.2)
Log- transformed	12.44 (0.34)

2.3.3.2. Means of Breast Density Measurements by Ethnicity

We summarized in Table 9 the breast density measurements by ethnicity [Table 9]. We observed similar means for that of the percent density (12.75% non-transformed for Hispanics and 12.30% non-transformed for Non-Hispanics) in both ethnic groups. In addition, we observed no significant differences for the means of the absolute dense volume between Hispanics (28,403.8 pixels non-transformed) and Non-Hispanics (30,913.94 pixels non-transformed). There was a trend for the Hispanics to have lower means for the non-dense volume (221,820.1 pixels non- transformed) and for the total breast volume (250,223.9 pixels non- transformed), than that of Non-Hispanics (247,631.0 pixels non- transformed for the non-dense volume; and 278,544.9 pixels non-transformed for the total breast volume).

Table 9. Means (SD) of Breast Density Measurements (non-transformed and log base 10 transformed) by Ethnicity.

Variable	Hispanics (n=41)	Non-Hispanics (n=64)	p-value
Percent Density (%), mean (SD)			
Non-transformed	12.75 (9.15)	12.30 (7.99)	0.7904
Log-transformed	2.31 (0.70)	2.30 (0.67)	0.9155
Absolute Dense Volume (pixels), mean (SD)			
Non-transformed	28,403.8 (15182.72)	30,913.94 (17741.22)	0.4567
Log-transformed	10.08 (0.62)	10.17 (0.59)	0.4843
Non-Dense Volume (pixels), mean (SD)			
Non-transformed	221,820.1 (79917.49)	247,631 (89332.2)	0.1357
Log-transformed	12.23 (0.40)	12.34 (0.41)	0.1970
Total Breast Volume (pixels), mean (SD)			
Non-transformed	250,223.9 (78385.26)	278,544.9 (90125.86)	0.1018
Log-transformed	12.37 (0.32)	12.48 (0.35)	0.1499

A 2-sample t-test was conducted to compare the breast density measurements between Hispanics and Non-Hispanics. R package version 3.4.1 [R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org].

2.4. Discussion

In this work, we aimed to describe the breast density measurements acquired by fat-water MRI in a cohort of premenopausal women with metabolic risk factors. Particularly, we sought to evaluate the potential heterogeneity in the breast parameters, the correlations among them, and the potential influence of ethnicity on the breast density measurements in our study cohort.

To our knowledge, this is the first study that evaluated the breast density measurements by fat-water MRI in a cohort of women with high adiposity and metabolic disorders. A major strength of MRI over mammography for breast density assessment lies in the number of non-compressed images/segments that can be taken and used in the analysis, yielding a three-dimensional image of the breast. Overweight/obese women have a greater compressed breast thickness, and this may lead to a decreased image contrast in conventional mammography. For this reason, the usage of fat-water MRI is especially relevant in our cohort for an accurate assessment of breast density [Conroy 2011].

Previous studies have evaluated the associations between breast density and breast cancer risk. Most studies showed that higher mammographic percent density and larger absolute dense area are related to increased breast cancer risk [Maskarinec 2000, Torres-Mejia 2005, Rauh 2012, Vachon 2007, Vachon 2007, Lokate 2011, Ursin 2005, Nagata 2005]. We generated the breast density measurements analogous to those reported in previous studies with mammography. Our data showed that the breast density parameters were non-normally distributed but rather were a mix of normally distributed subpopulations. We evaluated the correlations between the breast density measurements and showed that the percent density is positively correlated with the absolute dense volume and inversely correlated with the non-dense volume and total breast volume. We also showed that the absolute dense volume was not related to the non-dense volume and the total breast volume. This result suggests that the absolute dense volume was less affected by local breast adiposity and breast size. Similar to our results, a cross-sectional analysis from the IBIS-I trial reported a positive relationship between the absolute density and the percent density as assessed by mammography [Stone 2009]. Other studies indicate that overall adiposity can affect mammographic breast parameters. For this reason, overweight/obese women may present low mammographic percent density [Soguel 2017].

To the best of our knowledge, this is the first study that compared breast density acquired by fat-water MRI in Hispanics versus Non- Hispanics. As shown in the previous chapter, the anthropometric measures, elements of metabolic syndrome, and reproductive risk factors were similar between the ethnic groups. We found no statistically significant differences in the breast density measurements between the ethnic groups. Similarly, mammographic percent density was no different across races ($p < 0.0001$) in a retrospective study of Asians, African-Americans, and Caucasians [del Carmen 2006]. A cross-sectional study also found no differences in mammographic percent densities between Asians, African-Americans, and Caucasians [Chen 2004]. Although, Maskarinec et al. showed that Japanese and Chinese women have higher mammographic percent densities (20% higher) than Caucasians/Hawaiians [Maskarinec 2001]. However, those differences might be related to the breast size. Caucasians/Hawaiians have 50% larger breasts than Japanese/Chinese women [Maskarinec 2001]. Other study reported higher percent density in Chinese women (2.4 times) when compared to Malays and Indians [Chelliah 2013]. In addition, a higher proportion of Malays (50%) and Indians (65%) had fatty breasts in comparison to that of Chinese women (36%) [Chelliah 2013]. Therefore, local breast adiposity or breast size might influence the comparison of breast density between different races/ethnic groups.

In summary, we were able to identify subpopulations for each breast density measurement in a cohort of premenopausal women with elements of metabolic syndrome. The percent density was positively correlated with the absolute dense volume and inversely related to the non-dense volume and total breast volume. The absolute dense volume was not correlated with the non-dense volume and total breast volume. It is possible that the absolute dense volume is less affected by local adiposity and breast size. We did not find differences in the breast density parameters between Hispanics and Non-Hispanics in our study cohort. Further investigations are needed to confirm our findings.

CHAPTER III: Breast Density Measurements, Anthropometric Measurements of Adiposity, and Elements of Metabolic Syndrome

3.1. Introduction

Distinct studies suggested a link between obesity, metabolic disturbances, and breast cancer development [Soguel 2017]. Metabolic risk factors and obesity may increase breast cancer risk [Xue 2007, Vona-Davis 2007]. Those risk factors can alter the hormonal pathways, growth factors, pro-inflammatory cytokines, and in the insulin/IGF axis [Xue 2007, Vona-Davis 2007]. Even though obesity and metabolic disturbances are breast cancer risk factors, their relationship with breast density is not clear. The following review covers key findings on the associations between anthropometric measures and metabolic disturbances with mammographic breast parameters.

3.1.1. Anthropometric Measurements of Adiposity and Breast Density

Several cross-sectional studies evaluated the relationships between anthropometric measurements of adiposity and breast density [Table 10].

An inverse association of the mammographic percent density with high adiposity has been described consistently in the literature. Obese women present lower mammographic percent densities than their lean counterparts [Conroy 2011, Tehranifar 2011, Tseng 2010]. A potential explanation for this inverse relationship is that high adiposity and the proportion of breast parenchyma could be independent breast cancer risk factors [Soguel 2017]. Therefore, mammographic percent density should be BMI-adjusted when using percent density to assess breast cancer risk in overweight/obese women [Lokate 2011, Conroy 2012].

In contrast to the mammographic percent density, the association of the absolute dense area with high adiposity is not clear. Several studies showed that the absolute dense area is inversely related to adiposity [Kim 2015, Haars 2005]. Others showed that the absolute dense area is positively related to high BMI [Heng 2004, Shandu 2015]. Additional studies also suggested no association between the absolute dense area and BMI [Tseng 2010]. It must be noted, though, that breast density assessment by mammography might be inaccurate in women with high adiposity [Chen 2015]. Overweight and obese women have a greater compressed breast thickness, and this may lead to a decreased image contrast [Chen 2015]. Further studies are needed to elucidate the associations of breast parameters with high adiposity. Imaging modalities such as MRI on non-compressed breasts may better inform the relationship between adiposity and breast density.

Table 10. Cross-Sectional Studies Evaluating the Associations between the Anthropometric Measurements of Adiposity and Breast Density Measurements.

First author, Year	Sample Size	Cohort	Findings
Conroy, 2011	790	Multi-ethnic pre- and perimenopausal women	Abdominal adiposity is inversely related to the mammographic percent density (β = -4.8).
Tehranifar, 2011	191	Multi-ethnic postmenopausal women	BMI is inversely associated (β = -0.65) with the mammographic percent density.
Tseng, 2010	415	Premenopausal Chinese women	Mammographic percent density is inversely related to BMI (β = -1.8) in women with a BMI ≥ 23 kg/m^2. The non-dense area is positively related to BMI (β = 4.79) in women with a BMI ≥ 23 kg/m^2. No associations were observed between BMI and the absolute dense areas in women with a BMI ≥ 23 kg/m^2.
Kim, 2015	73974	Premenopausal Korean women	A large waist circumference (≥ 80 cm) is inversely associated with dense breasts (OR = 0.74).
Haars, 2005	418	Postmenopausal women from the Netherlands	The absolute dense areas are inversely associated with BMI (β = -2.12).
Heng, 2004	24609	Singaporean Chinese women ages 45 to 69	Weight (β = 0.16) and BMI (β = 0.28) were positively associated with the dense area after adjustment for age and reproductive factors.
Sandhu, 2015	266	Postmenopausal women between ages 35 – 75 years with a breast density ≥ 25% by BI-RADS	A positive correlation between BMI and absolute breast density (rho = 0.42).

3.1.2. Elements of Metabolic Syndrome and Breast Density

The metabolic syndrome, also called syndrome X or Reaven syndrome, refers to the presence of risk factors for cardiovascular disease and diabetes mellitus [Wani 2017]. There are several definitions of metabolic syndrome. Those are the World Health Organization (WHO), the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III), the European Group for the Study of Insulin Resistance (EGIR), and the International Diabetes Foundation (IDF) definitions. For the present work, we use the NCEP ATP III definition of metabolic syndrome. This one is the criteria most widely used and incorporates hyperglycemia (fasting glucose 100 mg/dl), hypertension (≥130 mmHg systolic blood pressure and/or ≥85 mmHg diastolic blood pressure), elevated triglycerides (≥150 mg/dl), reduced HDL-cholesterol (< 50 mg/dl), and visceral adiposity (for women, waist circumference ≥35 in or ≥31 in for Asians) [Huang 2009].

Several studies indicated that metabolic syndrome and its components as related to increased breast cancer risk [Bhandari 2014, Gezgen 2012]. The dysregulations in the molecular pathways, as seen in metabolic syndrome, may contribute to breast cancer development [Bhandari 2014]. Those include, but may not be limited to, increased breast epithelial cell proliferation, inhibition of apoptosis, increase in estradiol bioavailability and the production of pro-inflammatory cytokines [Bhandari 2014]. Even though metabolic syndrome or its components may contribute to increased breast cancer risk, their associations with other well-known breast cancer risk factors are unclear. An example of this is the relationship between metabolic syndrome and breast density.

Table 11 summarizes key cross-sectional studies on the associations between metabolic syndrome and breast density [Table 11]. Women with metabolic syndrome have higher absolute density than those without metabolic disturbances [Kim 2015, Rice 2013]. However, studies report inconsistent results across regions. Mexican women from Jalisco reported associations between breast density and metabolic disturbances, while Veracruz women do not present them [Rice 2013]. Although Tehranifar et al. found an inverse association between metabolic disturbances and the absolute dense areas [Tehranifar 2015].

Additional cross-sectional studies have examined the relationship between metabolic syndrome and its components and breast density. Conroy et al. found lower mammographic percent densities in a multi-ethnic cohort of premenopausal women with metabolic syndrome [Conroy 2011]. A study conducted in African-American and Hispanic women found lower percent densities in women with higher numbers of metabolic risk factors in comparison to individuals with no metabolic disorders [Tehranifar 2014]. They observed this association after adjusting for age at mammography and BMI [Tehranifar 2014]. A subsequent study by the same group showed that high levels of total cholesterol are related to a low mammographic percent density [Tehranifar 2015].

We can attribute the reported discrepancies in the relationships of metabolic syndrome and breast density to the use of different metabolic syndrome criteria, menopausal status and race/ethnicity of the cohort.

Table 11. Cross-Sectional Studies Evaluating the Associations between Metabolic Disturbances and Breast Density Measurements.

First author, Year	Sample Size	Cohort	Findings
Kim, 2015	73974	Pre- and postmenopausal women	Women with metabolic syndrome had a higher absolute density (OR = 1.22). Women with insulin resistance (HOMA-IR ≥ 2.7) had a higher absolute density (OR = 1.29 for premenopausal women, OR = 1.44 for postmenopausal women).
Rice, 2013	789	Premenopausal Mexican women from the states of Jalisco and Veracruz	Metabolic syndrome is associated with higher mammographic percent density in Jalisco women (OR = 4.76). Low HDL-C is associated with higher mammographic percent density in Jalisco women (OR = 4.62). High absolute density is associated with metabolic disturbances in Jalisco women. No significant relationships were observed for Veracruz women.
Tehranifar, 2014	191	Pre- and postmenopausal women from the New York City Multiethnic Breast Cancer Project	Multiple metabolic conditions are related to lower percent density in comparison to women with no metabolic disorders. 2 metabolic conditions related to a 6.4% of reduction (95% CI: -11.2, -1.6) in percent density and to 6.5 cm2 smaller dense area (95% CI: -13.1, -0.1) in comparison to women with no metabolic disorders. ≥ 3 metabolic conditions related to a 7.4% of reduction (95% CI: -12.9, -1.9) in percent density and to a 9.5 cm2 smaller dense area (95% CI: -17.1, -1.9) in comparison to women with no metabolic disorders.
Tehranifar, 2015	373	African-American and Hispanic women	The inverse association between metabolic risk factors with mammographic percent density and dense areas. High blood cholesterol is associated with lower percent density (β = -5.4) and absolute dense areas (β = -6.74).
Conroy, 2011	790	Multi-ethnic pre- and postmenopausal women	Lower mammographic percent density is related to metabolic syndrome and its components.

3.1.3. Objectives

The overall objective was to determine the association between adiposity and metabolic syndrome with breast density parameters acquired by fat-water MRI in overweight/obese women with metabolic syndrome components. The first objective was to determine the relationship between anthropometric measurements of adiposity and breast parameters. The second objective was to explore the relationship between the elements of metabolic syndrome and the breast parameters. The third objective was to evaluate potential differences in the associations by ethnicity.

3.2. Methodology

3.2.1. Anthropometric Measurements of Adiposity

Trained staff measured and calculated the body mass index, waist circumference, and the waist-hip ratio of the participants at the baseline visit. We asked the participant to stand on a calibrated scale with minimal movement, hands at their side, and shoes and excessive clothing removed. We measured waist and hip circumferences in a standing position by using a flexible metallic tape. We measured waist circumference at the midpoint between the lowest rib and the top of the iliac crest on the bare abdomen. We measured hip circumference at the level of the greatest protrusion of the gluteal muscles with minimal clothing. We performed all body measurements after a 12 h fast.

3.2.2. Biochemical Analysis of Serum Samples

We collected fasting blood samples at the baseline visit and sent to Sonora Quest Laboratories for comprehensive metabolic panel including serum glucose, HDL-cholesterol, and triglycerides.

3.2.3. Statistical Analysis

Several variables presented non-normal distributions and were log base 10 transformed. We calculated the body mass index (BMI) as the weight (kg)/ height^2 (m^2). We used the BMI categories of overweight (25.0 – 29.9 kg/m^2), obese class 1 (30.0 – 34.9 kg/m^2), obese class 2 (35.0 – 39.9 kg/m^2), and obese class 3 (≥ 40.0 kg/m^2) for categorical data analysis. We used analysis of variance to analyze the differences in breast density measures among BMI categories and was followed by Tukey's multiple comparison tests. We applied student t-tests to assess the differences between ethnic groups. We performed the analysis by using the R package version 3.4.1 [R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org]. We used multiple linear regression to determine the relationship of anthropometric measures and elements of metabolic syndrome with breast density measures. We performed the regression analysis using STATA version 15.1. StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC. We considered *P*-values equal or less than 0.05 as statistically significant.

3.3. Results

3.3.1. Correlations between Breast Density Measurements and Anthropometric Measurements of Adiposity

3.3.1.1. Anthropometric Measurements of Adiposity and Breast Density Measurements in the Overall Cohort

We summarized in Table 12 the correlations between anthropometric measurements of adiposity (BMI (kg/m^2), waist circumference (cm), and waist-hip ratio (fraction)) and the breast density measurements [Table 12].

The percent density was inversely associated with BMI ($r = -0.41$, $p < 0.00001$, Table 12, Fig. 4A), waist circumference ($r = -0.48$, $p < 0.00001$, Table 13, Fig. 4B), and waist-hip ratio ($r = -0.33$, $p = 0.0010$, Table 11, Fig. 4C). The absolute dense volume showed a similar correlation with the anthropometric measurements ($r = -0.21$, -0.24 and -0.26 for BMI, waist circumference, and waist-hip ratio, respectively, all $p \leq 0.05$, see Table 12, Fig. 5). In addition, the non-dense volume was positively correlated with all the anthropometric measurements ($r = 0.47$, 0.58, and 0.24 for BMI, waist circumference, and waist-hip ratio, respectively, all $p < 0.05$, see Table 12, Fig. 6). These strong associations were also observed for that of the total breast volume with BMI ($r = 0.45$, $p < 0.00001$, Table 12, Fig. 7A) and waist circumference ($r = 0.54$, $p < 0.00001$, Table 12, Fig. 7B). The association of the total breast volume with the waist-hip ratio was borderline significant ($r = 0.19$, $p = 0.0504$, Table 12, Figure 7C).

Overall, the correlations indicate that the anthropometric measurements of adiposity were associated with the breast density measurements of local adiposity and the overall breast size. The absolute dense volume and the percent density, on the other hand, were inversely correlated with the anthropometric measurements of adiposity [Table 12].

Table 12. Pearson Correlations between the Breast Density Measurements* and the Anthropometric Measurements of Adiposity.

Breast Density Measurement*	BMI*	Waist Circumference*	Waist-Hip Ratio*
Percent Density	-0.41 (p<0.00001)	-0.48 (p<0.00001)	-0.33 (p=0.0006)
Absolute Dense Volume	-0.21 (p=0.0325)	-0.24 (p=0.0135)	-0.26 (p=0.0075)
Non-Dense Volume	0.47 (p<0.00001)	0.58 (p<0.00001)	0.24 (p=0.0143)
Total Breast Volume	0.45 (p<0.00001)	0.54 (p<0.00001)	0.19 (p=0.0504)

*Variables had a non-normal distribution and were log base 10 transformed.

Figure 4. Percent Density and Anthropometric Measurements of Adiposity.

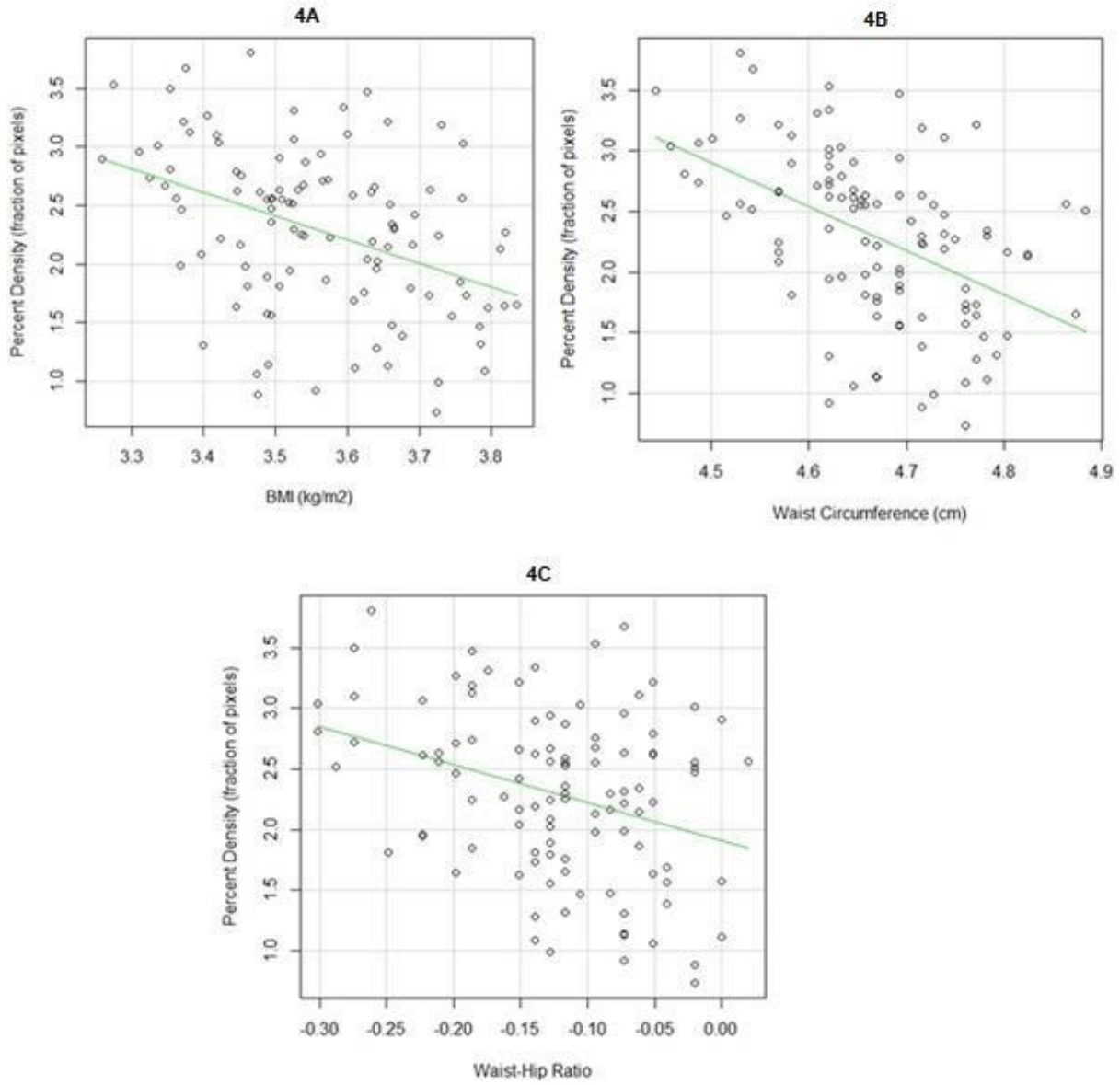


Figure 4A. Percent Density and BMI. There is a negative correlation between percent density and BMI. Pearson r= -0.41, p<0.00001.
Figure 4B. Percent Density and Waist Circumference. There is a negative correlation between percent density and waist circumference. Pearson r= -0.48, p<0.00001.
Figure 4C. Percent Density and Waist-Hip Ratio. There is a negative correlation between percent density and waist-hip ratio. Pearson r= -0.33, p=0.0006.
The percent density and the anthropometric measurements followed a non-normal distribution and were log base 10 transformed.

Figure 5. Absolute Dense Volume and Anthropometric Measurements of Adiposity.

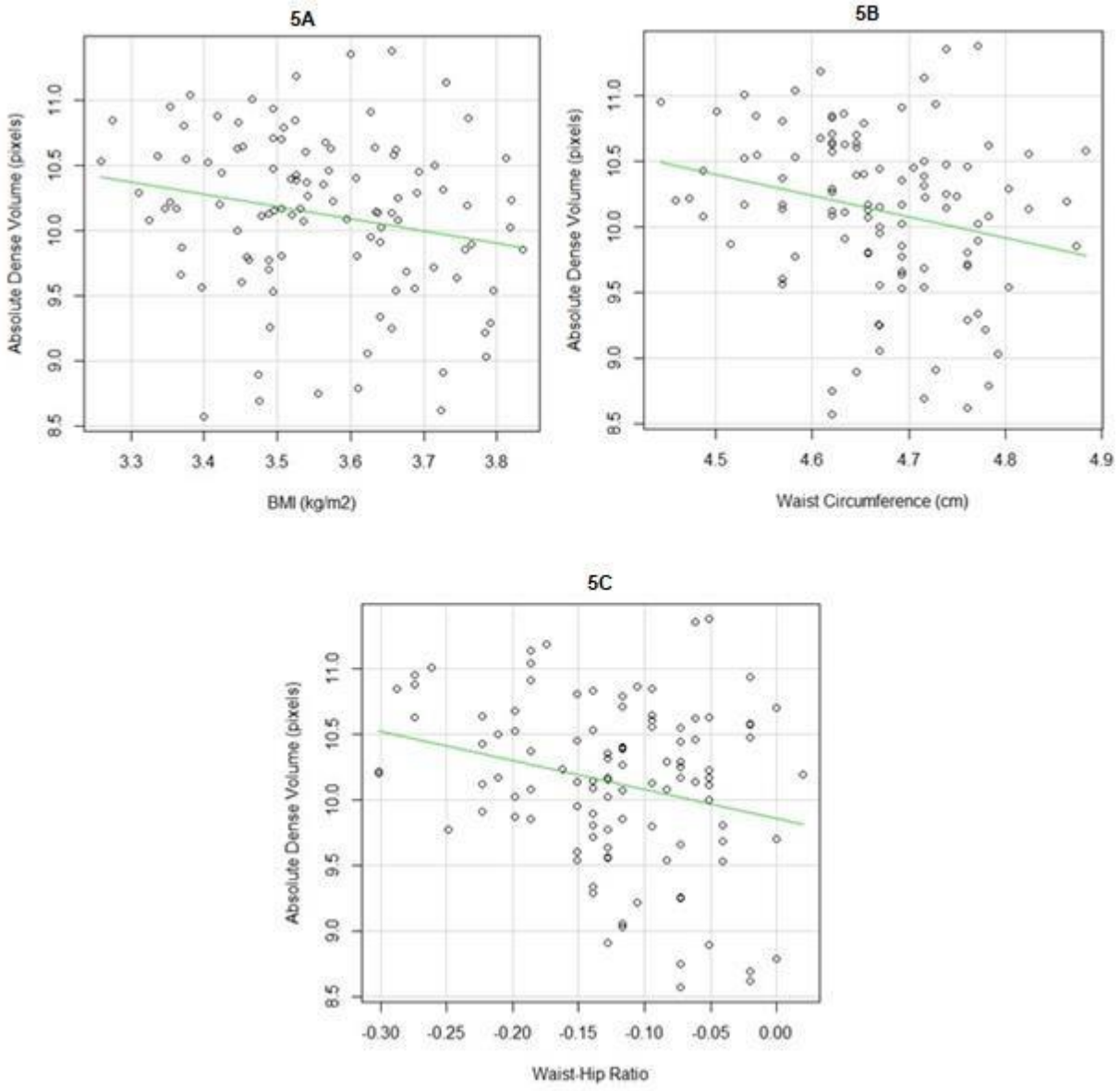


Figure 5A. Absolute Dense Volume and BMI. There is a negative correlation between absolute dense volume and BMI. Pearson r = -0.21, p = 0.0325.
Figure 5B. Absolute Dense Volume and Waist Circumference. There is a negative correlation between absolute dense volume and waist circumference. Pearson r = -0.24, p = 0.0135.
Figure 5C. Absolute Dense Volume and Waist-Hip Ratio. There is a negative correlation between absolute dense volume and waist-hip ratio. Pearson r = -0.26, p = 0.0075.
The absolute dense volume and the anthropometric measurements followed a non-normal distribution and were log base 10 transformed.

Figure 6. Non-Dense Volume and Anthropometric Measurements of Adiposity.

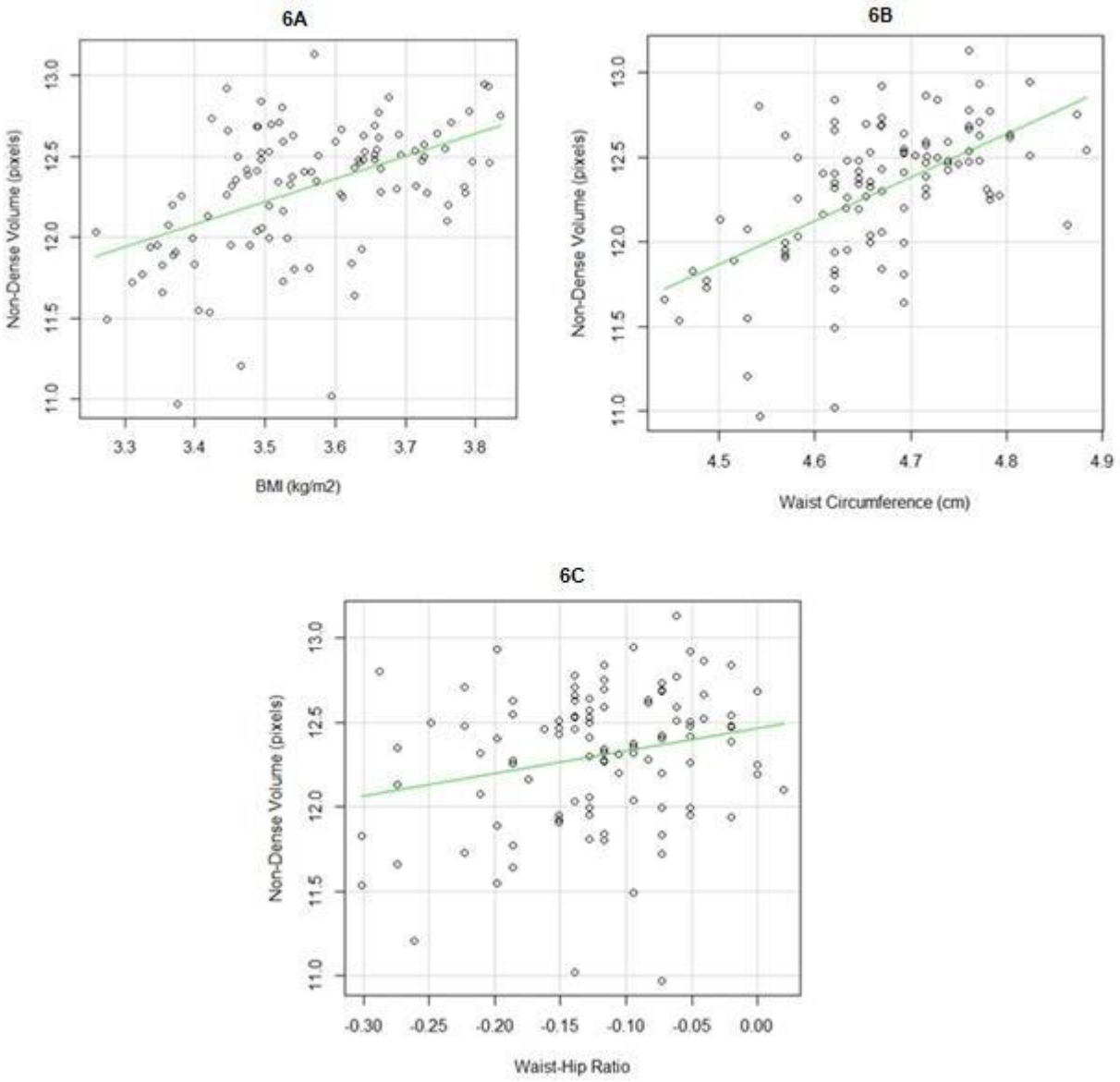


Figure 7A. Non-Dense Volume and BMI. There is a positive correlation between non-dense volume and BMI. Pearson $r = 0.47$, $p < 0.00001$.
Figure 7B. Non-Dense Volume and Waist Circumference. There is a positive correlation between non-dense volume and waist circumference. Pearson $r = 0.58$, $p < 0.00001$.
Figure 7C. Non-Dense Volume and Waist-Hip Ratio. There is a positive correlation between non-dense volume and waist-hip ratio. Pearson $r = 0.24$, $p = 0.0143$.
The non-dense volume and the anthropometric measurements followed a non-normal distribution and were log base 10 transformed.

Figure 7. Total Breast Volume and Anthropometric Measurements of Adiposity.

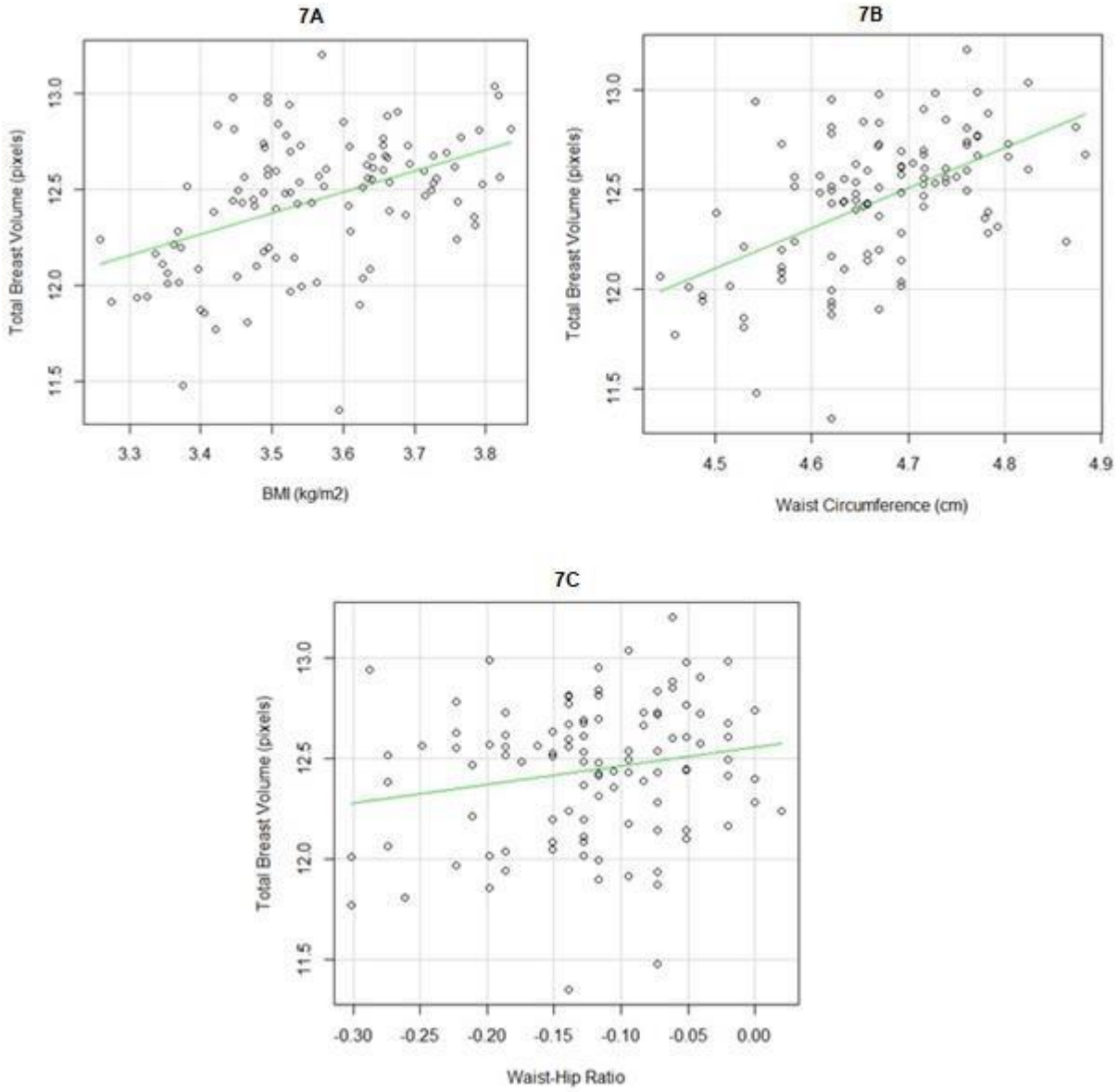


Figure 7A. Total Breast Volume and BMI. There is a positive correlation between total breast volume and BMI. Pearson $r = 0.45$, $p < 0.00001$.
Figure 7B. Total Breast Volume and Waist Circumference. There is a positive correlation between total breast volume and waist circumference. Pearson $r = 0.54$, $p < 0.00001$.
Figure 7C. Total Breast Volume and Waist-Hip Ratio. There is a positive correlation, although not statistically significant, between total breast volume and waist-hip ratio. Pearson $r = 0.19$, $p = 0.0504$.
The total breast volume and the anthropometric measurements followed a non-normal distribution and were log base 10 transformed.

3.3.1.2. Anthropometric Measurements of Adiposity and Breast Density Measurements by Ethnicity

We summarized in Tables 13 and 14 the correlation between breast density measurements and anthropometric measurements by ethnicity [Table 13 and Table 14].

For the Hispanics, the percent density was inversely correlated with BMI ($r = -0.33$, $p = 0.0379$), waist circumference ($r = -0.44$, $p = 0.0040$), and waist-hip ratio ($r = -0.45$, $p = 0.0030$) [Table 13, See Appendix A, Fig. A2]. For the Non-Hispanics, the percent density was also inversely correlated with BMI ($r = -0.50$, $p < 0.00001$) and waist circumference ($r = -0.52$, $p < 0.00001$) but the correlation with the waist-hip ratio was not statistically significant [Table 14, See Appendix A, Fig. A2].

For the Hispanics, the absolute dense volume was inversely correlated with BMI ($r = -0.12$, $p = 0.4688$), waist circumference ($r = -0.19$, $p = 0.2463$), and waist-hip ratio ($r = -0.34$, $p = 0.0308$) [Table 13, See Appendix A, Fig. A3]. For the Non-Hispanics, an inverse correlation was observed for the absolute dense volume with BMI ($r = -0.30$, $p = 0.0164$) and waist circumference ($r = -0.28$, $p = 0.0252$). No statistically significant correlations were observed for the waist-hip ratio [Table 14, See Appendix A, Fig. A3].

The non-dense volume was positively correlated with the BMI for both Hispanics ($r = 0.47$, $p = 0.0017$) and Non-Hispanics ($r = 0.49$, $p < 0.00001$) [Table 13 and 14, See Appendix A, Fig. A4]. Hispanics showed a positive correlation of the non-dense volume with waist circumference ($r = 0.62$, $p < 0.0001$) and waist-hip ratio ($r = 0.41$, $p = 0.0070$) [Table 13, See Appendix A, Fig. A4]. Non-Hispanics also presented a positive correlation of the non-dense volume with waist circumference ($r = 0.56$, $p < 0.00001$) but the correlation was not statistically significant for the waist-hip ratio [Table 14, See Appendix A, Fig. A4].

Both ethnic groups presented positive correlations between total breast volume with BMI ($r = 0.48$ Hispanics, $r = 0.44$ Non-Hispanics, all $p < 0.005$) and waist circumference ($r = 0.59$ Hispanics, $r = 0.52$ Non-Hispanics, all $p < 0.0001$). Hispanics presented a positive correlation of total breast volume with waist-hip ratio ($r = 0.33$, $p = 0.0374$) [Table 13, Fig. 12] but no statistically significant correlations were observed for the waist-hip ratio in Non-Hispanics [Table 14, See Appendix A, Fig. A5].

Table 13. Pearson Correlations between the Breast Density Measures* and the Anthropometric Measurements of Adiposity in Hispanics (n = 41).

Breast Density Measurement*	**BMI***	**Waist Circumference***	**Waist-Hip Ratio***
Percent Density	-0.33 (p=0.0379)	-0.44 (p=0.0040)	-0.45 (p=0.0030)
Absolute Dense Volume	-0.12 (p=0.4688)	-0.19 (p=0.2463)	-0.34 (p=0.0308)
Non-Dense Volume	0.47 (p=0.0017)	0.62 (p<0.0001)	0.41 (p=0.0070)
Total Breast Volume	0.48 (p=0.0015)	0.59 (p<0.0001)	0.33 (p=0.0374)

*Variables followed a non-normal distribution and were log base 10 transformed.

Table 14. Pearson Correlations between the Breast Density Measurements* and the Anthropometric Measurements of Adiposity in Non-Hispanics (n = 64).

Breast Density Measurement*	BMI*	Waist Circumference*	Waist-Hip Ratio*
Percent Density	-0.50 (p<0.00001)	-0.52 (p<0.00001)	-0.20 (p=0.1159)
Absolute Dense Volume	-0.30 (p=0.0164)	-0.28 (p=0.0252)	-0.16 (p=0.1963)
Non-Dense Volume	0.49 (p<0.00001)	0.56 (p<0.00001)	0.11 (p=0.3973)
Total Breast Volume	0.44 (p=0.0003)	0.52 (p<0.00001)	0.10 (p=0.4308)

*Variables followed a non-normal distribution and were base log 10 transformed.

3.3.2. Association between Breast Density Measurements and BMI Categories

We further compared the density measures among BMI categories of overweight (25.0 – 29.9 kg/m^2), obese class 1 (30.0 – 34.9 kg/m^2), obese class 2 (35.0 – 39.9 kg/m^2), and obese class 3 (≥ 40.0 kg/m^2).

3.3.2.1. BMI Categories and Percent Density

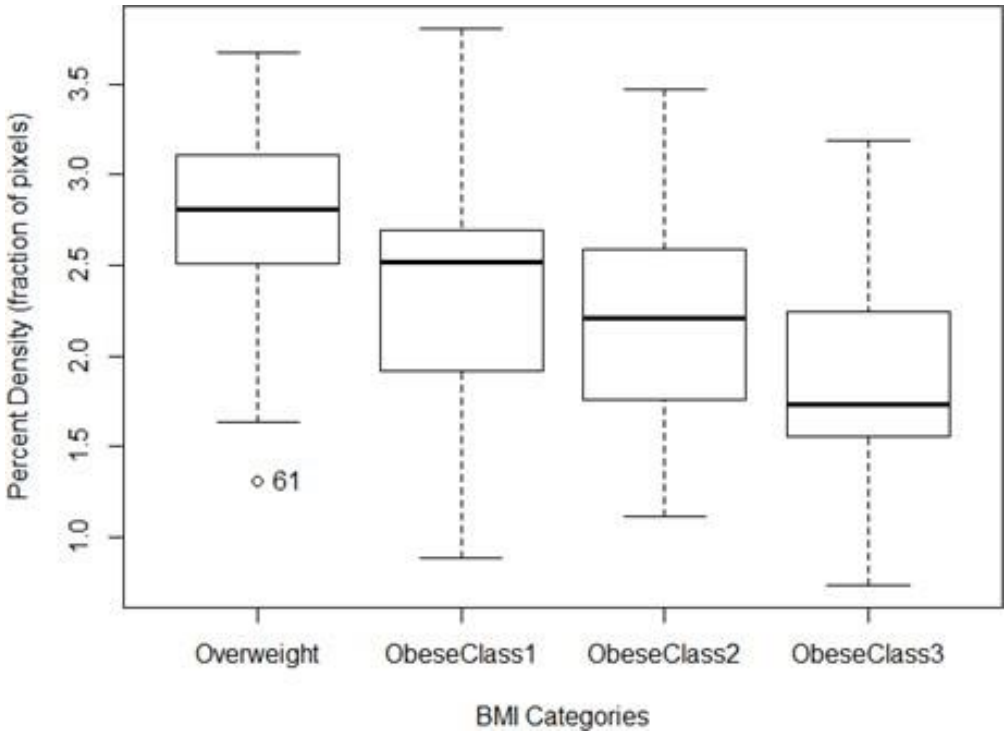
We summarized in Table 15 the percent density by BMI category [Table 15] and Figure 8 illustrates the percent density by BMI category [Figure 8]. We analyzed data by a one-way ANOVA, where the percent density was different among BMI categories (p-value = 2.232e-04) [Table 15]. Tukey's test showed that the overweight class was different from the obese class 1 (p-value = 0.05), the obese class 2 (p-value = 0.01), and the obese class 3 (p-value < 0.01) [Appendix A, Fig. A6, Appendix B, Table B1]. We observed no significant differences between the obese categories.

Table 15. Means (SD) of the Percent Density* (%) by BMI Categories in the Study Cohort (N = 107).

BMI category	Mean (%)	SD	N
Overweight (25.0 – 29.9 kg/m^2)	2.74**	0.59	23
Obese Class 1 (30.0 – 34.9 kg/m^2)	2.31	0.65	36
Obese Class 2 (35.0 – 39.9 kg/m^2)	2.19	0.65	26
Obese Class 3 (≥ 40.0 kg/m^2)	1.87	0.63	21

* Breast density measurement was non-normally distributed and was log base 10 transformed.
** Significantly different from obese BMI categories 1-3. Statistical significance was determined by one-way ANOVA (p-value = 2.232e-04) followed by Tukey's test, where the overweight class was different from the obese class 1 (p-value = 0.05), the obese class 2 (p-value = 0.01), and the obese class 3 (p-value < 0.01).

Figure 8. Boxplot of the Percent Density by BMI Category in the Study Cohort (N = 107).



Outliers are indicated by the participant's ID number.

3.3.2.2. BMI Categories and Absolute Dense Volume

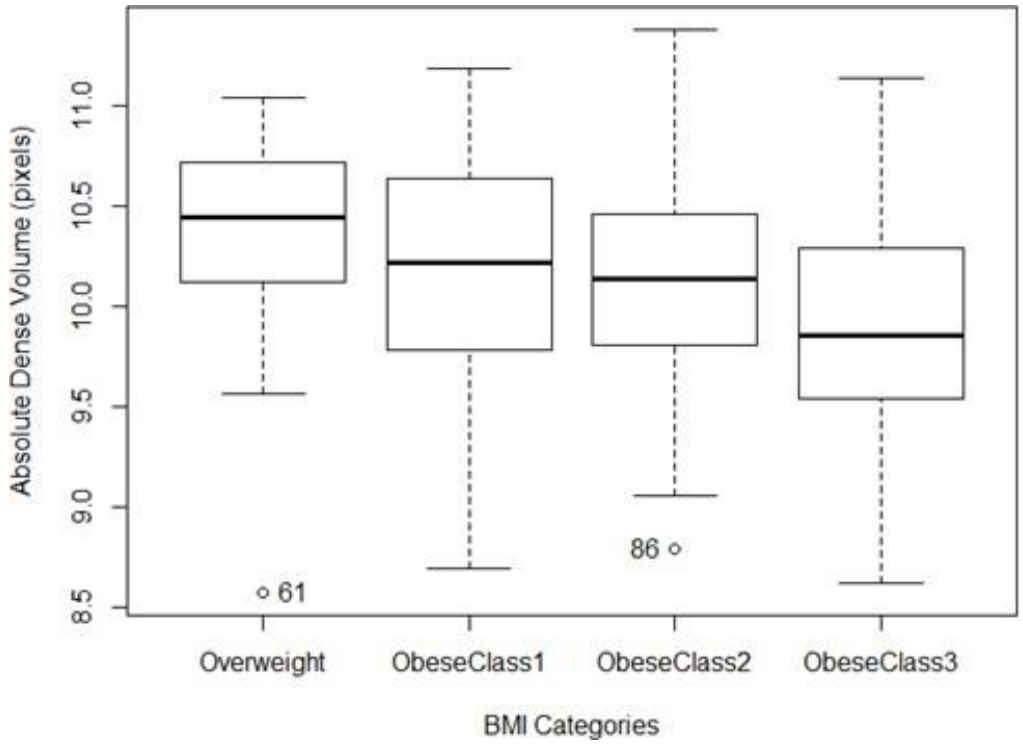
We summarized in Table 16 the absolute dense volume by BMI category [Table 16]. In Figure 9 we illustrate the absolute dense volume by BMI category [Figure 9]. The absolute dense volume was not different among the BMI categories when analyzed by one-way analysis of variance (p-value = 0.1077).

Table 16. Means (SD) of the Absolute Dense Volume* (pixels) by BMI categories in the Study Cohort (N = 107).

BMI category	Mean (pixels)	SD	n
Overweight (25.0 – 29.9 kg/m^2)	10.31	0.55	23
Obese Class 1 (30.0 – 34.9 kg/m^2)	10.16	0.61	36
Obese Class 2 (35.0 -39.9 kg/m^2)	10.12	0.62	26
Obese Class 3 (≥ 40.0 kg/m^2)	9.86	0.64	21

* Breast density measurement was non-normally distributed and was log base 10 transformed.

Figure 9. Boxplot of the Absolute Dense Volume by BMI Category in the Study Cohort (N = 107).



Outliers are indicated by the participant's ID number.

3.3.2.3. BMI Categories and Non-Dense Volume

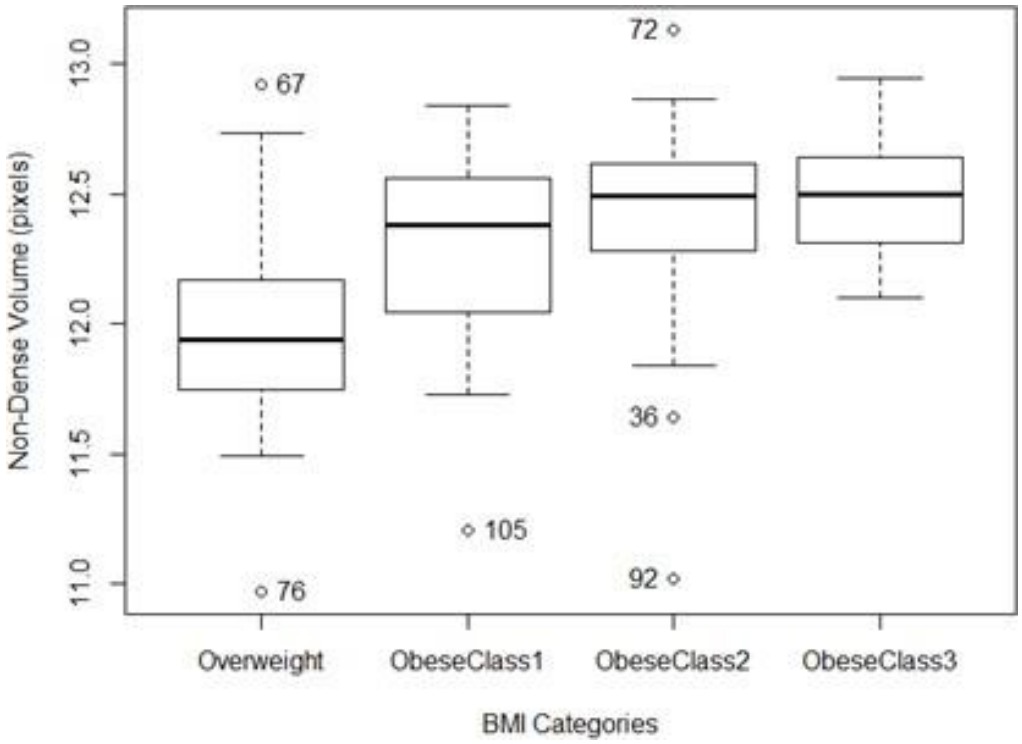
We summarized in Table 17 the non-dense volume by BMI category [Table 17]. We illustrate in Figure 10 the non-dense volume by BMI category [Figure 10]. One-way ANOVA showed that the non-dense volume was different among the groups (p-value = 2.232e-05) [Table 17]. Tukey's test showed that the overweight class was different from the obese class 1 (p-value = 0.009), the obese class 2 (p-value = 0.001), and the obese class 3 (p-value < 0.001) [See Appendix A, Fig. A7, Appendix B, Table B2]. We observed no significant differences between obese BMI categories.

Table 17. Means (SD) of the Non-Dense Volume* (pixels) by BMI categories in the Study Cohort (N = 107).

BMI category	Mean (pixels)	SD	N
Overweight (25.0 – 29.9 kg/m^2)	11.97**	0.43	23
Obese Class 1 (30.0 – 34.9 kg/m^2)	12.31	0.35	36
Obese Class 2 (35.0 -39.9 kg/m^2)	12.40	0.41	26
Obese Class 3 (≥ 40.0 kg/m^2)	12.51	0.23	21

* Breast density measurement was non-normally distributed and was log base 10 transformed.
** Significantly different from obese BMI categories 1-3. Statistical significance was determined by one-way ANOVA (p-value = 2.232e-05), followed by Tukey's test, where the overweight class was different from the obese class 1 (p-value = 0.009), the obese class 2 (p-value = 0.001), and the obese class 3 (p-value < 0.001).

Figure 10. Boxplot of the Non-Dense Volume by BMI Category in the Study Cohort (N = 107).



Outliers are indicated by the participant's ID number.

3.3.2.4. BMI Categories and the Total Breast Volume

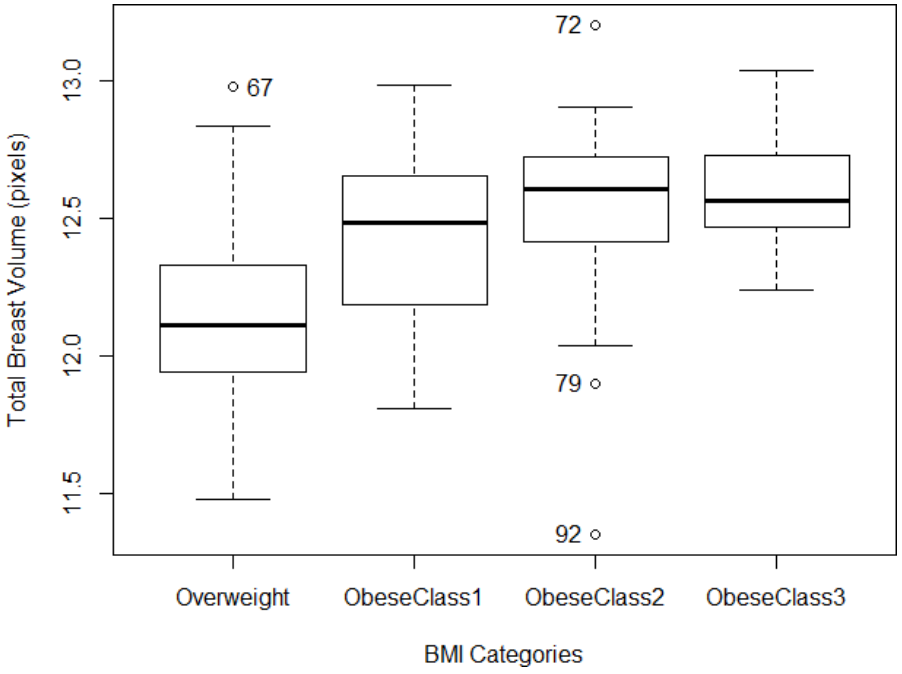
We summarized in Table 18 the total breast volume by BMI category [Table 18]. We illustrate in Figure 11 the total breast volume by BMI category [Figure 11]. One-way ANOVA showed that the total breast volume was different among the groups (p-value = 1.056e-04) [Table 18]. Tukey's test showed that the overweight class was different from the obese class 1 (p-value = 0.007), the obese class 2 (p-value = 0.001), and the obese class 3 (p- value < 0.001) [See Appendix A, Fig. A8, Appendix B, Table B3]. We observed no significant differences between obese BMI categories.

Table 18. Means (SD) of the Total Breast Volume* (pixels) by BMI categories in the Study Cohort (N = 107).

BMI category	Mean (pixels)	SD	n
Overweight (25.0 – 29.9 kg/m^2)	12.17**	0.35	23
Obese Class 1 (30.0 – 34.9 kg/m^2)	12.45	0.29	36
Obese Class 2 (35.0 -39.9 kg/m^2)	12.52	0.36	26
Obese Class 3 (≥ 40.0 kg/m^2)	12.59	0.21	21

* Breast density measurement was non-normally distributed and was log base 10 transformed.
** Significantly different from obese BMI categories 1-3. Statistical significance was determined by one-way ANOVA (p-value = 1.056e-04), followed by Tukey's test, where the overweight class showed to be different than the obese class 1 (p-value = 0.007), the obese class 2 (p-value = 0.001), and the obese class 3 (p-value < 0.001).

Figure 11. Boxplot of the Total Breast Volume by BMI Category in the Study Cohort (N =107).



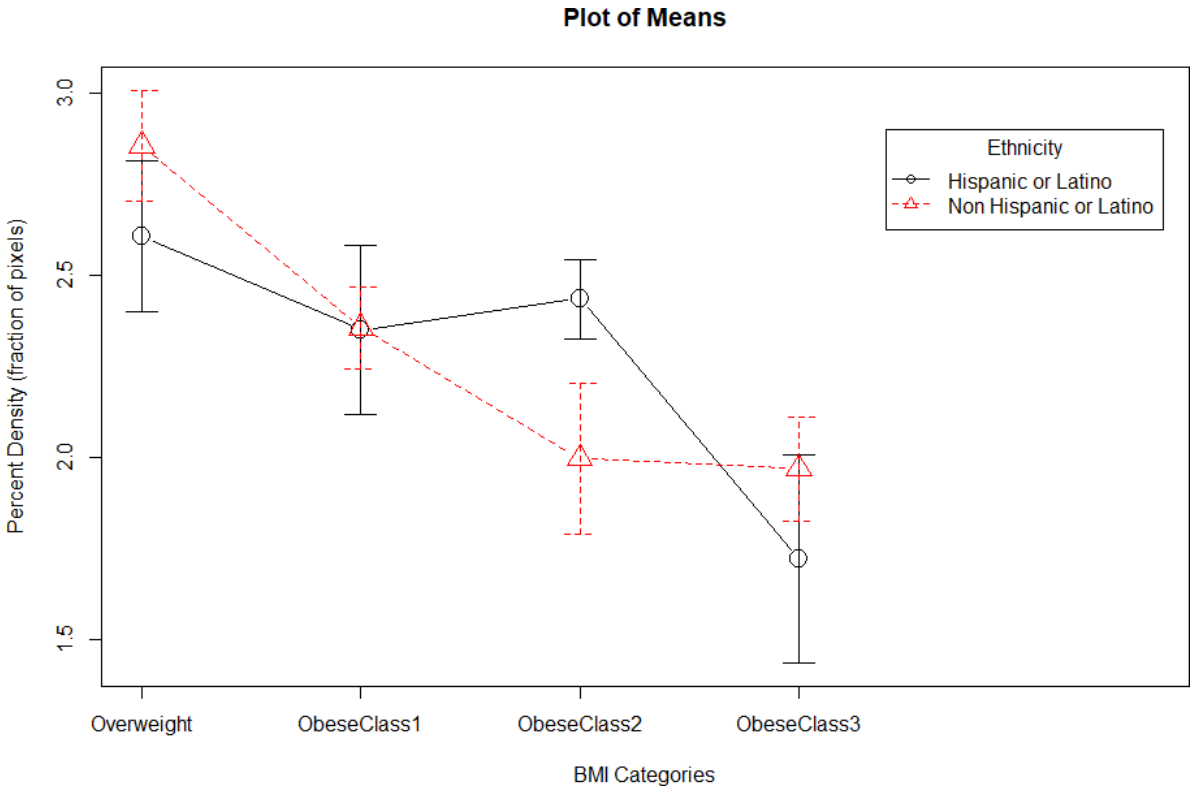
Outliers are indicated by the participant's ID number.

3.3.3. Associations of Breast Density Measurements by BMI Category and Ethnicity

3.3.3.1. The Percent Density, BMI Category, and Ethnicity

We further compared the percent density by ethnicity and BMI category [Figure 12]. We conducted a two-way ANOVA. The percent density was affected by BMI category (p-value = 0.0219) but not by ethnicity (p-value= 0.4557) nor the interaction of BMI category and ethnicity (p-value= 0.4511).

Figure 12. Plot of Means of the Percent Density stratified by BMI category and Ethnicity.

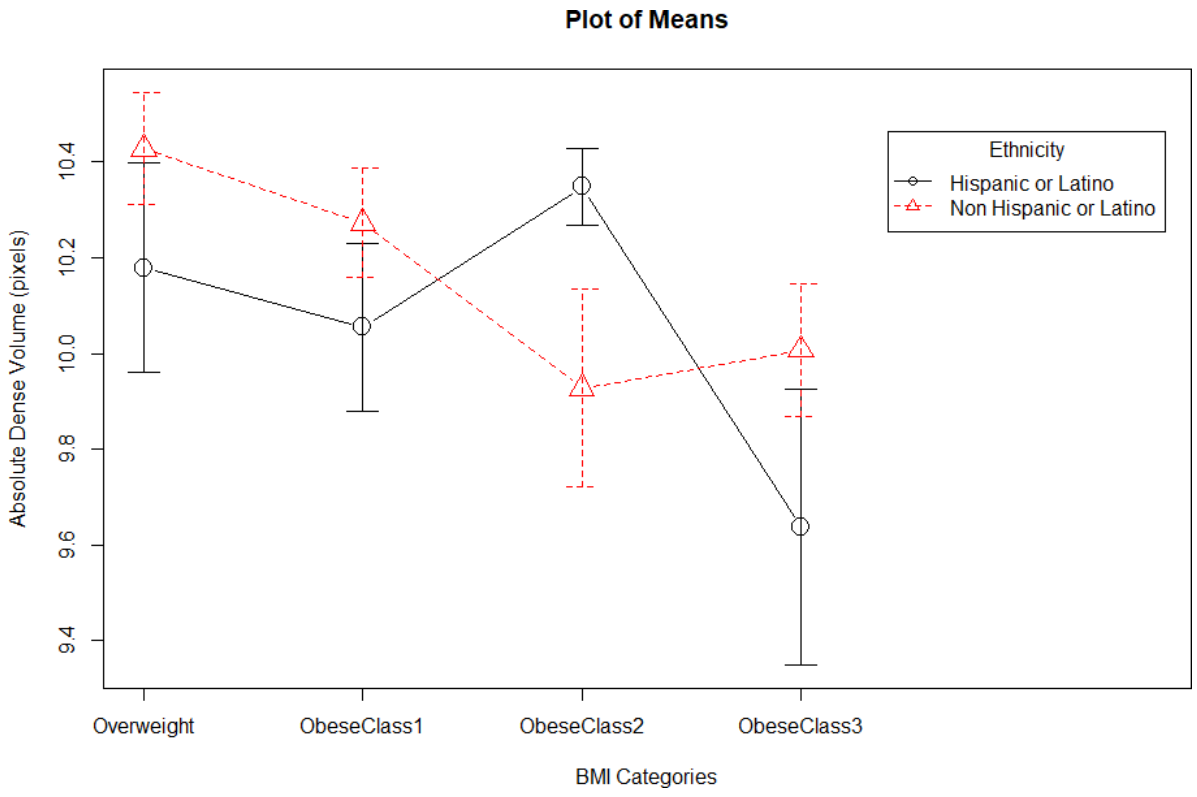


BMI categories: Overweight (25.0 – 29.9 kg/m^2), Obese Class 1 (30.0 – 34.9 kg/m^2), Obese Class 2 (35.0 – 39.9 kg/m^2), Obese Class 3 (≥ 40.0 kg/m^2). BMI (p-value) = 0.0219. Ethnicity (p-value) = 0.4557. BMI: Ethnicity (p-value) = 0.4511.

3.3.3.2. The Absolute Dense Volume, BMI Category, and Ethnicity

We compared the absolute dense volume between ethnic groups stratified by BMI category of obesity [Figure 13]. Data analysis by two-way ANOVA showed that the absolute density was not affected by the BMI category (p-value = 0.4409), ethnicity (p-value = 0.3909), and the interaction of BMI category and ethnicity (p-value = 0.4052).

Figure 13. Plot of Means of the Absolute Dense Volume stratified by BMI category and Ethnicity.

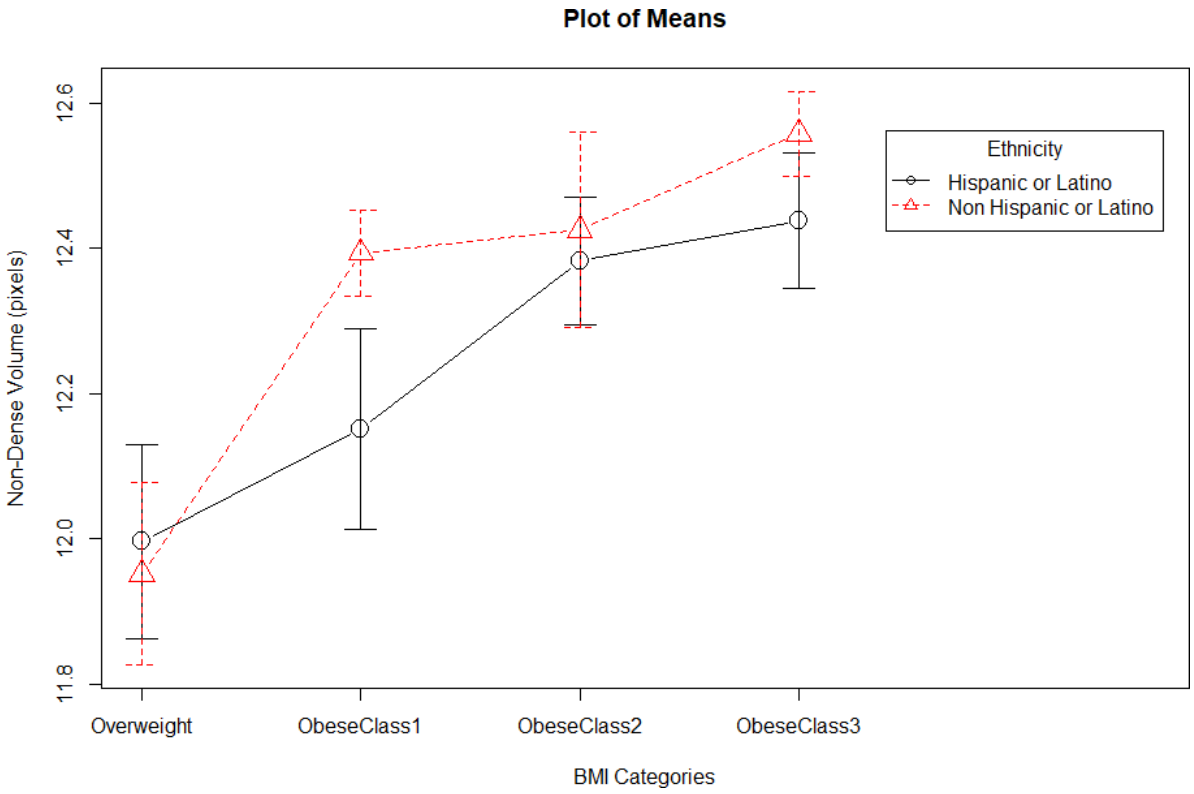


BMI categories: Overweight (25.0 – 29.9 kg/m^2), Obese Class 1 (30.0 – 34.9 kg/m^2), Obese Class 2 (35.0 – 39.9 kg/m^2), Obese Class 3 (≥ 40.0 kg/m^2). BMI (p-value) = 0.4409. Ethnicity (p-value) = 0.3909. BMI: Ethnicity (p-value) = 0.4052.

3.3.3.3. The Non-Dense Volume, BMI Category, and Ethnicity

We compared the non-dense volume between ethnic groups and stratified by BMI category of obesity [Figure 14]. The data analysis by two-way ANOVA showed that the non-dense volume was affected by BMI category (p-value = 0.00132), but not by ethnicity (p-value = 0.98495) nor the interaction of BMI category and ethnicity (p-value = 0.93754).

Figure 14. Plot of Means of the Non-Dense Volume stratified by BMI category and Ethnicity.

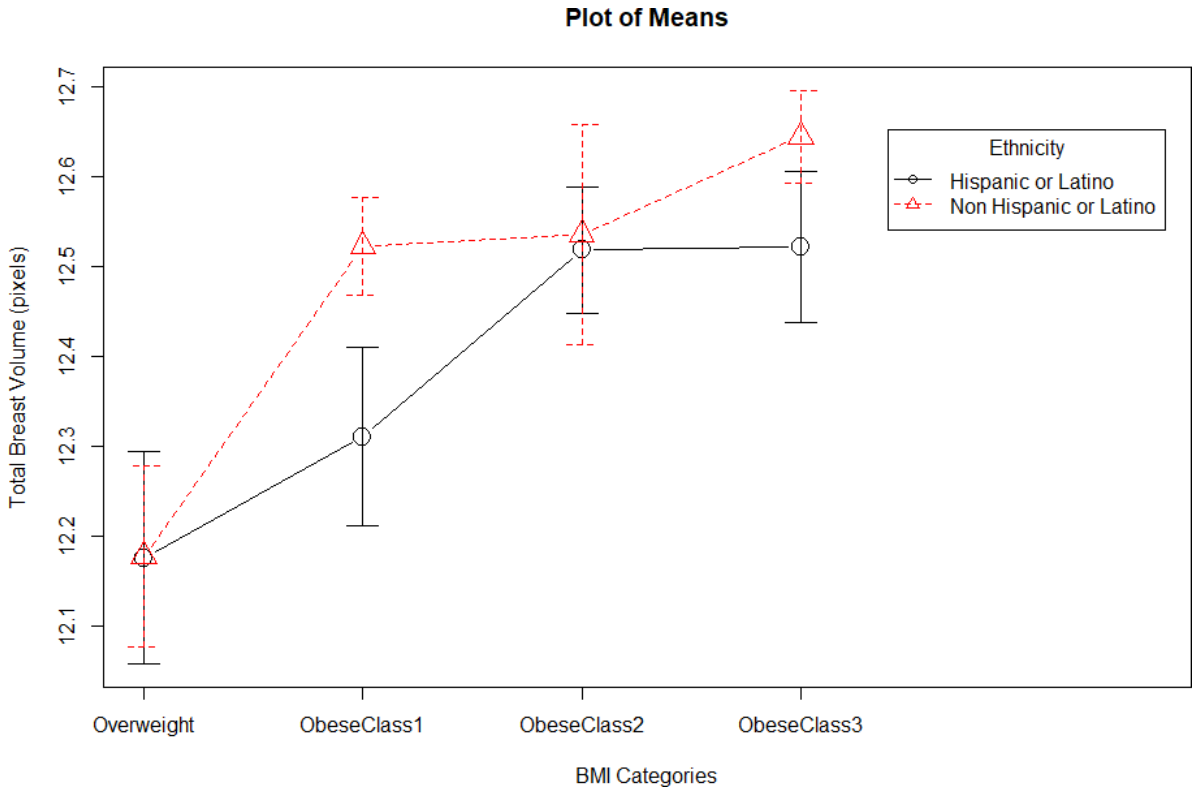


BMI categories: Overweight (25.0 – 29.9 kg/m^2), Obese Class 1 (30.0 – 34.9 kg/m^2), Obese Class 2 (35.0 – 39.9 kg/m^2), Obese Class 3 (≥ 40.0 kg/m^2). BMI (p-value) = 0.00132. Ethnicity (p-value) = 0.98495. BMI: Ethnicity (p-value) = 0.93754.

3.3.3.4. The Total Breast Volume, BMI Category, and Ethnicity

We compared the total breast volume between ethnic groups and stratified by BMI category of obesity [Figure 15]. The data analysis by two-way ANOVA showed that the total breast volume was affected BMI category (p-value = 0.00195), but not by ethnicity (p-value = 0.8777) nor the interaction of BMI category and ethnicity (p-value = 0.92892).

Figure 15. Plot of Means of the Total Breast Volume stratified by BMI category and Ethnicity.



BMI categories: Overweight (25.0 – 29.9 kg/m^2), Obese Class 1 (30.0 – 34.9 kg/m^2), Obese Class 2 (35.0 – 39.9 kg/m^2), Obese Class 3 (≥ 40.0 kg/m^2). BMI (p-value) = 0.00195. Ethnicity (p-value) = 0.8777. BMI: Ethnicity (p-value) = 0.92892.

3.3.4. Regression Analysis of Breast Density Measurements with the Anthropometric Measurements of Adiposity in the Study Cohort

We conducted regression analysis in order to evaluate the relationships of the anthropometric measurements with breast density measurements by adjusting for potential confounders. We conducted the regression analysis with three models: the unadjusted model, which included only the breast parameter with the anthropometric measurement (BMI, waist circumference, or waist-hip ratio); the partially adjusted model, adjusting for BMI; and the fully adjusted model to adjust for covariates of age, BMI, waist circumference, waist-hip ratio, ethnicity and reproductive factors [Table 19].

3.3.4.1. Percent Density and the Absolute Dense Volume

Similar to the correlation analysis, the regression analysis showed that both the percent density and the absolute dense volume were inversely related to BMI (β = -2.0487 for the percent density, and β = -0.9478 for the absolute dense volume, all $p < 0.05$). The relationships were not statistically significant in the fully adjusted model. The percent density and the absolute dense volume were also inversely related to the waist circumference (β = -3.6644 for the percent density, and β = - 1.6807 for the absolute dense volume, all $p < 0.01$), and this relationship remained statistically significant only for the percent density when including BMI as a covariate (β = -3.0028, $p = 0.0013$). However, neither the percent density nor the absolute dense volume was related to the waist circumference in the fully adjusted model. In addition, the waist-hip ratio was inversely related to the percent density and absolute dense volume (β = -3.1849 for the percent density, and β = -2.3102 for the absolute dense volume, all $p < 0.005$). This relationship remained significant for both parameters when including the BMI as a covariate (β = -2.8220 for the percent density, and β = -2.1509 for the absolute dense volume, all $p < 0.01$). Similarly, neither the percent density nor the absolute dense volume was related to the waist-hip ratio in the fully adjusted model [Table 19].

3.3.4.2. Non-Dense Volume and the Total Breast Volume

Similar to the correlation analysis, the regression analysis showed that the non-dense volume and the total breast volume were positively related to BMI (β = 1.3969 for the non-dense volume and β = 1.1009 for the total breast volume, all $p < 0.0001$). However, they were not related to BMI in the fully adjusted model. In addition, both parameters were positively related to the waist circumference (β = 2.5359 for the non-dense volume, and β = 1.9837 for the total breast volume, all $p < 0.00001$) and remained statistically significant in the fully adjusted model (β = 3.3061 for the non-dense volume, and β = 2.8155 for the total breast volume, all $p < 0.001$). Additionally, the non-dense volume was related to the waist-hip ratio (β = 1.3310, $p = 0.0132$), remaining statistically significant for the partially adjusted model (β = 1.0748, $p = 0.0248$). The total breast volume had a borderline relationship with the waist-hip ratio (β = 0.8748, $p = 0.0535$), but this relationship was lost for the partially adjusted model.

Table 19. Linear regression analysis comparing the breast density measurements [a] with the anthropometric measurements of adiposity [a] in the study cohort (N = 107).

Models of Explicative Variables	Breast Density Measurements [a] (β, 95% CI, p-value)			
	Percent Density	**Absolute Dense Volume**	**Non-Dense Volume**	**Total Breast Volume**
BMI				
Unadjusted model				
β	-2.0487	-0.9478	1.3969	1.1009
95% CI	(-2.9294, -1.1681)	(-1.7956, -0.1001)	(0.8938, 1.8999)	(0.6717, 1.5301)
p-value	<0.00001	0.0288	<0.00001	<0.00001
Fully adjusted model [c]				
β	-1.2009	-1.4254	-0.1562	-0.2244
95% CI	(-2.8143, 0.4124)	(-3.0504, 0.1996)	(-1.1315, 0.8192)	(-1.0742, 0.6253)
p-value	0.1423	0.0846	0.7506	0.6003
Waist circumference				
Unadjusted model				
β	-3.6644	-1.6807	2.5359	1.9837
95% CI	(-4.9250, -2.4037)	(-2.9382, -0.4231)	(1.8355, 3.2364)	(1.3775, 2.5900)
p-value	<0.00001	0.0093	<0.00001	<0.00001
Partially adjusted model [b]				
β	-3.0028	-1.3595	2.1235	1.6433
95% CI	(-4.7994, -1.2061)	(-3.1586, 0.4397)	(1.1266, 3.1204)	(0.7799, 2.5067)
p-value	0.0013	0.1371	0.0001	0.0003
Fully adjusted model [c]				
β	-1.9195	0.8959	3.3061	2.8155
95% CI	(-4.9424, 1.1033)	(-2.1487, 3.9406)	(1.4786, 5.1336)	(1.2234, 4.4076)
p-value	0.2097	0.5594	0.0006	0.0007
Waist-hip ratio				
Unadjusted model				
β	-3.1849	-2.3102	1.3310	0.8748
95% CI	(-4.900, -1.4699)	(-3.8846, -0.7357)	(0.2847, 2.3774)	(-0.0134, 1.7629)
p-value	0.0004	0.0044	0.0132	0.0535
Partially adjusted model [b]				
β	-2.8220	-2.1509	1.0748	0.6710
95% CI	(-4.4084, -1.2356)	(-3.7117, -0.5902)	(0.1391, 2.0106)	(-0.1365, 1.4786)
p-value	0.0006	0.0074	0.0248	0.1024
Fully adjusted model [c]				
β	-0.5679	-1.9076	-1.4280	-1.3397
95% CI	(-3.4787, 2.3429)	(-4.8393, 1.0241)	(-3.1877, 0.3318)	(-2.8727, 0.1934)
p-value	0.6986	0.1988	0.1102	0.0858

(a) Variables followed a non-normal distribution and were log base 10 transformed.
(b) The partially adjusted model includes BMI.
(c) The fully adjusted model includes anthropometric measurements (BMI, waist circumference, and waist- hip ratio), ethnicity, age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

3.3.5. Elements of Metabolic Syndrome in the Study Cohort

We used the NCEP ATP III criteria for metabolic syndrome for this study cohort [Huang 2009]. For study entry, all participants were required to have a large waist circumference (≥35 inches or ≥31 inches for Asians and women with polycystic ovary syndrome; one component of metabolic syndrome) to meet eligibility. Participants were also required to have a second component of metabolic syndrome. The most frequent second component in our study cohort was the presence of reduced HDL-C (64.48%), followed by high blood pressure (52.33%), elevated triglycerides (35.51%) and elevated fasting glucose (29.91%) [See Table 20]. Most participants had only two elements of metabolic syndrome (48.59%), followed by three elements (24.29%) and four or more elements of metabolic syndrome (26.17%) [See Table 21].

Table 20. Elements of Metabolic Syndrome Present in the Study Cohort (N = 106).

Element of Metabolic Syndrome	N (%)
Elevated Triglycerides (≥ 150 mg/dl or on drug treatment for it)	38 (35.51)
Reduced HDL-C (< 50 mg/dl or on drug treatment for it)	69 (64.48)
Elevated Blood Pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic or on drug treatment for it)	56 (52.33)
Elevated Fasting Glucose (≥ 100 mg/dl)	32 (29.91)

Table 21. Number of Elements of Metabolic Syndrome in the Study Cohort (N = 106).

Number of Elements of Metabolic Syndrome	N (%)
Two Elements of Metabolic Syndrome	52 (48.59)
Three Elements of Metabolic Syndrome	26 (24.29)
Four or More Elements of Metabolic Syndrome	28 (26.17)

3.3.5.1. Means of Breast Density Measurements by the Additional Element of Metabolic Syndrome

We compared the breast density measurements by the presence of each additional element of metabolic syndrome [Table 22]. The presence or absence of elevated triglycerides (≥150 mg/dl or on drug treatment for it) did not affect the breast density measurements. Similarly, the reduced HDL-C (<50 mg/dl) had no influence on the breast parameters. However, participants with elevated blood pressure (≥130 mmHg systolic or ≥85 mmHg diastolic or on drug treatment for it) had higher non-dense volume and larger breasts than those without hypertension. Elevated blood pressure had no effects on the absolute dense volume nor the percent density. Moreover, participants with elevated fasting glucose (≥100 mg/dl) had statistically significant lower absolute dense volume than those without hyperglycemia. Additionally, participants with hyperglycemia had lower percent densities than those without it, although it was not statistically significant. Elevated fasting glucose was not related to the non-dense volume or the total breast volume.

Table 22. Means (SD) of Breast Density Measurements with Elements of Metabolic Syndrome (N = 106).

Element of Metabolic Syndrome	Overall Study Cohort (N=106)			
	Absolute Dense Volume [a] (pixels)	Percent Density [a] (%)	Non-Dense Volume [a] (pixels)	Total Breast Volume [a] (pixels)
Elevated triglycerides (≥ 150 mg/dl) or on drug treatment for it				
Yes (n=38)	10.21 (0.62)	2.40 (0.72)	12.26 (0.50)	12.42 (0.41)
No (n=68)	10.07 (0.61)	2.23 (0.67)	12.32 (0.35)	12.45 (0.30)
P-value [b]	0.2653	0.2251	0.4395	0.6693
Reduced HDL-C (< 50 mg/dl) or on drug treatment for it				
Yes (n=69)	10.06 (0.66)	2.21 (0.73)	12.31 (0.44)	12.44 (0.37)
No (n=37)	10.24 (0.50)	2.43 (0.58)	12.27 (0.34)	12.42 (0.28)
P-value [b]	0.1424	0.1351	0.6496	0.7212
Elevated blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic) or on drug treatment for it				
Yes (n=56)	10.17 (0.56)	2.26 (0.60)	12.39 (0.37)	12.51 (0.32)
No (n=50)	10.07 (0.67)	2.32 (0.78)	12.20 (0.42)	12.35 (0.34)
P-value [b]	0.3876	0.6615	0.01791	0.01369
Elevated Fasting Glucose (≥ 100 mg/dl)				
Yes (n=32)	9.93 (0.68)	2.10 (0.73)	12.32 (0.34)	12.43 (0.29)
No (n=74)	10.20 (0.57)	2.37 (0.66)	12.29 (0.43)	12.44 (0.36)
P-value [b]	0.03944	0.06972	0.7571	0.9543

(a) Variables were non-normally distributed and were log base 10 transformed.
(b) Unpaired t-test was conducted to assess differences between the groups.

3.3.5.2. Regression Analysis of Breast Density Measurements with the Additional Element of Metabolic Syndrome

In order to further evaluate the potential relationships between each additional element of metabolic syndrome with breast density measurements, we performed regression analysis. We used three models for the regression analysis: the unadjusted model; the partially adjusted model, which was adjusted for BMI; and the fully adjusted model to adjust for covariates of age, BMI, waist circumference, waist-hip ratio, ethnicity, and reproductive factors [Table 23].

As shown in Table 23, elevated triglycerides (≥ 150 mg/dl or on drug treatment for it) were not related to the breast density measurements. Similarly, the reduced HDL-C (< 50 mg/dl or on drug treatment for it) was not related to the breast parameters in this study cohort. There was a relationship between the non-dense volume with elevated blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic or on drug treatment for it) in the unadjusted model (β = 0.1855, p = 0.0185), although this relationship was borderline significant in the partially adjusted (β = 0.1229, p-value = 0.0845) and in the fully adjusted model (β = 0.1662, p-value = 0.0546). Similar to that of the non-dense volume, the total breast volume was related to elevated blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic or on drug treatment for it) in the unadjusted model (β = 0.1633, p = 0.0134). However, this relationship showed borderline significance in the partially adjusted (β = 0.1144, p- value = 0.0594) and in the fully adjusted model (β = 0.1408, p-value = 0.0625) [Table 23]. In addition, an inverse relationship was found for the elevated fasting glucose with the absolute dense volume (β = -0.2781, p-value = 0.0334) in the unadjusted model, and this relationship remained statistically significant in the partially adjusted (β = -0.2835 p-value = 0.0271) and in the fully adjusted models (β = -0.4441, p-value = 0.0025). The relationship between elevated fasting glucose and percent density was borderline significant in the unadjusted model (β = -0.2735, p- value = 0.0609) but was statistically significant in the partially adjusted (β = -0.2852 p-value = 0.0325) and in the fully adjusted models (β = -0.4379, p-value = 0.0024) [Table 23]

Table 23. Linear regression analysis comparing the relationships of the additional element of metabolic syndrome with the breast density measurements [a] in the study cohort (N = 106).

Models of Explicative Variables	Breast Density Measurements [a] (β, 95% CI, p-value)			
	Percent Density	**Absolute Dense Volume**	**Non-Dense Volume**	**Total Breast Volume**
Elevated triglycerides (≥ 150 mg/dl) or on drug treatment for it				
Unadjusted model				
β	0.1597	0.1295	-0.0635	-0.0302
95% CI	(-0.1171, 0.4365)	(-0.1194, 0.3783)	(-0.2277, 0.1007)	(-0.1683, 0.1078)
p-value	0.2553	0.3047	0.4448	0.6651
Partially adjusted model [b]				
β	0.1458	0.1231	-0.0540	-0.0227
95% CI	(-0.1079, 0.3995)	(-0.1215, 0.3676)	(-0.1995, 0.0914)	(-0.1471, 0.1016)
p-value	0.2571	0.3207	0.4630	0.7177
Fully adjusted model [c]				
β	0.1478	0.1455	-0.0313	-0.0024
95% CI	(-0.1351, 0.4308)	(-0.1426, 0.4335)	(-0.2063, 0.1437)	(-0.1554, 0.1506)
p-value	0.3012	0.3176	0.7226	0.9754
Reduced HDL-C (< 50 mg/dl) or on drug treatment for it				
Unadjusted model				

β	-0.2258	-0.2016	0.0392	0.0242
95% CI	(-0.5008, 0.0493)	(-0.4486, 0.0455)	(-0.1253, 0.2037)	(-0.1139, 0.1623)
p-value	0.1066	0.1087	0.6372	0.7288
Partially adjusted model [b]				
β	-0.0727	-0.1382	-0.0740	-0.0656
95% CI	(-0.3372, 0.1918)	(-0.3918, 0.1153)	(-0.2246, 0.0766)	(-0.1940, 0.0629)
p-value	0.5870	0.2821	0.3320	0.3138
Fully adjusted model [c]				
β	-0.1518	-0.2177	-0.0637	-0.0659
95% CI	(-0.4552, 0.1516)	(-0.5243, 0.0890)	(-0.2508, 0.1234)	(-0.2291, 0.0974)
p-value	0.3220	0.1614	0.4996	0.4239
Elevated blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic) or on drug treatment for it				
Unadjusted model				
β	-0.0467	0.1166	0.1855	0.1633
95% CI	(-0.3137, 0.2203)	(-0.1222, 0.3554)	(0.0317, 0.3394)	(0.0346, 0.2920)
p-value	0.7295	0.3352	0.0185	0.0134
Partially adjusted model [b]				
β	0.0521	0.1665	0.1229	0.1144
95% CI	(-0.1962, 0.3004)	(-0.0705, 0.4035)	(-0.0170, 0.2628)	(-0.0046, 0.2334)
p-value	0.6782	0.1665	0.0845	0.0594
Fully adjusted model [c]				
β	-0.1192	0.0215	0.1662	0.1408
95% CI	(-0.4007, 0.1622)	(-0.2662, 0.3093)	(-0.0033, 0.3357)	(-0.0075, 0.2891)
p-value	0.4013	0.8820	0.0546	0.0625
Elevated fasting glucose (≥ 100 mg/dl)				
Unadjusted model				
β	-0.2735	-0.2781	0.0278	-0.0045
95% CI	(-0.5598, 0.0127)	(-0.5339, -0.0222)	(-0.1443, 0.1998)	(-0.1489, 0.1399)
p-value	0.0609	0.0334	0.7495	0.9507
Partially adjusted model [b]				
β	-0.2852	-0.2835	0.0357	0.0017
95% CI	(-0.5462, -0.0242)	(-0.5343, -0.0327)	(-0.1166, 0.1879)	(-0.1283, 0.1317)
p-value	0.0325	0.0271	0.6431	0.9793
Fully adjusted model [c]				
β	-0.4379	-0.4441	0.0382	-0.0062
95% CI	(-0.7159, -0.1598)	(-0.7271, -0.1610)	(-0.1435, 0.2199)	(-0.1651, 0.1527)
p-value	0.0024	0.0025	0.6765	0.9380

(a) Variables followed a non-normal distribution and were log base 10 transformed.
(b) The partially adjusted model includes BMI.
(c) The fully adjusted model includes anthropometric measurements (BMI, waist circumference, and waist- hip ratio), ethnicity, age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

3.3.5.3. Means of Breast Density Measurements by the Additional Element of Metabolic Syndrome and Ethnicity

3.3.5.3.1. Means of Breast Density Measurements by the Additional Element of Metabolic Syndrome in Hispanics

We further evaluated whether breast density measurements differ by the presence of the additional element of metabolic syndrome in Hispanics [Table 24]. Elevated triglycerides (≥150 mg/dl or on drug treatment for it) were not related to breast parameters. Similar reduced HDL-C (<50 mg/dl or on drug treatment for it) had no relationship with breast density measurements. Elevated blood pressure (≥130 mmHg systolic or ≥85 mmHg diastolic or on drug treatment for it) was not related to breast density measurements. However, Hispanics with elevated fasting glucose (≥100 mg/dl) had lower percent densities and absolute dense volume than those without hyperglycemia [Table 24].

Table 24. Means (SD) of Breast Density Measurements with the Additional Element of Metabolic Syndrome in Hispanics (n = 41).

Element of Metabolic Syndrome	Hispanics (n=41)			
	Percent Density (%)[a]	**Absolute Dense Volume (pixels)[a]**	**Non-Dense Volume (pixels)[a]**	**Total Breast Volume (pixels)[a]**
Elevated triglycerides (≥ 150 mg/dl) or on drug treatment for it				
Yes (n=15)	2.51 (0.65)	10.23 (0.61)	12.15 (0.43)	12.32 (0.33)
No (n= 26)	2.19 (0.71)	10.00 (0.63)	12.28 (0.38)	12.41 (0.32)
p-value	0.166	0.257	0.302	0.423
Reduced HDL-C (< 50 mg/dl) or on drug treatment for it				
Yes (n= 28)	2.28 (0.75)	10.06 (0.66)	12.24 (0.43)	12.39 (0.35)
No (n= 13)	2.38 (0.59)	10.13 (0.54)	12.21 (0.32)	12.35 (0.26)
p-value	0.662	0.733	0.845	0.775
Elevated blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic) or on drug treatment for it				
Yes (n=21)	2.36 (0.57)	10.20 (0.50)	12.29 (0.34)	12.43 (0.28)
No (n=20)	2.25 (0.82)	9.97 (0.72)	12.17 (0.44)	12.31 (0.36)
p-value	0.616	0.239	0.322	0.241
Elevated fasting glucose (≥ 100 mg/dl)				
Yes (n= 10)	1.60 (0.55)	9.45 (0.64)	12.39 (0.28)	12.45 (0.28)
No (n= 31)	2.54 (0.58)	10.29 (0.47)	12.18 (0.42)	12.35 (0.33)
p-value	<0.0001	<0.0001	0.147	0.398

(a) Variables followed a non-normal distribution and were log base 10 transformed.
(b) Unpaired t-test was conducted to assess differences between the groups.

3.3.5.3.2. Means of Breast Density Measurements by the Additional Element of Metabolic Syndrome in Non-Hispanics

We further evaluated whether breast density measurements differed by the presence of additional elements of metabolic syndrome in Non-Hispanics [Table 25]. The presence of elevated triglycerides, reduced HDL-C, or elevated fasting glucose had no influence on the breast parameters. However, Non-Hispanics with high blood pressure had higher non-dense volume ($p = 0.027$) and total breast volume ($p = 0.033$) than those without hypertension [Table 25].

Table 25. Means (SD) of Breast Density Measurements with the Additional Element of Metabolic Syndrome in Non-Hispanics (n = 64).

Element of Metabolic Syndrome	Non-Hispanics (n=64)			
	Percent Density (%)[a]	**Absolute Dense Volume (pixels)[a]**	**Non-Dense Volume (pixels)[a]**	**Total Breast Volume (pixels)[a]**
Elevated triglycerides (≥ 150 mg/dl) or on drug treatment for it				
Yes (n=22)	2.39 (0.71)	10.27 (0.56)	12.33 (0.54)	12.48 (0.45)
No (n= 42)	2.25 (0.64)	10.12 (0.61)	12.35 (0.33)	12.47 (0.29)
p-value	0.429	0.337	0.861	0.909
Reduced HDL-C (< 50 mg/dl) or on drug treatment for it				
Yes (n= 40)	2.20 (0.70)	10.09 (0.64)	12.36 (0.45)	12.49 (0.38)
No (n= 24)	2.45 (0.59)	10.30 (0.48)	12.31 (0.36)	12.45 (0.29)
p-value	0.159	0.168	0.620	0.730
Elevated blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic) or on drug treatment for it				
Yes (n= 35)	2.20 (0.62)	10.16 (0.60)	12.44 (0.38)	12.56 (0.34)
No (n=29)	2.41 (0.71)	10.19 (0.59)	12.21 (0.42)	12.37 (0.34)
p-value	0.198	0.850	0.027	0.033
Elevated fasting glucose (≥ 100 mg/dl)				
Yes (n= 22)	2.33 (0.69)	10.15 (0.59)	12.28 (0.36)	12.42 (0.30)
No (n= 42)	2.28 (0.66)	10.18 (0.60)	12.37 (0.44)	12.50 (0.38)
p-value	0.7622	0.8831	0.4384	0.413

(a) Variables were non-normally distributed and were log base 10 transformed.
(b) Unpaired t-test was conducted to assess differences between the groups.

3.3.5.4. Regression Analysis of Breast Density Measurements with the Additional Element of Metabolic Syndrome by Ethnicity

3.3.5.4.1. Regression Analysis of Breast Density Measurements with the Additional Element of Metabolic Syndrome in Hispanics

We analyzed the relationships between the additional element of metabolic syndrome with breast density measurements by regression analysis in the Hispanic cohort (n=41). We used similar regression models as described earlier.

As seen in Table 26, elevated triglycerides (≥150 or on drug treatment for it) were not related to the breast parameters. Likewise, the reduced HDL-C (<50 or on drug treatment for it) was not related to the breast density measurements. In terms of the elevated blood pressure (≥130 systolic or ≥85 diastolic or on drug treatment for it), there was a borderline relationship with the absolute dense volume in the partially adjusted model (β= 0.3606, p-value= 0.0836) but no other significant relationships were observed for this additional element of metabolic syndrome. The elevated fasting glucose, on the other hand, showed to be related to the percent density in the unadjusted (β= -0.9532, p-value< 0.00001), in the partially adjusted (β= -0.8810, p-value= 0.0001), and in the fully adjusted model (β= -0.7273, p-value= 0.0107). Similar to that of the percent density, the elevated fasting glucose was related to the absolute dense volume in the unadjusted (β= -0.8552, p-value< 0.00001), in the partially adjusted (β= -0.8508, p-value= 0.0001), and in the fully adjusted model (β= -0.8126, p-value= 0.0034) [Table 26].

Table 26. Linear regression analysis comparing the breast density measurements [a] by the additional element of metabolic syndrome in Hispanics (n = 41).

Models of Explicative Variables	Breast Density Measurements [a] (β, 95% CI, p-value)			
	Percent Density	**Absolute Dense Volume**	**Non-Dense Volume**	**Total Breast Volume**
Elevated triglycerides (≥ 150 mg/dl) or on drug treatment for it				
Unadjusted model				
β	0.2891	0.2015	-0.1344	-0.0877
95% CI	(-0.1649, 0.7432)	(-0.2089, 0.6119)	(-0.3921, 0.1234)	(-0.2979, 0.1226)
p-value	0.2055	0.3270	0.2984	0.4045
Partially adjusted model [b]				
β	0.2405	0.1858	-0.0942	-0.0547
95% CI	(-0.1982, 0.6791)	(-0.2298, 0.6014)	(-0.3270, 0.1385)	(-0.2442, 0.1348)
p-value	0.2743	0.3715	0.4179	0.5629
Fully adjusted model [c]				
β	-0.0289	0.0407	0.0850	0.0696
95% CI	(-0.6156, 0.5578)	(-0.5433, 0.6247)	(-0.2196, 0.3896)	(-0.1835, 0.3227)
p-value	0.9195	0.8864	0.5686	0.5742
Reduced HDL-C (< 50 mg/dl) or on drug treatment for it				
Unadjusted model				
β	-0.1478	-0.1236	0.0251	0.0242
95% CI	(-0.6165, 0.3208)	(-0.5440, 0.2968)	(-0.2404, 0.2906)	(-0.1913, 0.2397)

p-value	0.5274	0.5557	0.8495	0.8215
Partially adjusted model [b]				
β	0.0122	-0.0766	-0.1141	-0.0889
95% CI	(-0.4637, 0.4881)	(-0.5246, 0.3713)	(-0.3622, 0.1339)	(-0.2901, 0.1124)
p-value	0.9589	0.7312	0.3578	0.3774
Fully adjusted model [c]				
β	-0.2877	-0.3237	-0.0045	-0.0360
95% CI	(-0.8510, 0.2756)	(-0.8805, 0.2332)	(-0.3065, 0.2975)	(-0.2864, 0.2144)
p-value	0.3011	0.2408	0.9758	0.7684
Elevated blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic) or on drug treatment for it				
Unadjusted model				
β	0.1403	0.2619	0.1231	0.1216
95% CI	(-0.3020, 0.5827)	(-0.1277, 0.6516)	(-0.1245, 0.3707)	(-0.0782, 0.3214)
p-value	0.5250	0.1819	0.3210	0.2258
Partially adjusted model [b]				
β	0.3413	0.3606	-0.0059	0.0193
95% CI	(-0.0980, 0.7805)	(-0.0501, 0.7713)	(-0.2445, 0.2328)	(-0.1741, 0.2127)
p-value	0.1242	0.0836	0.9607	0.8409
Fully adjusted model [c]				
β	0.3620	0.4103	0.0249	0.0483
95% CI	(-0.2330, 0.9569)	(-0.1757, 0.9962)	(-0.2971, 0.3470)	(-0.2186, 0.3152)
p-value	0.2203	0.1606	0.8739	0.7111
Elevated fasting glucose (≥ 100 mg/dl)				
Unadjusted model				
β	-0.9532	-0.8552	0.2107	0.0980
95% CI	(-1.3762, -0.5301)	(-1.2343, -0.4761)	(-0.0755, 0.4968)	(-0.1386, 0.3345)
p-value	<0.00001	<0.00001	0.1446	0.4077
Partially adjusted model [b]				
β	-0.8810	-0.8508	0.1311	0.0302
95% CI	(-1.3013, -0.4607)	(-1.2425, -0.4592)	(-0.1332, 0.3954)	(-0.1866, 0.2470)
p-value	0.0001	0.0001	0.3218	0.7796
Fully adjusted model [c]				
β	-0.7273	-0.8126	-0.0234	-0.0852
95% CI	(-1.2686, -0.1861)	(-1.3262, -0.2989)	(-0.3525, 0.3057)	(-0.3562, 0.1857)
p-value	0.0107	0.0034	0.8841	0.5209

(a) Variables followed a non-normal distribution and were log base 10 transformed.
(b) The partially adjusted model includes BMI.
(c) The fully adjusted model includes anthropometric measurements (BMI, waist circumference, and waist- hip ratio), ethnicity, age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

3.3.5.4.2. Regression Analysis of Breast Density Measurements with the Additional Element of Metabolic Syndrome in Non-Hispanics

We further conducted regression analysis to evaluate the potential relationships of the additional elements of metabolic syndrome with breast parameters in the Non-Hispanic cohort (n=64). We used similar regression models as described earlier.

Table 27 shows that there was no relationship between elevated triglycerides and breast density measurements. In addition, no relationships were observed between reduced HDL-C and breast parameters. For elevated blood pressure, there was a relationship with the non-dense volume in the unadjusted (β= 0.2293, p-value= 0.0277), in the partially adjusted (β= 0.2078, p-value= 0.0236), and in the fully adjusted model (β= 0.3075, p-value= 0.0150). In addition, the elevated blood pressure was related to increased total breast volume in the unadjusted (β= 0.1893, p-value= 0.0332), in the partially adjusted (β= 0.1729, p-value= 0.0322), and in the fully adjusted model (β= 0.2480, p-value= 0.0285). A relationship was observed between the percent density with elevated blood pressure in the fully adjusted model (β= -0.3923, p-value= 0.0313). The absolute dense volume, however, was not related to the elevated blood pressure. The elevated fasting glucose showed to be borderline related to the percent density but only in the fully adjusted model (β= -0.2967, p-value= 0.0997). We observed no other statistically significant relationships for the elevated fasting glucose with other breast density parameters [Table 27].

Table 27. Linear regression analysis comparing the breast density measurements [a] by the additional element of metabolic syndrome in Non-Hispanics (n = 64).

Models of Explicative Variables	Breast Density Measurements [a] (β, 95% CI, p-value)			
	Percent Density	**Absolute Dense Volume**	**Non-Dense Volume**	**Total Breast Volume**
Elevated triglycerides (≥ 150 mg/dl) or on drug treatment for it				
Unadjusted model				
β	0.1409	0.1518	-0.0194	0.0108
95% CI	(-0.2136, 0.4955)	(-0.1623, 0.4659)	(-0.2410, 0.2022)	(-0.1781, 0.1998)
p-value	0.4299	0.3378	0.8617	0.9090
Partially adjusted model [b]				
β	0.1697	0.1673	-0.0367	-0.0024
95% CI	(-0.1393, 0.4787)	(-0.1345, 0.4690)	(-0.2321, 0.1586)	(-0.1740, 0.1691)
p-value	0.2764	0.2720	0.7082	0.9774
Fully adjusted model [c]				
β	0.0955	0.0719	-0.0508	-0.0236
95% CI	(-0.2948, 0.4858)	(-0.3365, 0.4803)	(-0.3241, 0.2225)	(-0.2671, 0.2200)
p-value	0.6237	0.7239	0.7094	0.8459
Reduced HDL-C (< 50 mg/dl) or on drug treatment for it				
Unadjusted model				
β	-0.2452	-0.2131	0.0540	0.0321
95% CI	(-0.5892, 0.0988)	(-0.5188, 0.0925)	(-0.1630, 0.2710)	(-0.1531, 0.2173)
p-value	0.1592	0.1684	0.6207	0.7303
Partially adjusted model [b]				
β	-0.0841	-0.1323	-0.0496	-0.0481
95% CI	(-0.3987, 0.2304)	(-0.4383, 0.1738)	(-0.2469, 0.1476)	(-0.2210, 0.1248)
p-value	0.5947	0.3909	0.6165	0.5797
Fully adjusted model [c]				

β	-0.1651	-0.2874	-0.1255	-0.1222
95% CI	(-0.5675, 0.2373)	(-0.7015, 0.1268)	(-0.4065, 0.1555)	(-0.3719, 0.1274)
p-value	0.4120	0.1686	0.3724	0.3284
Elevated blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic) or on drug treatment for it				
Unadjusted model				
β	-0.2179	-0.0286	0.2293	0.1893
95% CI	(-0.5534, 0.1175)	(-0.3304, 0.2732)	(0.0260, 0.4326)	(0.0155, 0.3631)
p-value	0.1989	0.8504	0.0277	0.0332
Partially adjusted model [b]				
β	-0.1821	-0.0092	0.2078	0.1729
95% CI	(-0.4764, 0.1121)	(-0.3002, 0.2817)	(0.0288, 0.3869)	(0.0152, 0.3306)
p-value	0.2205	0.9496	0.0236	0.0322
Fully adjusted model [c]				
β	-0.3923	-0.1442	0.3075	0.2480
95% CI	(-0.7475, -0.0370)	(-0.5346, 0.2462)	(0.0629, 0.5521)	(0.0274, 0.4687)
p-value	0.0313	0.4599	0.0150	0.0285
Elevated fasting glucose (≥ 100 mg/dl)				
Unadjusted model				
β	0.0541	-0.0234	-0.0861	-0.0775
95% CI	(-0.3019, 0.4102)	(-0.3397, 0.2930)	(-0.3066, 0.1345)	(-0.2655, 0.1105)
p-value	0.7622	0.8831	0.4384	0.4130
Partially adjusted model [b]				
β	-0.0467	-0.0783	-0.0260	-0.0316
95% CI	(-0.3615, 0.2680)	(-0.3853, 0.2287)	(-0.2233, 0.1714)	(-0.2046, 0.1414)
p-value	0.7675	0.6118	0.7933	0.7165
Fully adjusted model [c]				
β	-0.2967	-0.2969	0.0321	-0.0001
95% CI	(-0.6525, 0.0590)	(-0.6697, 0.0759)	(-0.2250, 0.2892)	(-0.2291, 0.2289)
p-value	0.0997	0.1155	0.8020	0.9991

(a) Variables followed a non-normal distribution and were log base 10 transformed.
(b) The partially adjusted model includes BMI.
(c) The fully adjusted model includes anthropometric measurements (BMI, waist circumference, and waist- hip ratio), ethnicity, age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

3.3.5.5. Means of Breast Density Measurements Stratified by the Presence of Metabolic Syndrome

According to the NCEP ATP III criteria, we considered subjects that presented three or more metabolic risk factors as having metabolic syndrome [Huang 2009]. We compared the means of the breast density measurements between those who had three or more components of metabolic syndrome with those who had two components of metabolic syndrome [Table 28]. As seen in Table 28, there was no statistically significant difference in the means of breast density measurements when analyzed by the presence of metabolic syndrome. Because there was no association with the breast parameters, we conducted no further statistical evaluation.

Table 28. Means (SD) of Breast Density Measurements Stratified by the Presence of Metabolic Syndrome.

Number of Elements of Metabolic Syndrome	Number of Study Subjects	Percent Density [a] (%)	Absolute Dense Volume [a] (pixels)	Non-Dense Volume [a] (pixels)	Total Breast Volume [a] (pixels)
2 elements	52	2.33 (0.62)	10.13 (0.53)	12.26 (0.37)	12.39 (0.31)
≥3 elements	54	2.24 (0.75)	10.12 (0.70)	12.34 (0.44)	12.48 (0.36)
p-value		0.4995	0.9339	0.3274	0.2269

(a) Variables followed a non-normal distribution and were log base 10 transformed.
(b) Unpaired t-test was conducted for assessing differences between the groups.

3.4. Discussion

To the best of our knowledge, this is the first work that aimed to evaluate the relationship between metabolic disturbances and breast density measurements, using fat-water MRI, in a cohort of premenopausal women with elements of metabolic syndrome. In this cross-sectional analysis, we found an inverse correlation between the absolute dense volume and the percent density with anthropometric measurements of adiposity. In addition, we found a positive association between the non-dense volume and the total breast volume with the anthropometric measurements of adiposity. After adjustment for covariates, the non-dense volume and total breast volume were positively related to the waist circumference. The non-dense volume and the total breast volume were not related to BMI or waist-hip ratio. The percent density and absolute dense volume were not related to the anthropometric measurements of adiposity. Some cross-sectional studies showed an inverse association between percent density and anthropometric measurements of adiposity [Conroy 2011, Tehranifar 2011, Tseng 2010]. Other authors report inconsistent results for the association between absolute dense area and anthropometric measurements of adiposity [Tseng 2010, Kim 2015, Haars 2005, Heng 2004, Sandhu 2015]. We attribute the discrepancies between our findings and others in the literature to the use of fat-water MRI, whereas other studies used mammography for breast density assessment. Different findings can also be due to differences in the study cohort and potential confounders.

Additionally, we explored the relationships between breast density parameters and BMI categories. We found that the overweight class is different from the other BMI categories of obesity in the percent density, non-dense volume, and in the total breast volume. The absolute dense volume was not different among the BMI categories of obesity.

In our study cohort, we could not detect an influence of ethnicity or the potential interaction of ethnicity with BMI, in the association between breast parameters and BMI. However, we must acknowledge that our sample size is small, and may not have the power to detect differences by ethnic groups. More clinical trials with larger multi-ethnic cohorts are needed to confirm our findings.

Our data showed that having elevated blood pressure (≥ 130 systolic or ≥ 85 diastolic or on drug treatment for it) in women with large waist was related to a larger non-dense volume and total breast volume. This relationship was specific to Non-Hispanics in our cohort. Having elevated fasting glucose (≥ 100 mg/dl) in women with large waist was related to a smaller absolute dense volume and the percent density. This relationship was specific to Hispanics in our study cohort. Those relationships remained significant after adjusting for anthropometric measurements, age, or reproductive factors.

Limited studies examined the associations between the breast density measurements with metabolic syndrome. Some studies showed that women with metabolic syndrome had higher absolute dense area [Kim 2015] or higher percent density [Rice 2013]. Other authors showed an inverse association between percent density, metabolic syndrome, and its components [Conroy 2011, Tehranifar 2015]. We can attribute discrepancies among these studies and ours to differences in imaging modality, the metabolic syndrome criteria, and the study cohort.

Strengths of our study were the usage of fat-water MRI to assess breast density. Mammography overestimates breast density by a factor of 2 in comparison to MRI [Klifa 2010]. In addition, fat-water MRI was performed on the non-compressed breasts. This imaging modality can overcome the potential issues of breast thickness and tissue overlapping in women with high adiposity [Thomson 2015, Li 2007]. Additionally, we conducted our study within the context of a rigorously designed clinical trial. We characterized the study populations in the potential confounders. Nonetheless, our study had some limitations. The cross-sectional design does not allow us to infer for causality in any of the associations described in this work. In addition, our study subjects were overweight/obese women with at least 2 elements of metabolic syndrome. Thus, we could not compare our study subjects with women without metabolic disturbances. More studies are needed to elucidate the underlying mechanisms for the associations between metabolic disturbances and breast density.

In summary, our work showed that percent density and absolute density were not related to anthropometric measurements of adiposity. In addition, the non-dense volume and total breast volume were only positively related to waist circumference following adjustment of potential confounders. Hispanic women with a large waist and elevated fasting glucose had lower percent and absolute densities. Non-Hispanic women with elevated blood pressure and a large waist had larger non-dense volume and total breast volume. Breast density needs to be adjusted for adiposity and metabolic risk factors when used for risk assessment. Additional studies are needed to confirm the observed associations and to understand the mechanisms responsible for the associations.

CHAPTER IV: Breast Density Measurements and the Insulin/IGF Axis

4.1. Introduction

4.1.1. The Insulin/IGF Axis and Breast Cancer Risk

4.1.1.1. Insulin and Breast Cancer Risk

Insulin is a hormone produced by the β-pancreatic cells of the islands of Langerhans. The main function of insulin is to modulate glucose homeostasis. Insulin resistance is a condition where the cells from distinct tissues are unable to respond to this hormone, leading to high blood sugar [Chiu 2006]. Insulin resistance can be estimated by models that evaluate β-cell function, such as the homeostatic model assessment of insulin resistance (HOMA-IR) [Renehan 2006, Gutch 2015, Wallace 2004]. Additionally, insulin resistance is one of the key features of metabolic disturbances associated with obesity [Shanik 2008, Goodwin 2011]. Several studies suggest that obesity could be involved in breast carcinogenesis [Shanik 2008, Goodwin 2011].

Perturbations in the insulin signaling pathway can lead to insulin resistance [Boucher 2014]. Several molecular mechanisms are altered in the insulin-resistant state [Boucher 2014]. Some of them are the activation of Ser/Thr kinases, inducing the inhibitory phosphorylation of insulin-signaling molecules [Powell 2003, Boura-Halfon 2009]. Reduced phosphorylation of insulin receptor (IR) and other signaling molecules diminish ligand affinity [Kowluru 2012, Boucher 2014]. The altered insulin/IGF pathway activates the PI3K/Akt and MAPK signaling pathways [Kowluru 2012, Belfiore 2008, Stoll 2002, Finalyson 2003, Rostoker 2015]. In addition, hyperinsulinemia and a higher bioavailability of IGF-1 inhibit the hepatic synthesis of sex hormone binding globulin (SHBG) [Arcidiacono 2012]. The decrease in SHBG contributes to a higher bioavailability of estradiol and promotes higher rates of epithelial cell growth and proliferation in the breast [Fortunati 2010]. Moreover, the dysregulations in the insulin signaling pathway, combined with visceral obesity, can increase breast cancer risk [Boucher 2014, Arcidiacono 2012].

4.1.1.2. Insulin-like Growth Factors (IGFs) and Breast Cancer Risk

Insulin acts in conjunction with the insulin-growth factors (IGF) and with the growth hormone (GH) axes to modulate glucose metabolism [Lewitt 2014]. The insulin-like growth factors (IGFs) are insulin-related peptide hormones that promote cell growth, cell proliferation and inhibit apoptosis [Laron 2001]. There are two IGFs: IGF-1 and IGF-2 [Rinderknecht 1976]. The GH stimulates distinct tissues (e.g. the liver) to synthesize and secrete IGFs, which in turn exerts paracrine or autocrine effects in the tissue [Rosenfeld 1999]. The IGFs act through cell surface receptors, like the IGF-1R and the IGF-2R, although they can also bind with low affinity to the insulin receptor [LeRoith 1995]. GH mainly induces IGF-1 secretion, whose concentrations increase during childhood, reach a peak during puberty, and then progressively decrease with age [Laron 1996]. The production of IGF-2 is less dependent on GH than for that of IGF-1 and has less impact on cell growth and cell proliferation [Laron 2001].

Several studies evaluated the role of the IGFs in breast carcinogenesis. Prolonged hyperinsulinemia can reduce the production of IGF-binding proteins which leads to increased levels of bioavailable IGF-1. IGF-1 signaling plays a role in breast carcinogenesis, acting in synergy with

the activities induced by the estrogen receptor [Kaaks 2014], through the promotion of cell proliferation and anti-apoptotic activities [Renehan 2006, Coughlin 2012].

Several epidemiological studies showed that higher levels of bioavailable IGF-1 were associating with higher tumor burdens in breast cancer cases [Christopoulos 2015]. Moreover, alterations in the IGF axis can increase breast cancer risk [Coughlin 2015]. Those alterations may worsen the outcome for breast cancer prognosis [Coughlin 2015].

4.1.1.3. IGF-binding proteins (IGFBPs) and Breast Cancer Risk

The IGF-binding proteins (IGFBPs) mediate the bioavailability of IGF-1 and its interaction with IGF-1R [Laron 2001, Guler 1989, LeRoth 1992]. The IGFBPs are a family of six binding proteins, which are found in the circulation, and in the extracellular fluids. The IGFBPs modulate the bioavailability of IGFs in the tissue [Laron 2001]. The IGFBPs bind to more than the 99% of the free IGFs [Laron 2001]. Among those, the IGFBP-3 binds to more than 75% of circulating IGF-1 [Burger 2005]. The IGFBP-3, which forms a ternary complex with IGF- 1 and with the insulin-like growth factor acid-labile subunit (IGFALS), circulates in plasma and aids to prolong the half-life of IGF-1 up to 12-15 h [Guler 1989]. In addition, phosphorylated IGFBP-3 alters the IGF-1/IGF-1R interaction [Burger 2005, LeRoith 1992, Hwa 1999, Lewitt 1994]. This can impair the mitogenic activities of IGF-1 [Burger 2005, LeRoith 1992, Hwa 1999, Lewitt 1994]. The IGFBP-degrading proteases can modulate the IGFBP-3 activities [Firth 2002]. This proteolysis can affect the IGF-dependent actions of the IGFBP-3 [Firth 2002]. In addition, the growth hormone (GH) controls the levels of IGFBP-3 [Lewitt 2014]. This regulation can be affected by factors like ethnicity, diet, gender, and age [Lewitt 2014].

Studies have shown that high circulating levels of IGFBP-3 are protective against breast carcinogenesis in premenopausal women [Hankinson 1998, Krajcik 2002]. In addition, IGFBP-3 exerted anti-proliferative and pro-apoptotic activities *in vitro* [Renehan 2004]. However, IGFBP-3 can also exert anti-apoptotic activities depending on the cellular microenvironment [Renehan 2004]. Further studies are needed to elucidate whether the intact, fragmented or the post-translationally modified IGFBP-3 affects breast cancer risk [Burger 2005]. More prospective studies with standardized assays are required to evaluate the role of circulating IGFBP-3 on breast cancer risk.

4.1.2. The Insulin/IGF Axis and Breast Density

Several studies have evaluated the association between insulin and breast density, with conflicting results, as some indicated no association while others showed a modest correlation [Table 28]. Hispanic women showed no association between percent density and fasting serum insulin or HOMA-IR. [Wolin 2007]. Similarly, Ahern et al found no association between the circulating levels of C-peptide and the mammographic percent density [Ahern 2013]. Diorio et al. showed an inverse association between high levels of fasting C-peptide and percent density ($r = -0.21$) [Diorio 2005]. However, this correlation lost significance after BMI and waist-hip ratio adjustment [Diorio 2005]. In contrast, Korean premenopausal women showed a positive correlation between mammographic percent density and HOMA-IR [Kim 2015]. Potential explanations for those discrepancies are the characteristics of the study cohort, such as the menopausal status, age, ethnic background, overall adiposity, and the presence of diabetes mellitus type 2 [Kim 2015].

Similar to that of insulin, the association between IGFs and breast density is not clear [Table 29]. A number of studies show associations between high levels of IGF-1, high mammographic percent density, and absolute dense areas [dos Santos Silva 2006, Izzo 2012, Diorio 2005, Diorio 2008, Byrne 2000]. In addition, most of the studies suggested that low levels of IGFBP-3 are related to high mammographic percent densities [Izzo 2012, Diorio 2005, Diorio 2008, Byrne 2000]. However, others have found no association between the IGFs and breast density measurements, or else, indicated that the relationship is limited to normal-weight women [Maskarinec 2003, Rice 2013]. Rinaldi et al. also found no significant associations between circulating levels of IGFs and mammographic percent density in a cohort of premenopausal Mexican women [Rinaldi 2014]. Distinct findings in the literature might be due to the criteria used for IGF-1 assessment because bioavailable IGF-1 can differ from that of the total IGF-1 in peripheral blood [Kucera 2015]. IGF-1 decrease with age, confounding the associations between IGF-1 and breast density for older women. [Laron 1996].

Table 29. Cross-Sectional Studies Evaluating the Association between Insulin and Breast Density Measurements.

First Author, Year	Sample size	Cohort	Findings
Wolin, 2007	95	Hispanic women between 40-77 years old	Insulin and HOMA are not associated with mammographic percent density
Diorio, 2005	1574	Pre- and postmenopausal women (Canada)	C-peptide inversely correlated with mammographic percent densities (r= -0.21) but this correlation was lost after adjusting for overall adiposity.
Ahern, 2013	2869	Pre- and postmenopausal women from the NHS I and NHS II	C-peptide is not associated with mammographic percent density
Kim, 2015	73974	Pre- and postmenopausal women	Insulin resistance (HOMA-IR) is positively associated with absolute dense areas in premenopausal women [OR = 1.29] and in postmenopausal women [OR = 1.44]

Table 30. Cross-Sectional Studies Evaluating the Associations between the IGFs and Breast Density Measurements.

First Author, Year	Sample size	Cohort	Findings
Diorio, 2005	1574	Pre- and postmenopausal women (Canada)	High levels of circulating IGF-1 and low levels of circulating IGFBP-3 are associated with high mammographic percent density.
Dos Santos Silva, 2006	456	Pre- and postmenopausal women from Guernsey study	High absolute dense areas are related to high serum levels of IGF-I, IGF-II, and IGFBP-3 but only in premenopausal women.
Izzo, 2012	341	Pre- and postmenopausal women	Crude association between breast density and plasma levels of IGF-1 and the molar ratio IGF-1/IGFBP-3.
Diorio, 2008	737	Premenopausal women	Different isoforms of circulating IGFBP-3 may bear a different relationship to mammographic percent density.
Byrne, 2000	65	Premenopausal women from the NHS	Mammographic percent density is correlated with high levels of IGF-1 and inversely correlated with IGFBP-3.
Rinaldi, 2014	593	Premenopausal Mexican women from ESMaestras Cohort	High levels of IGF-1 and the molar ratio IGF-1/IGFBP-3 are not related to mammographic percent density.
Maskarinec, 2013	240	Premenopausal women entering a nutritional intervention	Mammographic absolute dense areas were not associated with IGF-1 and were inversely related with IGFBP-3 ($r = -0.15$) in lean women (BMI < 25 kg/m^2)

4.1.3. Objectives

In this work, we aimed to evaluate the association between the insulin/IGF axis and the breast density measurements, acquired by fat-water MRI, in a cohort of overweight/obese premenopausal women with elements of metabolic syndrome. We hypothesized that higher levels of insulin or bioavailable IGF-1 were related to increased breast density in our cohort. Our first objective was to evaluate the relationship between insulin/IGF axis and anthropometric measurements of adiposity. The second objective was to determine the relationship between insulin/IGF axis and breast density measurements. Our third objective was to evaluate those relationships by ethnic groups.

4.2. Methodology

4.2.1. Analysis of Serum Levels of Insulin, IGF-1, and IGFBP-3

We measured insulin by using an enzyme-linked immunoassay by Calbiotech Inc. (California, USA). We measured IGF-1 and IGFBP-3 by using an enzyme-linked immunoassay by R&D (R&D Systems, Minneapolis, USA). These immunoassays have been validated and showed no cross-reactivity with other members of the IGF family. The calibrators used in the assays ranged between 6.25 uIU/ml to 50 uIU/ml for insulin, 0.375 ng/ml to 1.5 ng/ml for IGF-1, and 6.25 ng/ml to 25 ng/ml for IGFBP-3. For IGFBP-3, we diluted samples 1:100 in an assay buffer prior to the analysis. We analyzed serum samples and standards in duplicate for each immunoassay, and we averaged the measures. We inserted two quality controls in each batch of analysis. The intra-assay CVs were 11.5%, 21.7%, and 12.3%, and inter-assay CVs were 14.1%, 21.9%, and 12.2%, respectively, for insulin, IGF-1, and IGFBP-3. We calculated the molar ratio IGF-1/IGFBP-3 as an indicator of bioavailable IGF-1 [Kucera 2015]. The equivalents for conversions are 1 ng/mL IGF-1 = 0.13 nmol/L IGF-1, and 1 ng/mL IGFBP-3 = 0.035 nmol/L IGFBP-3. We calculated the HOMA-IR score [Homeostatic Model Assessment for Insulin Resistance] as fasting insulin (uIU/ml) x fasting glucose (mg/dl)/405 [Wallace 2004].

4.2.2. Statistical Analyses

We transformed non-normally distributed variables by using their logarithm. We performed correlation analysis between breast density measurements and the analytes. We used a t-test to compare the potential differences in the circulating levels of insulin/IGF axis by ethnicity. We used the R package version 3.4.1 [R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria] for correlation analyses and t-test. We used linear regression to explore the associations between breast density measurements and serum levels of insulin/IGF axis, which were adjusted for BMI, age, and reproductive variables. We performed regression analysis by using STATA version 15.1. StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC. We considered P-values equal or less than 0.05 as statistically significant.

4.3. Results

4.3.1. Baseline Characteristics of the Insulin/IGF Axis in the Study Cohort

We performed the analysis of circulating levels of the insulin/IGF axis from 106 participants with baseline breast fat-water MRI images. We summarized in Table 30 the circulating levels of insulin/IGF axis and the derived HOMA-IR and IGF-1/IGFBP-3 molar ratio, for the overall population and by ethnicity.

The overall cohort presented fasting levels of insulin of 7.01 ± 8.84 uIU/ml, HOMA-IR of 1.74 ± 2.32, fasting circulating levels of IGF-1 of 104.91 ± 45.97 ng/ml, fasting circulating levels of IGFBP-3 of 2129.96 ± 593.92 ng/ml, and the IGF-1/IGFBP-3 molar ratio of 0.19 ± 0.09. The fasting levels of insulin, HOMA-IR, the fasting circulating levels of IGF-1 or the molar ratio did not differ by ethnic group. The fasting circulating levels of IGFBP-3 tended to be lower in Hispanics than in Non-Hispanics but did not reach statistical significance [Table 30].

Table 31. Means (SD) of the Circulating Metabolites of the Insulin/IGF Axis in the Study Cohort (N = 106).

Variable [a]	Overall Cohort (N=106)	Hispanics (n=41)	Non-Hispanics (n=64)	P-value [b]
Insulin (uIU/mL)				
Mean (SD)	7.01 (8.84)	8.63 (11.17)	5.99 (6.94)	0.1368
Log-transformed	1.31 (1.18)	1.49 (1.24)	1.19 (1.14)	0.2075
HOMA-IR				
Mean (SD)	1.74 (2.32)	2.19 (3.07)	1.46(1.65)	0.1175
Log-transformed	-0.12 (1.22)	0.04 (1.29)	-0.24 (1.18)	0.2386
IGF-1 (ng/mL)				
Mean (SD)	104.91 (45.97)	106.18 (54.84)	104.74 (39.80)	0.8769
Log-transformed	4.56 (0.41)	4.54 (0.48)	4.58 (0.36)	0.6667
IGFBP-3 (ng/mL)				
Mean (SD)	2,129.96 (593.92)	2,010.50 (499.92)	2,225.28 (622.66)	0.0661
Log-transformed	7.62 (0.29)	7.57 (0.28)	7.67(0.27)	0.0716
Molar Ratio IGF-1/IGFBP-3				
Mean (SD)	0.19 (0.09)	0.20 (0.12)	0.18 (0.06)	0.1786
Log-transformed	-1.74 (0.42)	-1.70 (0.49)	-1.77 (0.36)	0.4397

(a) Variables followed a non-normal distribution and were log-transformed. Non-transformed values are shown by means of comparison with normal values as reported for a lean population.
(b) Two sample t-test was conducted to assess differences between Hispanics and Non-Hispanics.

4.3.2. Regression Analysis of the Insulin/IGF Axis with the Anthropometric Measurements of Adiposity in the Study Cohort

We used regression analysis to evaluate the relationship between insulin/IGF axis and anthropometric measures in the overall study cohort to allow for the adjustment of potential confounders [Table 31]. The partially adjusted model included the adjustment for BMI. The fully adjusted model included the anthropometric measurements of adiposity, age at baseline visit, age at menarche, menstrual phase, and age at first live birth of a child [Table 31].

The Insulin/IGF Axis and BMI

We found the fasting serum insulin to be related to BMI in the unadjusted model (β = 2.9996, p-value = 0.0002) and this relationship remained significant after adjusting for ethnicity, waist circumference, waist-hip ratio, age, and other reproductive factors (β = 2.6183, p-value = 0.0412). Similarly, the surrogate for insulin resistance, HOMA-IR, showed to be related to the BMI (β = 3.1052, p-value = 0.0002) and remained significant after adjusting for ethnicity, waist circumference, waist-hip ratio, age, and reproductive factors (β = 2.6312, p-value = 0.0490). We found the fasting serum IGF-1, IGFBP-3, or the molar ratio IGF-1/IGFBP-3 not related to BMI in any of the models [Table 31].

The Insulin/IGF Axis and Waist Circumference

We showed that the fasting serum insulin was related to the waist circumference in the unadjusted model (β = 3.3750, p-value = 0.0065). This relationship lost significance after adjusting for BMI. However, insulin was borderline related to the waist circumference in the fully adjusted model (β = -5.0129, p-value = 0.0907). Similar to that of insulin, HOMA-IR was related to the waist circumference in the unadjusted model (β = 3.5397, p-value = 0.0059) but this relationship lost significance after adjusting for BMI. Like insulin, HOMA-IR showed a borderline relationship to the waist circumference in the fully adjusted model (β = -5.3490, p-value = 0.0827). The fasting serum IGF-1 was related to the waist circumference but only in the fully adjusted model (β = -2.7317, p-value = 0.0044). Likewise, the fasting serum IGFBP-3 was borderline related to the waist circumference in the fully adjusted model (β = -1.2927, p-value = 0.0782). There were no statistically significant relationships between the IGF- 1/IGFBP-3 molar ratio and the waist circumference [Table 31].

The Insulin/IGF Axis and Waist-Hip Ratio

As shown in Table 31, there was a trend for an association between fasting serum insulin and the waist-hip ratio in the unadjusted model. This relationship lost significance when BMI is added as a covariate, but it showed statistical significance for the fully adjusted model (β = 6.3799, p-value = 0.0264). Similar to that of insulin, HOMA-IR was borderline related to the waist-hip ratio (β = 2.97, p-value = 0.0701), but this relationship lost statistical significance by the effects of overall adiposity. The waist-hip ratio, however, was related to the HOMA-IR for the fully adjusted model (β = 6.9865, p- value = 0.0196). The fasting serum IGF-1 showed a relationship with the waist-hip ratio but only for the fully adjusted model (β = 1.9740, p-value = 0.0304). Similarly, the fasting serum IGFBP-3 showed a borderline relationship with the waist-hip ratio in the fully adjusted model (β = 1.3041, p-value = 0.0653). There were no statistically significant relationships between the IGF-1/IGFBP- 3 molar ratio and the waist-hip ratio [Table 31].

Table 32. Linear regression analysis comparing the insulin/IGF axis [a] with the anthropometric measurements of high adiposity [a] in the study cohort (N = 106).

Models of Explicative Variables	Insulin/IGF Axis [a] (β, 95% CI, p-value)				
	Insulin	**HOMA-IR**	**IGF-1**	**IGFBP-3**	**Molar Ratio IGF-1/IGFBP-3**
BMI					
Unadjusted model					
β	2.9996	3.1052	-0.1198	0.0849	-0.2047
95% CI	(1.4500, 4.5491)	(1.4978, 4.7126)	(-0.6942, 0.4545)	(-0.3203, 0.4900)	(-0.7913, 0.3820)
p-value	0.0002	0.0002	0.6799	0.6788	0.4906
Fully adjusted model [c]					
β	2.6183	2.6312	0.0498	0.0677	-0.0179
95% CI	(0.1078, 5.1287)	(0.0155, 5.2509)	(-0.7460, 0.8455)	(-0.5471, 0.6825)	(-0.8228, 0.7870)
p-value	0.0412	0.0490	0.9012	0.8270	0.9647
Waist circumference					
Unadjusted model					
β	3.3750	3.5397	-0.6176	-0.0691	-0.5484
95% CI	(0.9640, 5.7861)	(1.0414, 6.0380)	(-1.4767, 0.2416)	(-0.6809, 0.5427)	(-1.4294, 0.3325)
p-value	0.0065	0.0059	0.1570	0.8232	0.2198
Partially adjusted model [b]					
β	0.2954	0.3991	-0.9952	-0.3269	-0.6682
95% CI	(-3.0584, 3.6492)	(-3.0795, 3.8777)	(-2.2231, 0.2328)	(-1.2016, 0.5477)	(-1.9314, 0.5949)
p-value	0.8617	0.8204	0.1111	0.4602	0.2965
Fully adjusted model [c]					
β	-5.0129	-5.3490	-2.7317	-1.2927	-1.4390
95% CI	(-10.8407, 0.8148)	(-11.4089, 0.7109)	(-4.5820, -0.8813)	(-2.7348, 0.1494)	(-3.3645, 0.4865)
p-value	0.0907	0.0827	0.0044	0.0782	0.1407
Waist-hip ratio					
Unadjusted model					
β	2.6944	2.9700	0.4347	0.1946	0.2401
95% CI	(-0.4143, 5.8032)	(-0.2480, 6.1880)	(-0.6565, 1.5259)	(-0.5766, 0.9657)	(-0.8785, 1.3587)
p-value	0.0886	0.0701	0.4314	0.6179	0.6712
Partially adjusted model [b]					
β	2.1663	2.4250	0.4608	0.1807	0.2801
95% CI	(-0.7823, 5.1149)	(-0.6285, 5.4785)	(-0.6396, 1.5612)	(-0.5973, 0.9588)	(-0.8463, 1.4065)
p-value	0.1481	0.1183	0.4082	0.6460	0.6230
Fully adjusted model [c]					
β	6.3799	6.9865	1.9740	1.3041	0.6699
95% CI	(0.7682, 11.9915)	(1.1513, 12.8216)	(0.1923, 3.7557)	(-0.0845, 2.6928)	(-1.1842, 2.5239)
p-value	0.0264	0.0196	0.0304	0.0653	0.4738

(a) Variables followed a non-normal distribution and were log base 10 transformed.

(b) The partially adjusted model includes BMI.

(c) The fully adjusted model includes anthropometric measures (BMI, waist circumference, waist-hip ratio) ethnicity, age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

4.3.3. Association between the Insulin/IGF Axis and Breast Density Measurements

4.3.3.1. Correlations between the Insulin/IGF Axis and Breast Density Measurements

We determined the potential correlation between the insulin/IGF axis and breast density measurements in the overall study cohort (N = 106). Table 32 shows there was a trend for an inverse correlation between the fasting serum insulin levels and the percent density (r = -0.18, p-value = 0.058, Figure 17). The absolute dense volume was inversely correlated with insulin (r = -0.21, p-value = 0.029, Figure 17). The HOMA-IR was also inversely correlated with the percent density (r = -0.20, p-value = 0.040, Figure 18) and with the absolute dense volume (r = -0.22, p-value = 0.020, Figure 18). We observed no other statistically significant correlations for the overall study cohort [Table 32, Figures 19, 20 and 21].

We conducted further correlation analysis by ethnic group. Hispanics present borderline correlations for the insulin with the percent density (r = -0.29, p-value = 0.062, See Appendix A, Fig. A9), for the HOMA-IR with the absolute dense volume (r = -0.30, p-value = 0.056, See Appendix A, Fig. A10), and for the IGFBP-3 with the absolute dense volume (r = -0.30, p-value = 0.053, See Appendix A, Fig. A12) [Table 33]. We observed a statistically significant correlation for the HOMA-IR with the percent density (r = -0.33, p-value = 0.033, See Appendix A, Fig. A10) in the Hispanic cohort [Table 33]. We observed no other statistically significant correlations for the Hispanic cohort [Table 33, See Appendix A, Fig. A11 and A13]. There were no statistically significant correlations observed for the Non-Hispanics [Table 34, See Appendix A, Fig. A9 to A13].

Table 33. Pearson Correlations [a] between the Breast Density Measurements and the Insulin/IGF Axis [a] (N = 106).

Breast Density Measurement [a]	**Insulin** [a]	**HOMA-IR** [a]	**IGF-1** [a]	**IGFBP-3** [a]	**Molar ratio** [a]
Percent Density	-0.18 (p=0.058)	-0.20 (p=0.040)	0.00 (p=0.967)	-0.15 (p=0.129)	0.10 (p=0.316)
Absolute Dense Volume	-0.21 (p=0.029)	-0.22 (p=0.020)	-0.03 (p=0.787)	-0.12 (p=0.238)	0.05 (p=0.584)
Non-Dense Volume	0.01 (p=0.916)	0.02 (p=0.839)	-0.02 (p=0.850)	0.13 (p=0.194)	-0.11 (p=0.281)
Total Breast Volume	-0.01 (p=0.918)	0.00 (p=0.962)	-0.04 (p=0.686)	0.09 (p=0.359)	-0.10 (p=0.304)

(a) Variables were non-normally distributed and were log base 10 transformed.

Table 34. Pearson Correlations [a] between the Breast Density Measurements and the Insulin/IGF Axis [a] in Hispanics (n = 41).

Breast Density Measurement [a]	**Insulin** [a]	**HOMA-IR** [a]	**IGF-1** [a]	**IGFBP-3** [a]	**Molar ratio** [a]
Percent Density	-0.29 (p=0.0624)	-0.33 (p=0.0331)	0.00 (p=0.994)	-0.25 (p=0.1077)	0.15 (p=0.3625)
Absolute Dense Volume	-0.25 (p=0.1131)	-0.30 (p=0.0561)	-0.10 (p=0.5283)	-0.30 (p=0.0534)	0.08 (p=0.6309)
Non-Dense Volume	0.17 (p=0.2951)	0.17 (p=0.2876)	-0.14 (p=0.3895)	0.05 (p=0.7590)	-0.16 (p=0.3115)
Total Breast Volume	0.15 (p=0.3458)	0.14 (p=0.3753)	-0.19 (p=0.2276)	-0.03 (p=0.8329)	-0.17 (p=0.2975)

(a) Variables were non-normally distributed and were log base 10 transformed.

Table 35. Pearson Correlations [a] between the Breast Density Measurements and the Insulin/IGF Axis [a] in Non-Hispanics (n=64).

Breast Density Measurement [a]	**Insulin** [a]	**HOMA-IR** [a]	**IGF-1** [a]	**IGFBP-3** [a]	**Molar ratio** [a]
Percent Density	-0.11 (p=0.403)	-0.11 (p=0.403)	-0.04 (p=0.728)	-0.19 (p=0.138)	0.10 (p=0.454)
Absolute Dense Volume	-0.17 (p=0.182)	-0.16 (p=0.202)	-0.01 (p=0.925)	-0.13 (p=0.294)	0.09 (p=0.495)
Non-Dense Volume	-0.07 (p=0.594)	-0.06 (p=0.664)	0.07 (p=0.578)	0.16 (p=0.200)	-0.05 (p=0.694)
Total Breast Volume	-0.08 (p=0.519)	-0.07 (p=0.582)	0.06 (p=0.619)	0.13 (p=0.304)	-0.03 (p=0.789)

(a) Variables were non-normally distributed and were log base 10 transformed.

Figure 16. The Circulating Fasting Levels of Insulin[a] and Breast Density Measurements[a] (N = 106).

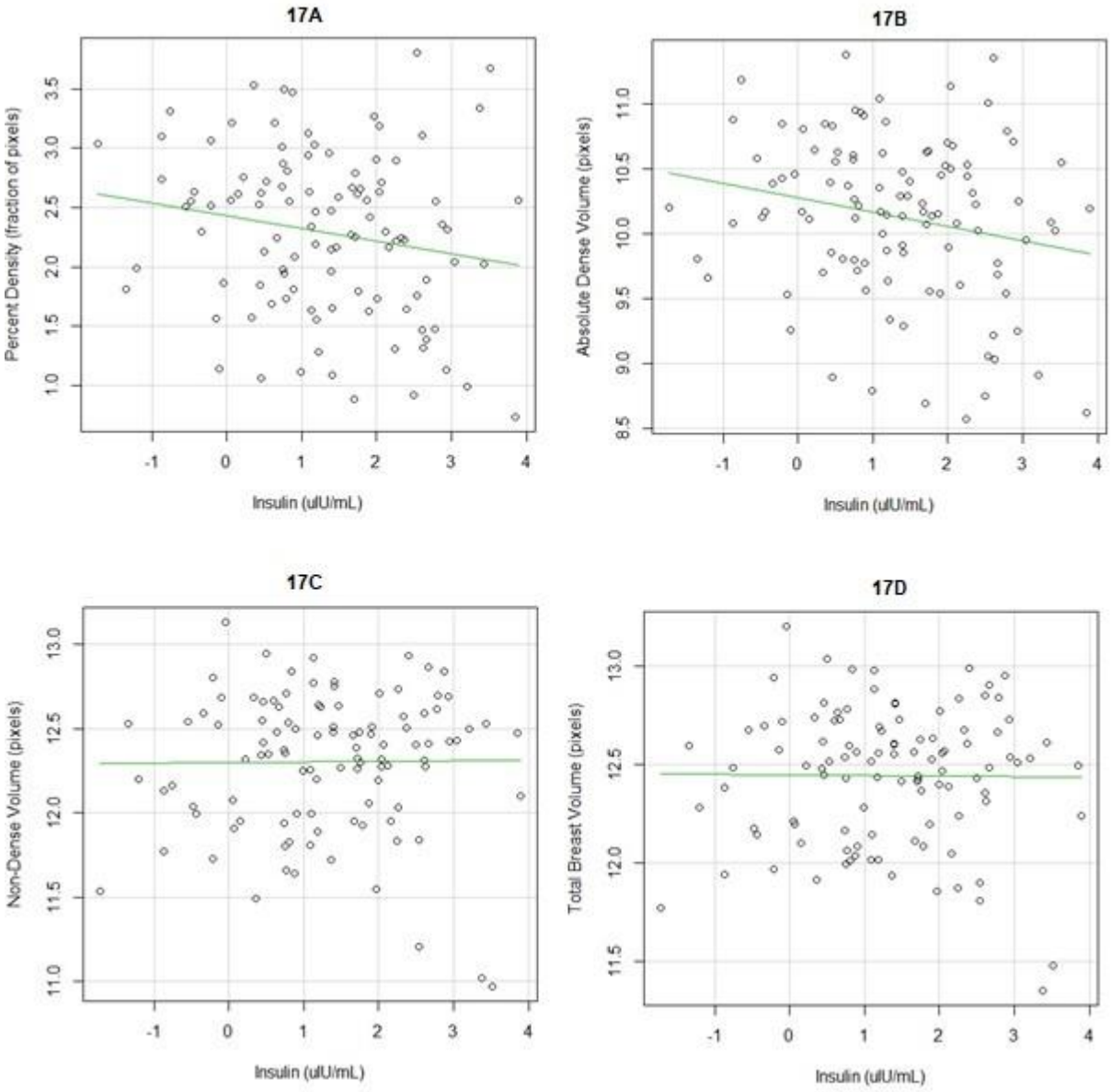


Figure 17A. Circulating fasting serum insulin (uIU/ml) and the percent density (fraction) in the overall study population.
Figure 17B. Circulating fasting serum insulin (uIU/ml) and the absolute dense volume (pixels) in the overall study population.
Figure 17C. Circulating fasting serum insulin (uIU/ml) and the non-dense volume (pixels) in the overall study population.
Figure 17D. Circulating fasting serum insulin (uIU/ml) and the total breast volume (pixels) in the overall study population.
(a) Variables followed a non-normal distribution and were base 10 log-transformed.

Figure 17. The Circulating Fasting Levels of HOMA-IR [a] and Breast Density Measurements[a] (N = 106).

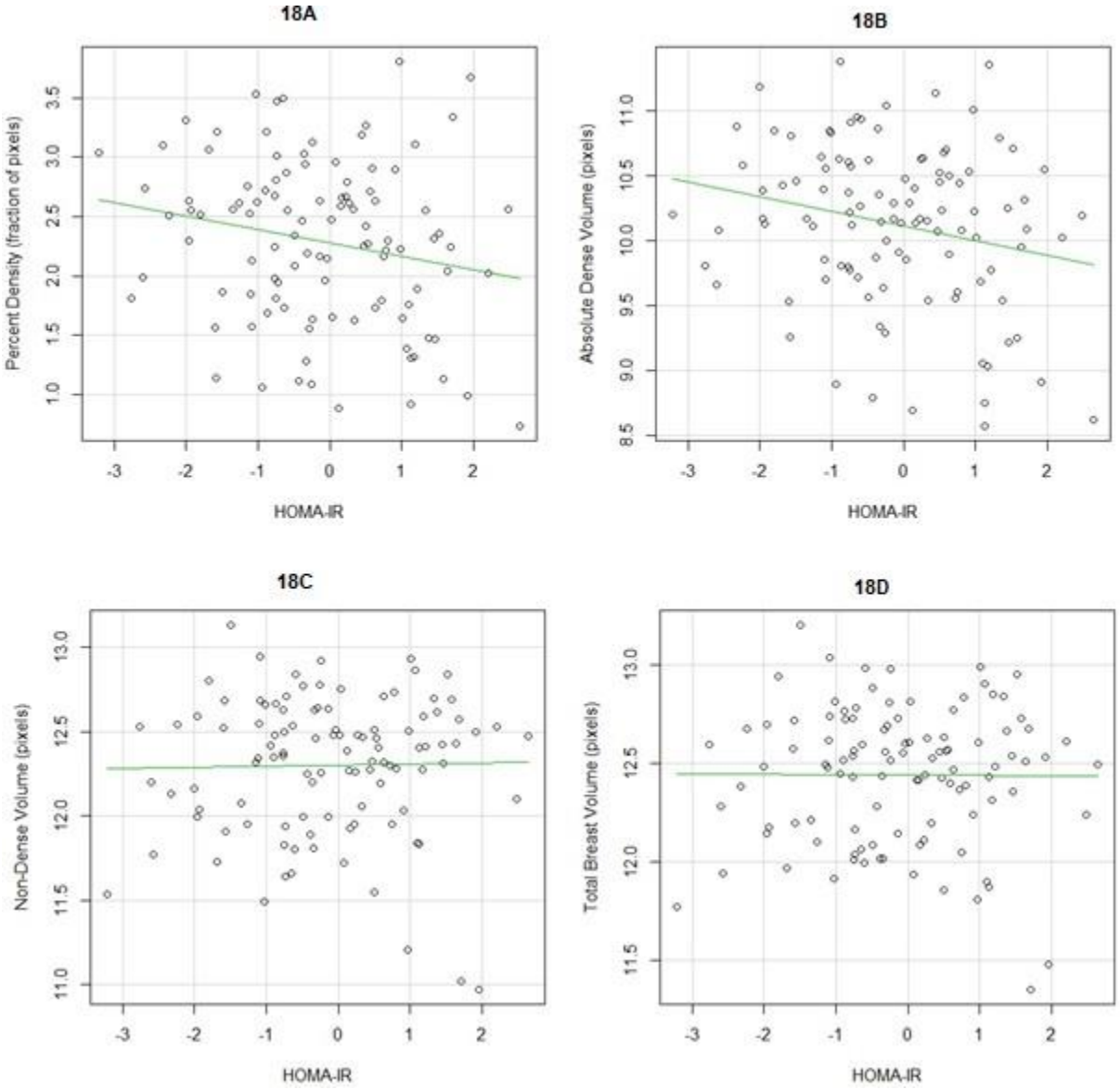


Figure 18A. The HOMA-IR and the percent density (fraction) in the overall study population.
Figure 18B. The HOMA-IR and the absolute dense volume (pixel units) in the overall study population.
Figure 18C. The HOMA-IR and the non-dense volume (pixel units) in the overall study population.
Figure 18D. The HOMA-IR and the total breast volume (pixel units) in the overall study population.
(a) Variables followed a non-normal distribution and were base 10 log-transformed.

Figure 18. The Circulating Fasting Levels of IGF-1[a] and Breast Density Measurements[a] (N = 106).

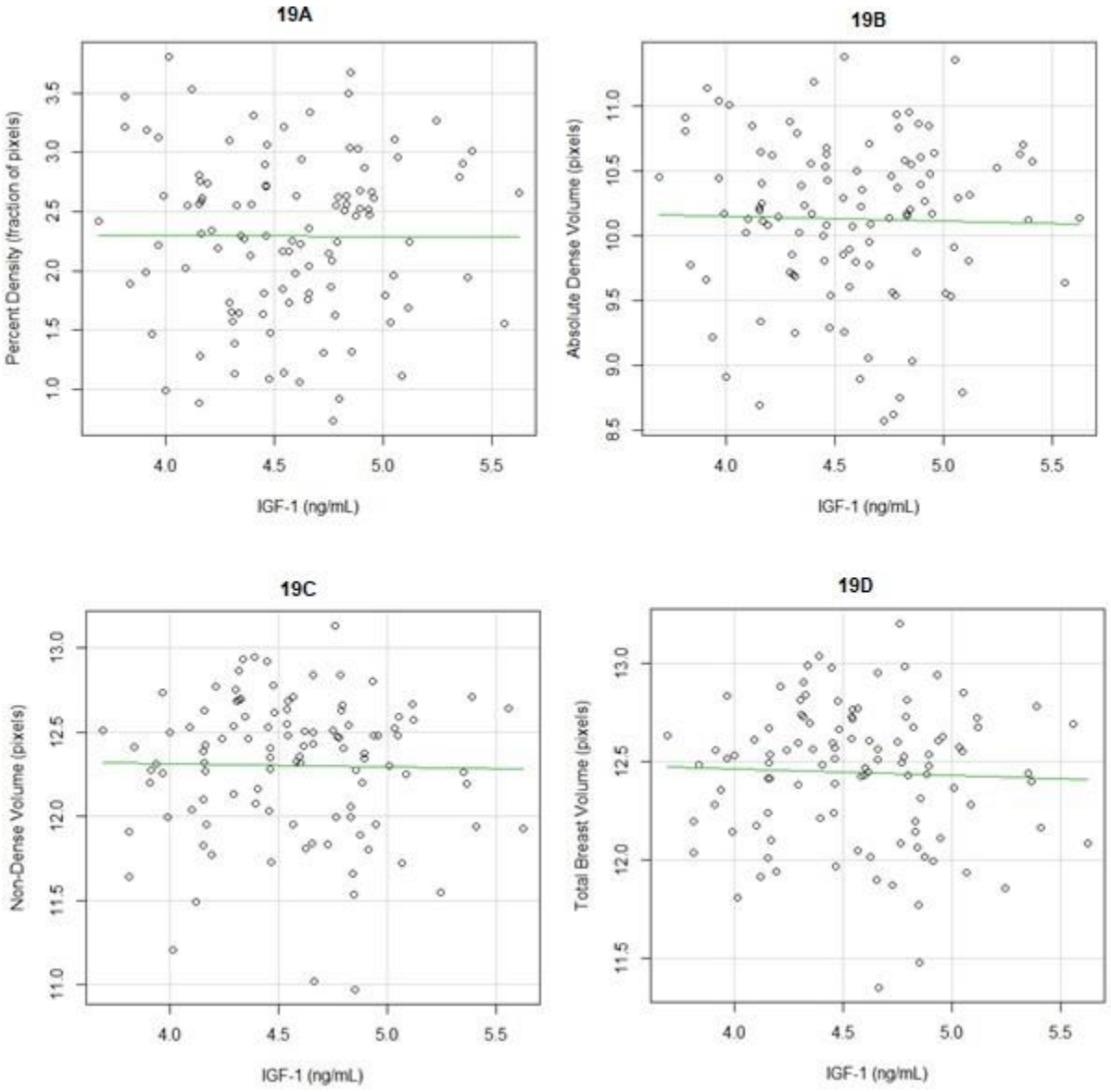


Figure 19A. Circulating fasting IGF-1 (ng/ml) and the percent density (fraction) in the overall study population.
Figure 19B. Circulating fasting serum IGF-1 (ng/ml) and the absolute dense volume (pixel units) in the overall study population.
Figure 19C. Circulating fasting serum IGF-1 (ng/ml) and the non-dense volume (pixel units) in the overall study population.
Figure 19D. Circulating fasting serum IGF-1 (ng/ml) and the total breast volume (pixel units) in the overall study population.
(a) Variables followed a non-normal distribution and were log base 10 transformed.

Figure 19. The Circulating Fasting Levels of IGFBP-3[a] and Breast Density Measurements[a] (N = 106).

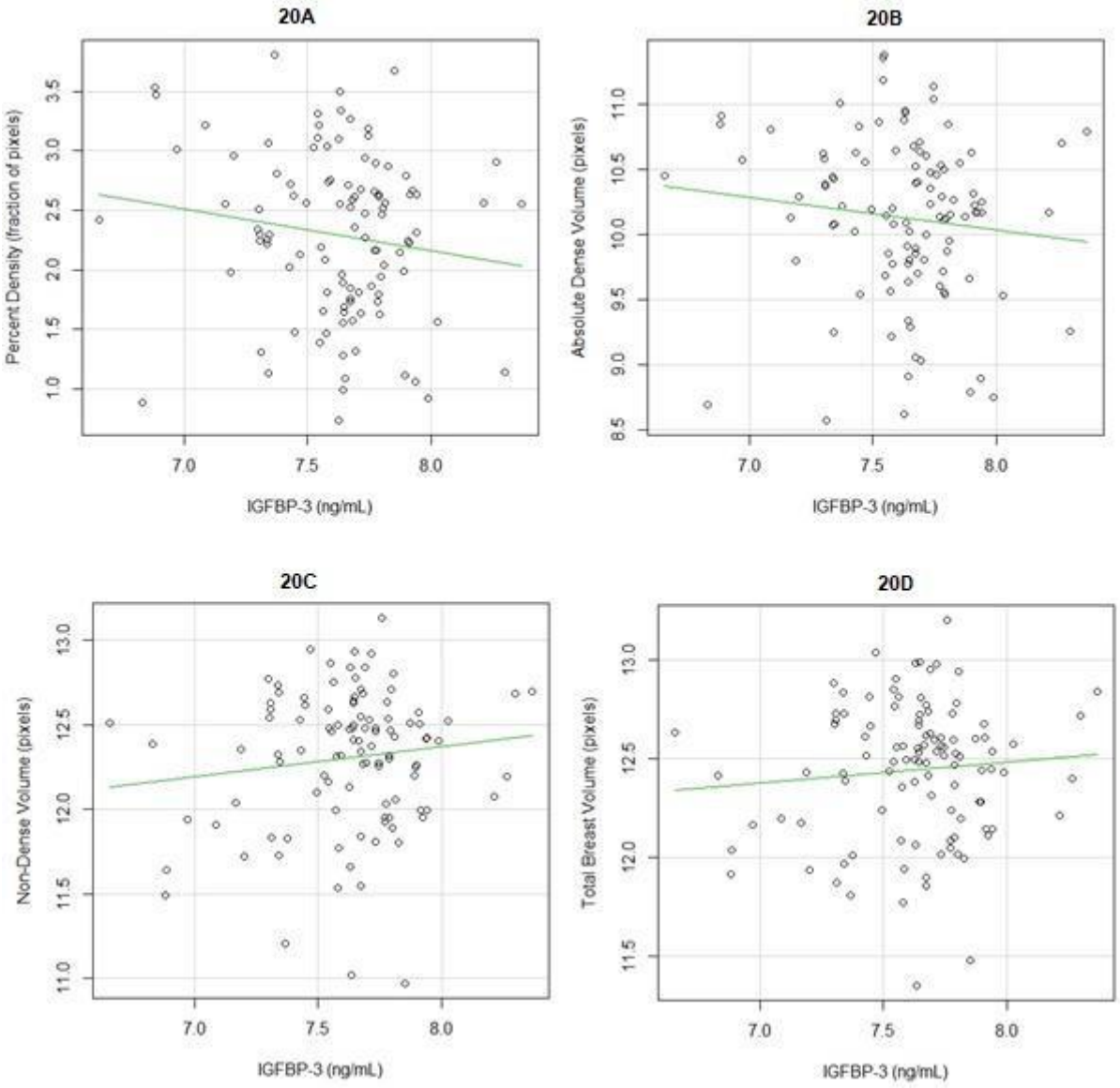


Figure 20A. Circulating fasting IGFBP-3 (ng/ml) and the percent density (fraction) in the overall study population.
Figure 20B. Circulating fasting serum IGFBP-3 (ng/ml) and the absolute dense volume (pixel units) in the overall study population.
Figure 20C. Circulating fasting serum IGFBP-3 (ng/ml) and the non-dense volume (pixel units) in the overall study population.
Figure 20D. Circulating fasting serum IGFBP-3 (ng/ml) and the total breast volume (pixel units) in the overall study population.
(a) Variables followed a non-normal distribution and were log base 10 transformed.

Figure 20. The molar ratio IGF-1/IGFBP-3[a] and Breast Density Measurements[a] (N = 106).

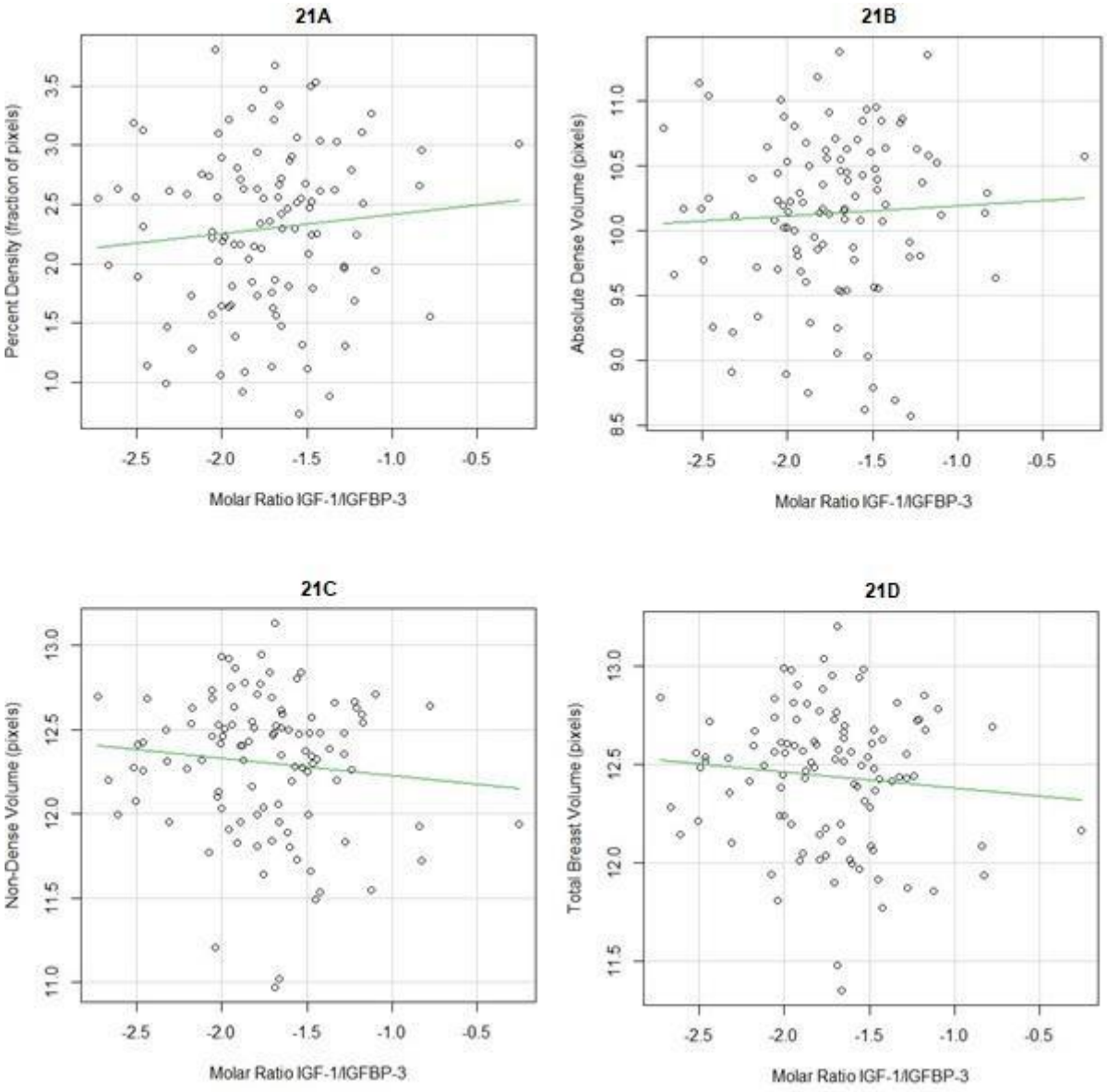


Figure 21A. The molar ratio IGF-1/IGFBP-3 and the percent density (fraction) in the overall study population.
Figure 21B. The molar ratio IGF-1/IGFBP-3 and the absolute dense volume (pixel units) in the overall study population.
Figure 21C. The molar ratio IGF-1/IGFBP-3 and the non-dense volume (pixel units) in the overall study population.
Figure 21D. The molar ratio IGF-1/IGFBP-3 and the total breast volume (pixel units) in the overall study population.
(a) Variables followed a non-normal distribution and were log base 10 transformed.

4.3.3.2. Regression Analysis of the Insulin/IGF Axis with Breast Density Measurements

4.3.3.2.1. The Insulin/IGF Axis and Breast Density Measurements in the Overall Study Cohort

We conducted regression analysis to evaluate the relationship between the insulin/IGF axis and the breast density measurements in the overall study cohort. We adjusted for potential confounders [Table 35]. We included BMI as a variable for the partially adjusted model. We included anthropometric measurements, age at baseline visit, age at menarche, menstrual phase, and age at first live birth of a child in the fully adjusted model.

Percent Density and the Insulin/IGF Axis

The percent density was borderline related to the fasting serum insulin levels in the unadjusted model (β = -0.1079, p-value = 0.0580). However, this relationship lost significance after adjustment for covariates in the partially and fully adjusted models. The percent density was related to HOMA-IR in the unadjusted model (β = -0.1122, p-value = 0.0406). However, this relationship lost significance after BMI adjustment. The percent density was borderline related to HOMA-IR in the fully adjusted model (β = -0.0934, p-value = 0.0929). In addition, the percent density was positively related to the IGF-1/IGFBP-3 molar ratio but only for the fully adjusted model (β = 0.3694, p-value = 0.0403). The percent density had no other statistically significant relationships with the insulin/IGF axis [Table 35].

Absolute Dense Volume and the Insulin/IGF Axis

The absolute dense volume was inversely related to the fasting serum insulin in the unadjusted (β = -0.1108, p-value = 0.0295) and in the fully adjusted models (β = -0.1396, p-value = 0.0169). However, this relationship lost significance after BMI adjustment. In addition, the absolute dense volume was inversely related to the HOMA-IR in the unadjusted model (β = -0.1135, p-value = 0.0205). The absolute dense volume was borderline related to HOMA-IR after BMI adjustment (β = -0.0874, p-value = 0.0913). However, this relationship was statistically significant in the fully adjusted model (β = -0.1438, p-value = 0.01). The absolute dense volume showed no other relationships with measures of IGF axis [Table 35].

Non-Dense Volume and the Insulin/IGF Axis

The non-dense volume was borderline related to the fasting serum insulin but only when the BMI was considered (β = -0.0620, p-value = 0.0526). Similarly, the non-dense volume was borderline related to HOMA-IR in the partially adjusted model (β = -0.0559, p-value = 0.0698). The non-dense volume showed no other statistically significant relationships with the insulin/IGF axis [Table 35].

Total Breast Volume and the Insulin/IGF Axis

The total breast volume was related to the fasting serum insulin after adjusting for adiposity (β = -0.0555, p-value = 0.0416). In addition, the total breast volume had a borderline relationship with insulin after adjusting for age, waist circumference, and reproductive factors (β = -0.0538, p-value = 0.0841). Similarly, the total breast volume was related to the HOMA-IR after BMI adjustment (β = -0.0516, p = 0.0493). The total breast volume was borderline related to HOMA-IR in the fully adjusted model (β = - 0.0504, p-value = 0.0914). Other relationships were not considered statistically significant [Table 35].

Table 36. Linear regression analysis comparing the insulin/IGF axis [a] with the breast density measurements [a] in the study cohort (N = 106).

Models of Explicative Variables [a]	Breast Density Measurements [a] (β, 95% CI, p-value)			
	Percent Density	**Absolute Dense Volume**	**Non-Dense Volume**	**Total Breast Volume**
Insulin				
Unadjusted model				
β	-0.1079	-0.1108	0.0036	-0.0029
95% CI	(-0.2195, 0.0037)	(-0.2103, -0.0113)	(-0.0639, 0.0710)	(-0.0595, 0.0537)
p-value	0.0580	0.0295	0.9167	0.9188
Partially adjusted model [b]				
β	-0.0272	-0.0827	-0.0620	-0.0555
95% CI	(-0.1384, 0.0840)	(-0.1885, 0.0230)	(-0.1246, 0.0007)	(-0.1088, -0.0021)
p-value	0.6281	0.1238	0.0526	0.0416
Fully adjusted model [c]				
β	-0.0859	-0.1396	-0.0566	-0.0538
95% CI	(-0.2005, 0.0287)	(-0.2535, -0.0258)	(-0.1268, 0.0137)	(-0.1149, 0.0074)
p-value	0.1397	0.0169	0.1129	0.0841
HOMA-IR				
Unadjusted model				
β	-0.1122	-0.1135	0.0067	-0.0013
95% CI	(-0.2195, -0.0049)	(-0.2092, -0.0178)	(-0.0584, 0.0717)	(-0.0559, 0.0533)
p-value	0.0406	0.0205	0.8395	0.9629
Partially adjusted model [b]				
β	-0.0358	-0.0874	-0.0559	-0.0516
95% CI	(-0.1458, 0.0713)	(-0.1891, 0.0143)	(-0.1165, 0.0046)	(-0.1031, -0.0002)
p-value	0.5093	0.0913	0.0698	0.0493
Fully adjusted model [c]				
β	-0.0934	-0.1438	-0.0511	-0.0504
95% CI	(-0.2028, 0.0159)	(-0.2522, -0.0354)	(-0.1186, 0.0163)	(-0.1091, 0.0083)
p-value	0.0929	0.0100	0.1354	0.0914
IGF-1				
Unadjusted model				
β	-0.0068	-0.0400	-0.0185	-0.0332
95% CI	(-0.3339, 0.3203)	(-0.3332, 0.2532)	(-0.2127, 0.1758)	(-0.1961, 0.1297)
p-value	0.9672	0.7873	0.8509	0.6869
Partially adjusted model [b]				
β	-0.0347	-0.0528	0.0007	-0.0181
95% CI	(-0.3350, 0.2656)	(-0.3412, 0.2356)	(-0.1715, 0.1729)	(-0.1649, 0.1287)
p-value	0.8192	0.7173	0.9935	0.8073
Fully adjusted model [c]				
β	0.1491	0.0953	-0.0556	-0.0539
95% CI	(-0.2161, 0.5144)	(-0.2774, 0.4679)	(-0.2807, 0.1695)	(-0.2505, 0.1427)
p-value	0.4185	0.6121	0.6243	0.5865
IGFBP-3				
Unadjusted model				

β	-0.3536	-0.2468	0.1799	0.1068
95% CI	(-0.8122, 0.1050)	(-0.6598, 0.1663)	(-0.0934, 0.4531)	(-0.1234, 0.3370)
p-value	0.1293	0.2388	0.1946	0.3597
Partially adjusted model [b]				
β	-0.3145	-0.2291	0.1529	0.0854
95% CI	(-0.7358, 0.1068)	(-0.6357, 0.1776)	(-0.0894, 0.3951)	(-0.1221, 0.2929)
p-value	0.1418	0.2665	0.2136	0.4162
Fully adjusted model [c]				
β	-0.3832	-0.3345	0.1256	0.0488
95% CI	(-0.8498, 0.0833)	(-0.8114, 0.1425)	(-0.1648, 0.4160)	(-0.2059, 0.3035)
p-value	0.1059	0.1666	0.3915	0.7039
Molar Ratio IGF-1/IGFBP-3				
Unadjusted model				
β	0.1616	0.0791	-0.1032	-0.0825
95% CI	(-0.1566, 0.4799)	(-0.2072, 0.3655)	(-0.2921, 0.0857)	(-0.2411, 0.0761)
p-value	0.3162	0.5849	0.2812	0.3045
Partially adjusted model [b]				
β	0.1167	0.0586	-0.0722	-0.0581
95% CI	(-0.1764, 0.4099)	(-0.2237, 0.3409)	(-0.2402, 0.0957)	(-0.2014, 0.0852)
p-value	0.4316	0.6812	0.3957	0.4234
Fully adjusted model [c]				
β	0.3694	0.2882	-0.1276	-0.0811
95% CI	(0.0168, 0.7219)	(-0.0748, 0.6513)	(-0.3485, 0.0933)	(-0.2749, 0.1127)
p-value	0.0403	0.1179	0.2536	0.4070

(a) Variables followed a non-normal distribution and were log base 10 transformed.
(b) The partially adjusted model included BMI.
(c) The fully adjusted model included anthropometric measures (BMI, waist circumference, waist-hip ratio), age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

4.3.3.2.2. Regression Analysis of the Insulin/IGF Axis with Breast Density Measurements by Ethnicity

4.3.3.2.2.1. The Insulin/IGF Axis and Breast Density Measurements in Hispanics

We used regression models to investigate the relationship between the insulin/IGF axis and the breast density measurements in the Hispanic cohort (n=41). Partial adjustment included overall adiposity (BMI). Fully adjustment included anthropometric measures (BMI and waist circumference), age (age at baseline visit), and reproductive factors (age at menarche, menstrual phases, age at first live birth of a child) [Table 36].

Percent Density and the Insulin/IGF Axis in Hispanics

The percent density showed a borderline relationship with the fasting serum levels of insulin for the unadjusted model only (β= -0.1661, p-value= 0.0624). In addition, the percent density was related to the HOMA-IR in the unadjusted model (β= -0.1810, p-value= 0.0331) and was borderline related in the fully adjusted model (β= -0.1752, p-value= 0.0757). There was a borderline relationship between the percent density and the fasting serum IGFBP-3 after BMI adjustment (β= -0.6687, p-value= 0.0718). The percent density showed no other significant relationships with the insulin/IGF axis in the Hispanic cohort [Table 36].

Absolute Dense Volume and the Insulin/IGF Axis in Hispanics

The absolute dense volume was borderline related to HOMA-IR in the unadjusted (β= -0.1453, p-value= 0.0561), the partially adjusted (β= -0.1509, p-value= 0.0801), and the fully adjusted models (β= -0.1772, p-value= 0.0709). In addition, the absolute dense volume showed a borderline relationship with the fasting serum IGFBP-3 in the unadjusted model (β= -0.6645, p- value= 0.0534). This relationship became statistically significant after BMI adjustment (β= -0.6794, p-value= 0.0497). The absolute dense volume showed no other statistically significant relationships with the insulin/IGF axis in the Hispanic cohort [Table 36].

Non-Dense Volume and the Insulin/IGF Axis in Hispanics

The non-dense volume was not related to the insulin/IGF axis in the Hispanic cohort [Table 36].

Total Breast Volume and the Insulin/IGF Axis in Hispanics

The total breast volume, similar to that of the non-dense volume, was not related to the insulin/IGF axis in the Hispanic cohort [Table 36].

Table 37. Linear regression analysis comparing the insulin/IGF axis [a] with the breast density measurements [a] in Hispanics (n = 41).

Models of Explicative Variables [a]	Breast Density Measurements [a] (β, 95% CI, p-value)			
	Percent Density	**Absolute Dense Volume**	**Non-Dense Volume**	**Total Breast Volume**
Insulin				
Unadjusted model				
β	-0.1661	-0.1266	0.0540	0.0395
95% CI	(-0.3413, 0.0090)	(-0.2846, 0.0314)	(-0.0490, 0.1570)	(-0.0442, 0.1233)
p-value	0.0624	0.1131	0.2951	0.3458
Partially adjusted model [b]				
β	-0.1029	-0.1266	-0.0214	-0.0237
95% CI	(-0.2983, 0.0925)	(-0.3074, 0.0542)	(-0.1265, 0.0836)	(-0.1085, 0.0610)
p-value	0.2930	0.1643	0.6818	0.5742
Fully adjusted model [c]				
β	-0.1613	-0.1586	0.0035	0.0027
95% CI	(-0.3692, 0.0465)	(-0.3659, 0.0486)	(-0.1114, 0.1184)	(-0.0928, 0.0982)
p-value	0.1217	0.1267	0.9506	0.9535
HOMA-IR				
Unadjusted model				
β	-0.1810	-0.1453	0.0526	0.0357
95% CI	(-0.3467, -0.0153)	(-0.2946, 0.0040)	(-0.0461, 0.1514)	(-0.0448, 0.1161)
p-value	0.0331	0.0561	0.2876	0.3753
Partially adjusted model [b]				
β	-0.1270	-0.1509	-0.0177	-0.0240
95% CI	(-0.3115, 0.0576)	(-0.3209, 0.0190)	(-0.1179, 0.0826)	(-0.1048, 0.0568)
p-value	0.1718	0.0801	0.7230	0.5515
Fully adjusted model [c]				
β	-0.1752	-0.1772	0.0015	-0.0019
95% CI	(-0.3702, 0.0197)	(-0.3708, 0.0165)	(-0.1082, 0.1112)	(-0.0931, 0.0892)
p-value	0.0757	0.0709	0.9782	0.9657
IGF-1				
Unadjusted model				
β	-0.0018	-0.1322	-0.1152	-0.1304
95% CI	(-0.4760, 0.4725)	(-0.5524, 0.2881)	(-0.3830, 0.1526)	(-0.3456, 0.0848)
p-value	0.9940	0.5283	0.3895	0.2276
Partially adjusted model [b]				
β	-0.0775	-0.1596	-0.0551	-0.0820
95% CI	(-0.5371, 0.3820)	(-0.5870, 0.2678)	(-0.2988, 0.1885)	(-0.2777, 0.1136)
p-value	0.7345	0.4544	0.6495	0.4014
Fully adjusted model [c]				
β	0.1775	0.0901	-0.0900	-0.0875
95% CI	(-0.5070, 0.8621)	(-0.5948, 0.7749)	(-0.4481, 0.2681)	(-0.3844, 0.2094)
p-value	0.5961	0.7876	0.6075	0.5474
IGFBP-3				
Unadjusted model				
β	-0.6259	-0.6645	0.0691	-0.0386
95% CI	(-1.3946, 0.1429)	(-1.3392, 0.0102)	(-0.3836, 0.5218)	(-0.4060, 0.3289)
p-value	0.1077	0.0534	0.7590	0.8329

Partially adjusted model [b]				
β 95% CI p-value	-0.6687 (-1.3997, 0.0622) 0.0718	-0.6794 (-1.3577, -0.0010) 0.0497	0.1035 (-0.3002, 0.5073) 0.6066	-0.0106 (-0.3381, 0.3169) 0.9481
Fully adjusted model [c]				
β 95% CI p-value	-0.3921 (-1.3076, 0.5233) 0.3840	-0.4660 (-1.3705, 0.4385) 0.2969	-0.0144 (-0.5016, 0.4729) 0.9518	-0.0739 (-0.4774, 0.3297) 0.7079
Molar Ratio IGF-1/IGFBP-3				
Unadjusted model				
β 95% CI p-value	0.2068 (-0.2471, 0.6606) 0.3625	0.0976 (-0.3099, 0.5050) 0.6309	-0.1308 (-0.3889, 0.1273) 0.3115	-0.1092 (-0.3184, 0.1000) 0.2975
Partially adjusted model [b]				
β 95% CI p-value	0.1535 (-0.2867, 0.5937) 0.4845	0.0811 (-0.3327, 0.4948) 0.6939	-0.0860 (-0.3195, 0.1475) 0.4605	-0.0725 (-0.2611, 0.1162) 0.4417
Fully adjusted model [c]				
β 95% CI p-value	0.4432 (-0.2628, 1.1492) 0.2064	0.3900 (-0.3188, 1.0988) 0.2661	-0.0929 (-0.4738, 0.2881) 0.6182	-0.0532 (-0.3708, 0.2643) 0.7313

(a) Variables followed a non-normal distribution and were log base 10 transformed.
(b) The partially adjusted model included BMI.
(c) The fully adjusted model included anthropometric measures (BMI, waist circumference, waist-hip ratio), age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

4.3.3.2.2.2. The Insulin/IGF Axis and Breast Density Measurements in Non-Hispanics

We used regression models to investigate the relationship between the insulin/IGF axis and the breast density measurements in the Non-Hispanic cohort (n = 64). We considered overall adiposity (BMI) for a partial adjustment in the regression analysis. Fully adjusted models included anthropometric measures (BMI and waist circumference), age (age at baseline visit), and reproductive factors (age at menarche, menstrual phases, age at first live birth of a child) [Table 37].

Percent Density and the Insulin/IGF Axis in Non-Hispanics

The percent density showed no statistically significant relationships with the insulin/IGF axis in the Non-Hispanic cohort [Table 37].

Absolute Dense Volume and the Insulin/IGF Axis in Non-Hispanics

Similar to that of the percent density, the absolute dense volume was not related to the insulin/IGF axis in the Non-Hispanic cohort [Table 37].

Non-Dense Volume and the Insulin/IGF Axis in Non-Hispanics

The non-dense volume showed an inverse borderline relation with the fasting serum insulin for the partially adjusted (β = -0.0818 p-value = 0.0517) and the fully adjusted models (β = -0.0999 p-value = 0.0548) [Table 37]. The non-dense volume showed no relations with HOMA-IR, IGF-1, IGFBP-3, or with the molar ratio IGF-1/IGFBP-3 [Table 37].

Total Breast Volume and the Insulin/IGF Axis in Non-Hispanics

Similar to that of the non-dense volume, the total breast volume showed an inverse borderline relation with the fasting serum insulin for the partially adjusted (β = -0.0699, p-value = 0.0581) and for the fully adjusted model (β = -0.0915, p-value = 0.0479). The total breast volume was not related to HOMA-IR, IGF-1, IGFBP-3, or to the molar ratio IGF-1/IGFBP-3 [Table 37].

Table 38. Linear regression analysis comparing the insulin/IGF axis [a] with the breast density measurements [a] in Non-Hispanics (n = 64).

Models of Explicative Variables [a]	Breast Density Measurements [a] (β, 95% CI, p-value)			
	Percent Density	**Absolute Dense Volume**	**Non-Dense Volume**	**Total Breast Volume**
Insulin				
Unadjusted model				
β	-0.0622	-0.0877	-0.0247	-0.0255
95% CI	(-0.2100, 0.0855)	(-0.2178, 0.0423)	(-0.1169, 0.0675)	(-0.1040, 0.0531)
p-value	0.4030	0.1825	0.5942	0.5191
Partially adjusted model [b]				
β	0.0227	-0.0472	-0.0818	-0.0699
95% CI	(-0.1128, 0.1583)	(-0.1791, 0.0848)	(-0.1642, 0.0006)	(-0.1423, 0.0025)
p-value	0.7385	0.4772	0.0517	0.0581
Fully adjusted model [c]				
β	-0.0491	-0.1406	-0.0999	-0.0915
95% CI	(-0.2011, 0.1028)	(-0.2939, 0.0127)	(-0.2020, 0.0022)	(-0.1820, -0.0009)
p-value	0.5175	0.0713	0.0548	0.0479
HOMA-IR				
Unadjusted model				
β	-0.0601	-0.0811	-0.0194	-0.0210
95% CI	(-0.2028, 0.0826)	(-0.2069, 0.0447)	(-0.1086, 0.0697)	(-0.0969, 0.0549)
p-value	0.4033	0.2023	0.6643	0.5823
Partially adjusted model [b]				
β	0.0229	-0.0412	-0.0748	-0.0641
95% CI	(-0.1081, 0.1539)	(-0.1689, 0.0864)	(-0.1548, 0.0051)	(-0.1343, 0.0061)
p-value	0.7280	0.5208	0.0660	0.0727
Fully adjusted model [c]				
β	-0.0514	-0.1350	-0.0900	-0.0836
95% CI	(-0.1971, 0.0943)	(-0.2822, 0.0121)	(-0.1886, 0.0085)	(-0.1710, 0.0038)
p-value	0.4800	0.0711	0.0723	0.0603
IGF-1				
Unadjusted model				
β	-0.0817	-0.0197	0.0815	0.0620
95% CI	(-0.5509, 0.3874)	(-0.4367, 0.3973)	(-0.2099, 0.3729)	(-0.1866, 0.3106)
p-value	0.7288	0.9250	0.5780	0.6198
Partially adjusted model [b]				
β	-0.0437	0.0006	0.0585	0.0443
95% CI	(-0.4548, 0.3674)	(-0.4010, 0.4023)	(-0.1989, 0.3159)	(-0.1815, 0.2701)
p-value	0.8324	0.9975	0.6511	0.6961
Fully adjusted model [c]				
β	0.2407	0.1857	-0.0741	-0.0550
95% CI	(-0.2498, 0.7312)	(-0.3296, 0.7010)	(-0.4205, 0.2723)	(-0.3635, 0.2535)
p-value	0.3275	0.4709	0.6681	0.7206
IGFBP-3				
Unadjusted model				
β	-0.4626	-0.2919	0.2490	0.1707
95% CI	(-1.0788, 0.1536)	(-0.8439, 0.2602)	(-0.136, 0.634)	(-0.1591, 0.5006)
p-value	0.1385	0.2947	0.2009	0.3048
Partially adjusted model [b]				

β	-0.3658	-0.2405	0.1900	0.1253
95% CI	(-0.9084, 0.1768)	(-0.7747, 0.2938)	(-0.1517, 0.5318)	(-0.1757, 0.4263)
p-value	0.1826	0.3716	0.2706	0.4084
Fully adjusted model [c]				
β	-0.2935	-0.1719	0.1843	0.1216
95% CI	(-0.9333, 0.3463)	(-0.8452, 0.5013)	(-0.2641, 0.6327)	(-0.2789, 0.5222)
p-value	0.3596	0.6088	0.4113	0.5432
Molar Ratio IGF-1/IGFBP-3				
Unadjusted model				
β	0.1746	0.1414	-0.0570	-0.0331
95% CI	(-0.289, 0.6381)	(-0.2705, 0.5534)	(-0.3463, 0.2322)	(-0.2799, 0.2137)
p-value	0.4545	0.4951	0.6948	0.7894
Partially adjusted model [b]				
β	0.1574	0.1323	-0.0466	-0.0251
95% CI	(-0.2481, 0.5629)	(-0.2642, 0.5289)	(-0.3018, 0.2085)	(-0.2491, 0.1988)
p-value	0.4405	0.5072	0.7160	0.8233
Fully adjusted model [c]				
β	0.4015	0.2785	-0.1773	-0.123
95% CI	(-0.0708, 0.8738)	(-0.2247, 0.7818)	(0.5147, 0.1601)	(-0.4248, 0.1789)
p-value	0.0935	0.2702	0.2948	0.4155

(a) Variables followed a non-normal distribution and were log base 10 transformed.
(b) The partially adjusted model included BMI.
(c) The fully adjusted model included anthropometric measures (BMI, waist circumference, waist-hip ratio), age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

4.4. Discussion

The dysregulation of the insulin/IGF axis can contribute to breast tumorigenesis [Goodwin 2011, Christopoulos 2015]. The presence of hyperinsulinemia, higher levels of bioavailable IGF-1, and low IGFBPs activity may lead to higher rates of epithelial breast cell proliferation [Izzo 2012, Byrne 2000, Ahern 2013, Rinaldi 2014] which might lead to increased breast density.

We showed that insulin and HOMA-IR are inversely related to the absolute dense volume and percent density. The inverse relationships with the absolute density remained significant after adjustment of potential confounders. Nonetheless, the associations with the percent density lost the significance after adjusting for covariates. Similar to our findings, Diorio et al reported an inverse association between the circulating levels of C-peptide (a surrogate of insulin levels) and mammographic percent density [Diorio 2005]. This relationship, however, was lost after adjusting for overall adiposity. Another study reported insulin was not related to the mammographic percent density in a cohort of Hispanic women after BMI adjustment, where the majority of the study subjects (88%) were overweight/obese (BMI > 25 kg/m2) [Wolin 2007].

Our data showed that circulating IGF-1 and IGFBP-3 were not related to the breast density measurements. In addition, we showed that the molar ratio IGF-1/IGFBP-3 (a surrogate for bioavailable IGF-1) was related to the percent density after adjustment of potential confounders. Prior studies evaluated the associations between IGF-axis and breast density measurements, with inconsistent findings. Some studies showed that the IGFs were not related to the mammographic percent densities [Rice 2012, Maskarinec 2003, Rinaldi 2014]. Other works reported a positive association between IGF-1 and mammographic percent density [Byrne 2000, Diorio 2005, dos Santos Silva 2006, Izzo 2012]. In addition, the IGF-1 was related to breast density but only in lean women (BMI < 25 kg/m2) [Maskarinec 2003, Rice 2012]. The IGFBP-3 has been linked to breast cancer risk, as several polymorphisms of IGFBP-3 were related to lower mammographic breast density [Verheus 2009]. IGFBP-3 was inversely related to mammographic percent density in some studies [Diorio 2008, Byrne 2000, Maskarinec 2003] but not in others [dos Santos Silva 2006]. The IGF-1/IGFBP-3 molar ratio (which indicates the bioavailable IGF-1) was related to mammographic percent density in premenopausal women from the NHS [Byrne 2000]. However, a study conducted in premenopausal Mexican women found no associations between the mammographic percent density and the bioavailable IGF-1 [Rinaldi 2014]. The reported discrepancies among the prior studies could be related to the varying confounders such as age, menopausal status, and adiposity in the study cohort.

We further analyzed the associations of the insulin/IGF axis with breast density by ethnicity. Our findings indicate a trend for an inverse link between IGFBP-3 and the percent density in Hispanics. We observed the same trend between IGFBP-3 and the absolute dense volume in Hispanics. However, these relationships had no statistical significance after adjusting for covariates. Non-Hispanics showed an inverse relationship between insulin and the non-dense volume after adjustment for confounders. In addition, Non-Hispanics showed an inverse association between insulin and the total breast volume after full adjustment. However, Non-Hispanics showed no other statistically significant associations between the insulin/IGF axis and the breast density measurements.

Our findings of the inverse associations between insulin/HOMA-IR and absolute dense volume were consistent with the observed inverse associations between elevated fasting glucose and absolute dense volume and percent density [Table 23 in Chapter 3]. The biological mechanisms for the inverse associations are not known. It is plausible that the insulin sensitivity in the breast tissue was compromised in those with elevated insulin/glucose, suggesting that hyperinsulinemia/hyperglycemia and breast density may be independent risk factors for breast

cancer. We suggest that higher bioavailable IGF-1 was related to higher percent density in our study cohort. However, we need further studies to determine the causal relationship.

The strengths of our study were the usage of fat-water MRI, which enables to acquire breast density measurements from non-compressed breasts. In addition, we could adjust for potential confounders given the collection of data from breast cancer risk questionnaires and demographics. However, the small sample size limited our study, and thus we may not detect potential differences by ethnicity.

In conclusion, the results of this cross-sectional study showed that insulin/HOMA-IR are inversely related to the absolute dense volume. In addition, the bioavailable IGF-1 is related to the percent density. These data suggest that the insulin and IGF axis may have a differential relationship to breast density. Further studies are needed to confirm our results and to determine the underlying biological mechanisms for the diverse associations.

CHAPTER V: Breast Density and the Adipokines

5.1. Introduction

Adipokines secreted from the adipose tissue, like leptin and adiponectin, can contribute to metabolic disorders and have been associated with breast cancer risk. However, the associations between circulating adipokines with other well-known risk factors, such as breast density, are not clear. The following sections highlight prior studies examining the associations between the circulating adipokines and mammographic breast density.

5.1.1. Leptin and Breast Cancer Risk

Leptin is a polypeptide hormone mainly produced by the white adipose tissue. It can act as a growth factor that promotes cell proliferation, pro-inflammatory responses, and angiogenesis [van Rossum 2000]. Moreover, leptin is a growth factor positively associated with body weight, as the circulating levels of leptin increase with increasing body fat [Sinha 1996]. Distinct *in vitro* and *in vivo* studies suggest that high circulating levels of leptin, as seen in obesity, could be related to epithelial breast cell tumorigenesis [Hu 2002]. Additionally, leptin can exert different effects, according to the menopausal status, in the mammary tissue [Dossus 2017]. Leptin impairs estradiol production in premenopausal women but stimulates aromatase activities in postmenopausal women [Nalabolu 2014, Brannian 2002]. Thus, the potential effects of leptin on obese women and its contribution to breast cancer risk may differ according to the menopausal status.

Epidemiological studies have evaluated the potential relationships between leptin and other known breast cancer risk factors, such as breast density. According to these [Table 38], most of the studies showed that leptin is inversely associated with mammographic percent density and positively associated with the non-dense area of the breast [Maskarinec 2010, Stuedal 2006, Woolcott 2013, Dossus 2017]. Findings are conflicting with regards to the relationship between leptin and the absolute dense areas with some studies showing a weak correlation [Stuedal 2006] while others finding an inverse correlation [Woolcott 2013]. Potential explanations for those discrepancies are the confounding effect of overall adiposity, as it may influence the relationship observed for leptin and the breast density measurements [Dossus 2017]. Distinct results could be due to the menopausal status of the cohort [Nalabolu 2014, Brannian 2002]. Hence, it is important to conduct further studies to explore the interplay between leptin, obesity, breast density, and breast cancer risk by menopausal status.

5.1.2. Adiponectin and Breast Cancer Risk

Adiponectin is a peptide mainly produced by the adipocytes that, in conjunction with leptin, regulates energy homeostasis [Fasshauer 2003]. Adiponectin exists in three different isoforms in the circulation: high molecular weight (HMW) adiponectin, middle molecular weight (MMW) adiponectin, and low molecular weight (LMW) adiponectin [Ebinuma 2006]. Among those three isoforms, the HMW adiponectin is the major active form [Zhuo 2009, Fasshauer 2003]. This last one relates to insulin sensitivity and metabolic syndrome [Zhuo 2009, Fasshauer 2003]. Adiponectin also exerts anti-proliferative, pro-apoptotic, and anti-estrogenic activities in the mammary tissue [Barb 2007]. For those properties, adiponectin may protect against breast carcinogenesis [Barb 2007]. In the case of obesity, though, those protective effects might be

reduced, as adiponectin concentrations decrease with increasing overall adiposity and circulating leptin concentrations [Arita 1999].

Limited studies have examined the associations between adiponectin and breast density [Table 38]. The associations between total adiponectin and breast density measurements, in most of the studies, were lost after BMI adjustment [Maskarinec 2010, Woolcott 2013, Dossus 2017].

Table 39. Cross-Sectional Studies Evaluating the Associations between the Adipokines and Breast Density Measurements.

First author, Year	Sample Size	Cohort	Findings
Maskarinec, 2010	183	Premenopausal women from a nutritional intervention trial	Leptin was inversely associated with the mammographic percent density, but this association was lost after BMI-adjustment. Adiponectin was associated with the mammographic percent density, but this association was reduced after adjusting for BMI.
Stuedal, 2006	967	Tromso Mammography and Breast Cancer Study	Leptin was positively associated with the non-dense area but weakly correlated with the absolute dense area and the mammographic percent density.
Woolcott, 2013	302	Postmenopausal women from the ALPHA trial	Leptin was negatively correlated with the percent dense volume (r = -0.20) and with the absolute dense volume (r = -0.19). Those correlations remained significant after adjusting for overall adiposity. Adiponectin was negatively correlated with the non-dense volume (r = -0.012), but this association was lost after BMI adjustment.
Dossus, 2017	574	Premenopausal Mexican women from the ESMaestras cohort	High circulating levels of leptin and a high leptin-to-adiponectin ratio (LAR) were associated with low mammographic percent density and with high absolute dense areas. Adiponectin was associated with low absolute dense areas and non-dense areas, but those correlations were lost after BMI-adjustment.

5.1.3. Objectives

In this cross-sectional analysis, we aimed to evaluate the potential associations between the adipokines and breast density measurements acquired by fat-water MRI in a cohort of premenopausal women with elements of metabolic syndrome. For this purpose, the first objective was to assess the relationships between the anthropometric measures and the circulating adipokines. The second objective was to evaluate the associations between leptin and HMW adiponectin with the breast density measurements, overall and by ethnicity. We hypothesized that high circulating levels of leptin or low circulating levels of high-molecular-weight (HMW) adiponectin were related to high breast densities in the study cohort.

5.2. Methodology

5.2.1. Analysis of Serum Levels of Leptin and High-Molecular Weight (HMW) Adiponectin

We measured fasting leptin and high-molecular-weight (HMW) adiponectin using an enzyme-linked immunoassay by R&D (R&D Systems, Minneapolis, USA). We decided to measure HMW adiponectin because it showed a better correlation with glucose tolerance and metabolic syndrome than total adiponectin [Trujillo 2005, Hirose 2010]. The calibrators that we used in the assays ranged from 125 pg/ml to 1000 pg/ml for leptin and from 3.90 ng/ml to 250 ng/ml for HMW adiponectin. For both assays, we diluted fasting serum samples 1:100 in an assay buffer prior their analysis. The immunoassays had less than 0.5% of cross-reactivity observed with available related molecules. We analyzed serum samples and standards in duplicate for each immunoassay, and we averaged measures. We inserted two quality controls in each analysis. The intra-assay CVs were 16.63% for leptin and 20.61% for HMW adiponectin. The inter-assay CVs were 16.38% for leptin and 18.32% for HMW adiponectin.

5.2.2. Statistical Analyses

We estimated the correlations between the adipokines and breast density measurements as Pearson's correlation coefficients. We transformed non-normally distributed variables by using their logarithm. We used a t-test to compare the differences in adipokines by ethnic groups. We used the R package version 3.4.1 [R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria] for statistical analyses. We used linear regression to explore the associations between breast density measurements and serum measurements of adipokines. We performed regression analysis by using STATA version 15.1. StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC. All statistical tests were two-sided, and we considered P-values equal or less than 0.05 as statistically significant.

5.3. Results

5.3.1. The Adipokines in the Study Cohort

We analyzed the serum levels of leptin and HMW adiponectin from 106 participants with baseline breast fat-water MRI images. We summarized in Table 39 the circulating levels of leptin and HMW adiponectin, for the overall population and by ethnicity. The mean circulating levels of leptin in the overall study subjects were 51.54 ± 31.71 ng/ml. Leptin showed no statistically significant difference by ethnicity. The mean circulating levels of HMW adiponectin in the study cohort were

4295.31 ± 2926.46 ng/ml. HMW adiponectin showed no statistically significant difference by ethnicity [Table 39].

Table 40. Mean (SD) of the Fasting Circulating Leptin and HMW Adiponectin in the Study Cohort (N = 106).

Variable [a]	**Overall Cohort (n=106)**	**Hispanics (n=41)**	**Non-Hispanics (n=64)**	**P-value** [b]
Fasting Serum Leptin (ng/mL)				
Non-transformed	51.54 (31.71)	53.33 (32.74)	50.45 (31.48)	0.6529
Log-transformed	3.76 (0.62)	3.8 (0.60)	3.73 (0.64)	0.6096
Fasting Serum HMW Adiponectin (ng/mL)				
Non-transformed	4,295.31 (2926.46)	3,741.75 (2222.25)	4,626.0 (3284.9)	0.1329
Log-transformed	8.12 (0.75)	8.04 (0.65)	8.17 (0.81)	0.3761

(a) Variables presented a non-normal distribution and were base 10 log-transformed.
(b) A two-sample t-test was conducted to assess differences between the groups.

5.3.2. Association between Adipokines and Anthropometric Measurements of Adiposity

We performed regression analyses to evaluate the relationship between circulating adipokines and the anthropometric measures of obesity in the study cohort. We included overall adiposity (BMI) for the partially adjusted models. We considered the anthropometric measures, age at baseline visit, age at menarche, menstrual phase, and age at first live birth of a child for the fully adjusted models [Table 40].

Leptin

The fasting circulating levels of leptin were positively related to the BMI (β = 2.0973, p-value < 0.00001). However, this relationship lost significance in the fully adjusted model [Table 40]. Leptin was related to waist circumference in the unadjusted model (β = 3.0927, p-value < 0.00001). This relationship remained statistically significant in the partially adjusted model (β = 1.6994, p-value = 0.0418). However, this relationship lost its significance in the fully adjusted model. Furthermore, leptin was borderline related to the waist-hip ratio in the unadjusted (β = 1.6370, p-value = 0.0490) and in the partially adjusted model (β = 1.2655, p-value= 0.0888). Leptin was not related to the waist-hip ratio in the fully adjusted model (β = -0.9017, p-value = 0.5279) [Table 40].

HMW Adiponectin

The fasting serum levels of HMW adiponectin were inversely related to BMI in the unadjusted model (β = -2.0497 p-value = 0.0001) and in the fully adjusted model (β = -4.3587 p-value < 0.00001). In addition, fasting serum levels of HMW adiponectin were inversely related to the waist circumference in the unadjusted model (β = -1.8277, p-value = 0.0211). This relationship was lost in the partially adjusted model (β = 0.7730, p-value = 0.4677) but was positively and statistically significant in the fully adjusted model (β = 4.8955, p-value = 0.0096). The HMW adiponectin was borderline related to the waist-hip ratio in the unadjusted model (β = -1.8858, p-value = 0.0604). The relationship of HMW adiponectin with the waist-hip ratio was not statistically significant in the

partially adjusted model (β = -1.5253, p-value = 0.1048). However, the HMW adiponectin was significantly related to the waist-hip ratio in the fully adjusted model (β = -5.3370, p-value = 0.0036) [Table 40].

Table 41. Linear regression analysis comparing the adipokines [a] with the anthropometric measurements of adiposity in the study cohort (N = 106).

Models of Explicative Variables [a]	Adipokines [a] (β, 95% CI, p-value)	
	Fasting Serum Leptin	**Fasting Serum HMW Adiponectin**
BMI		
Unadjusted model		
β	2.0973	-2.0497
95% CI	(1.3265, 2.8680)	(-3.0236, -1.0757)
p-value	<0.00001	0.0001
Fully adjusted model [c]		
β	0.5208	-4.3587
95% CI	(-1.0496, 2.0911)	(-6.3168, -2.4005)
p-value	0.5108	<0.00001
Waist circumference		
Unadjusted model		
β	3.0927	-1.8277
95% CI	(1.9221, 4.2632)	(-3.3756, -0.2798)
p-value	<0.00001	0.0211
Partially adjusted model [b]		
β	1.6994	0.7730
95% CI	(0.0643, 3.3345)	(-1.3299, 2.8758)
p-value	0.0418	0.4677
Fully adjusted model [c]		
β	2.4471	4.8955
95% CI	(-0.4952, 5.3893)	(1.2265, 8.5644)
p-value	0.1017	0.0096
Waist-hip ratio		
Unadjusted model		
β	1.6370	-1.8858
95% CI	(0.0075, 3.2666)	(-3.8557, 0.0841)
p-value	0.0490	0.0604
Partially adjusted model [b]		
β	1.2655	-1.5253
95% CI	(-0.1954, 2.7264)	(-3.3738, 0.3231)
p-value	0.0888	0.1048
Fully adjusted model [c]		
β	-0.9017	-5.3370
95% CI	(-3.7348, 1.9314)	(-8.8699, -1.8042)
p-value	0.5279	0.0036

(a) Variables followed a non-normal distribution and were base 10 log-transformed.
(b) The partially adjusted model included BMI.
(c) The fully adjusted model included anthropometric measures (BMI, waist circumference, waist-hip ratio), ethnicity, age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

5.3.3. Association between Adipokines and Breast Density Measurements

5.3.3.1. Correlation Analysis between Adipokines and Breast Density Measurements

We evaluated the potential correlations between circulating leptin and HMW adiponectin with breast density measurements. The circulating levels of leptin were positively correlated with both the non-dense volume ($r = 0.46$, p-value < 0.00001) and the total breast volume ($r = 0.46$, p-value < 0.00001) [Table 41, Fig. 22]. Leptin was inversely correlated with percent density ($r = -0.25$, p-value = 0.010) [Table 41, Fig. 22]. The HMW adiponectin was statistically significantly correlated with the percent density ($r = 0.22$, p-value = 0.026) [Table 41, Fig. 23]. HMW adiponectin was borderline correlated with the absolute dense volume ($r = 0.17$, p-value = 0.086) [Table 41, Fig. 23]. HMW adiponectin showed no other statistically significant correlations with the breast parameters [Table 41, Fig. 23].

We conducted further correlation analysis by ethnic group. Hispanics presented correlations between leptin and the non-dense volume ($r = 0.47$, p-value = 0.002) and with the total breast volume ($r = 0.48$, p-value = 0.001) [Table 42, See Appendix A, Fig. A14]. Hispanics presented no correlations between leptin and percent density or the absolute dense volume. In addition, Hispanics presented no statistically significant correlations between the HMW adiponectin and the breast density measurements [Table 42, See Appendix A, Fig. A15].

Non-Hispanics presented inverse correlations between leptin and percent density ($r = -0.27$, p-value = 0.029) and presented positive correlations between leptin, the non-dense volume ($r = 0.47$, $p = 0.0001$) and the total breast volume ($r = 0.47$, p-value < 0.0001) [Table 43, See Appendix A, Fig. A14]. Non-Hispanics presented no statistically significant correlations between leptin and the absolute dense volume [Table 43, See Appendix A, Fig. A14]. Non-Hispanics presented no statistically significant correlations between HMW adiponectin and the breast density measurements [Table 43, See Appendix A, Fig. A15].

Table 42. Pearson Correlations between the Adipokines[a] and Breast Density Measurements[a] in the Study Cohort (N = 106).

Variable [a]	**Fasting Serum Leptin**	**Fasting Serum HMW Adiponectin**
Percent Density	-0.25 (p = 0.010)	0.22 (p = 0.026)
Absolute Dense Volume	-0.02 (p = 0.854)	0.17 (p = 0.086)
Non-Dense Volume	0.46 (p < 0.00001)	-0.15 (p = 0.127)
Total Breast Volume	0.46 (p < 0.00001)	-0.13 (p = 0.181)

(a) Variables presented a non-normal distribution and were base 10 log-transformed.

Table 43. Pearson Correlations between the Adipokines[a] and Breast Density Measurements[a] in Hispanics (n = 41).

Variable [a]	**Fasting Serum Leptin**	**Fasting Serum HMW Adiponectin**
Percent Density	-0.22 (p=0.174)	0.11 (p=0.475)
Absolute Dense Volume	0.01 (p=0.965)	0.09 (p=0.564)
Non-Dense Volume	0.47 (p=0.002)	-0.09 (p=0.579)
Total Breast Volume	0.48 (p=0.001)	-0.07 (p=0.667)

(a) Variables presented a non-normal distribution and were base 10 log-transformed.

Table 44. Pearson Correlations between the Adipokines[a] and Breast Density Measurements[a] in Non-Hispanics (n = 64).

Variable [a]	**Fasting Serum Leptin**	**Fasting Serum HMW Adiponectin**
Percent Density	-0.27 (p = 0.029)	0.30 (p = 0.014)
Absolute Dense Volume	-0.02 (p = 0.849)	0.23 (p = 0.062)
Non-Dense Volume	0.47 (p = 0.0001)	-0.20 (p = 0.110)
Total Breast Volume	0.47 (p < 0.0001)	-0.18 (p = 0.153)

(a) Variables presented a non-normal distribution and were base 10 log-transformed.

Figure 21. The Fasting Circulating Levels of Leptin [a] and Breast Density Measurements [a] (N = 106).

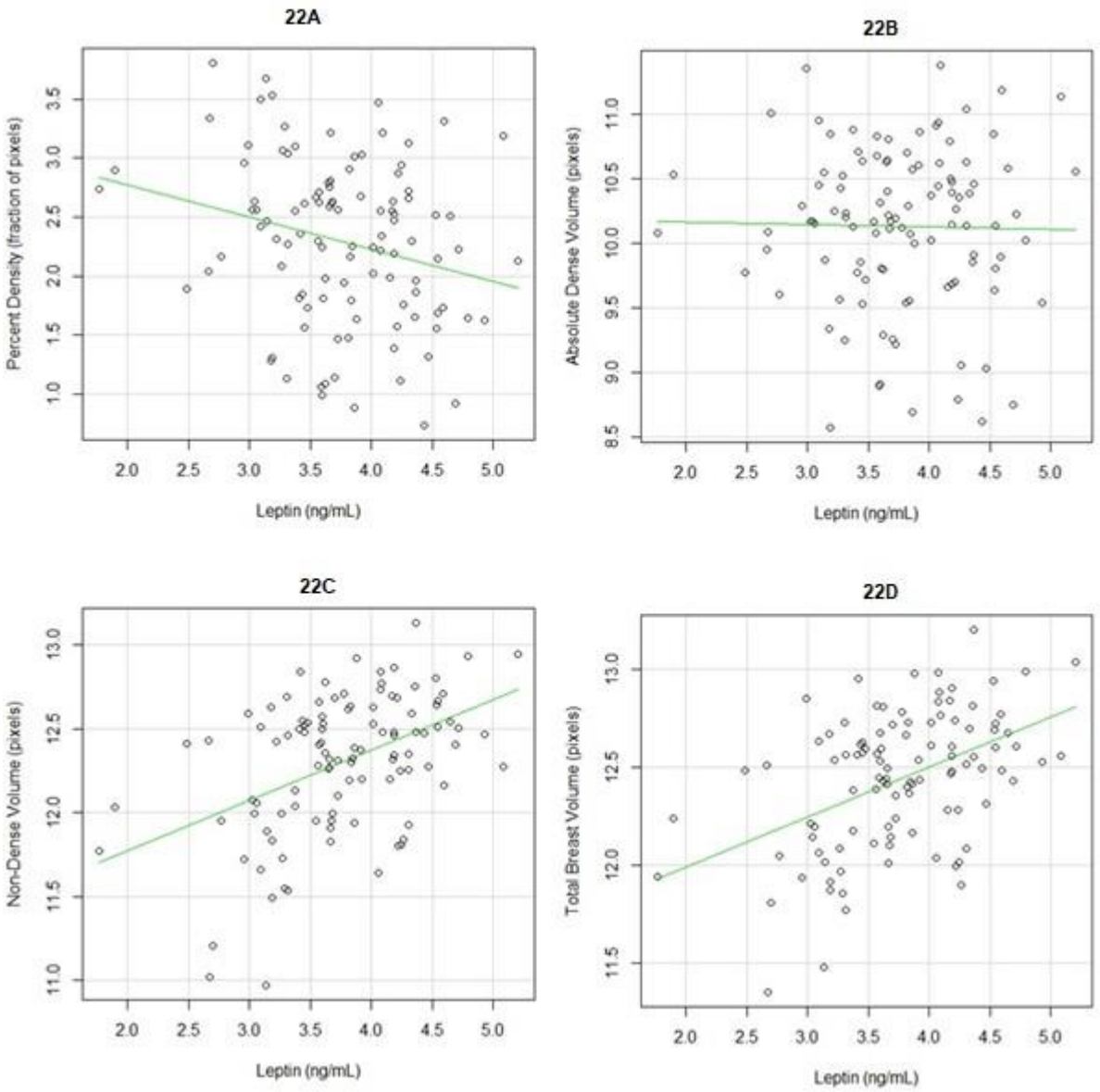


Figure 22A. Circulating fasting serum levels of leptin (ng/ml) and the percent density (fraction) in the overall study population.
Figure 22B. Circulating fasting serum levels of leptin (ng/ml) and the absolute dense volume (pixel units) in the overall study population.
Figure 22C. Circulating fasting serum levels of leptin (ng/ml) and the non-dense volume (pixel units) in the overall study population.
Figure 22D. Circulating fasting serum levels of leptin (ng/ml) and the total breast volume (pixel units) in the overall study population.
(a) Variables presented a non-normal distribution and were base 10 log-transformed.

Figure 22. The Fasting Circulating Levels of HMW Adiponectin [a] and Breast Density Measurements [a] (N = 106).

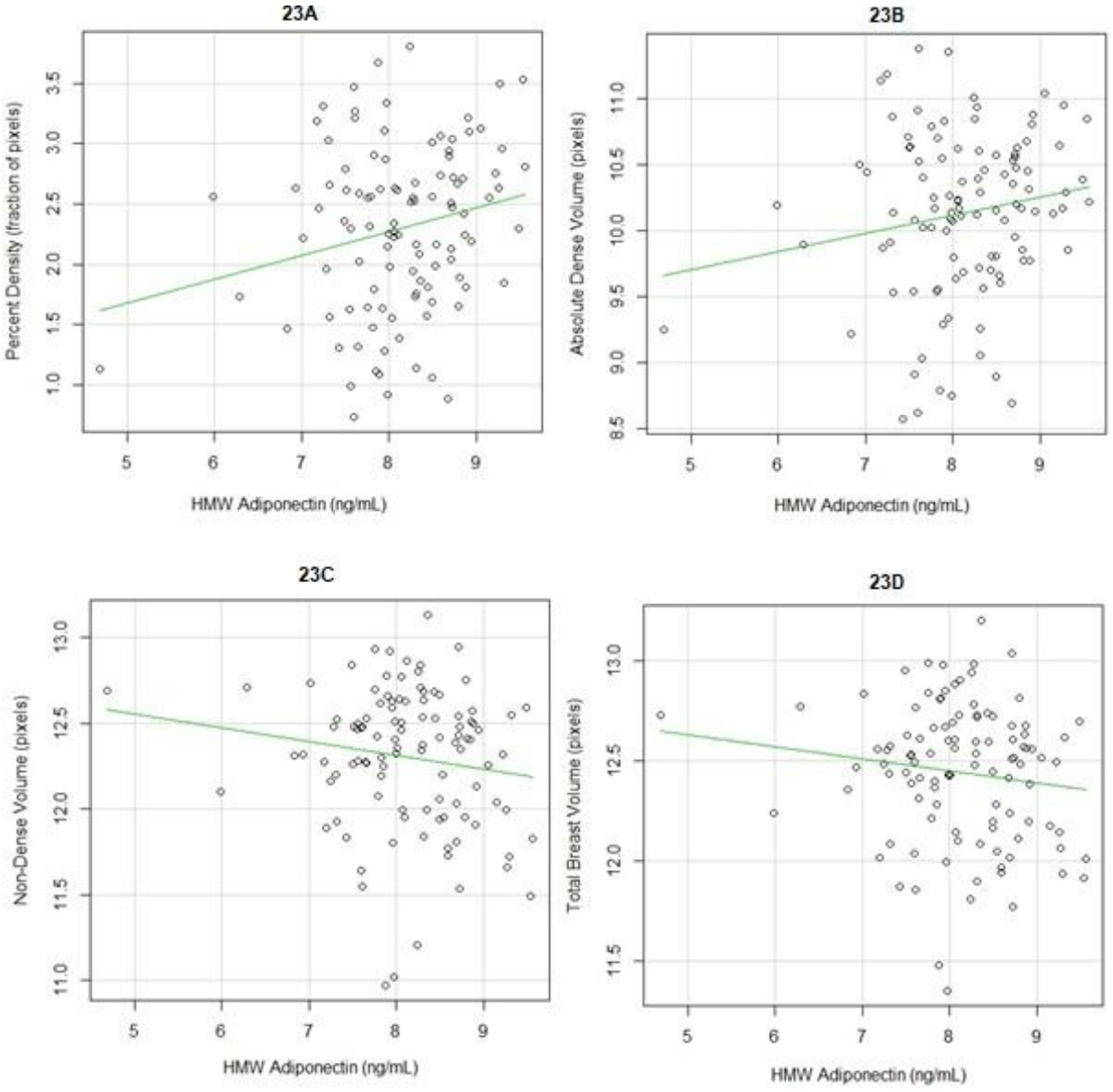


Figure 23A. Circulating fasting serum levels of HMW adiponectin (ng/ml) and the percent density (fraction) in the overall study population.
Figure 23B. Circulating fasting serum levels of HMW adiponectin (ng/ml) and the absolute dense volume (pixel units) in the overall study population.
Figure 23C. Circulating fasting serum levels of HMW adiponectin (ng/ml) and the non-dense volume (pixel units) in the overall study population.
Figure 23D. Circulating fasting serum levels of HMW adiponectin (ng/ml) and the total breast volume (pixel units) in the overall study population.
(a) Variables presented a non-normal distribution and were base 10 log-transformed.

5.3.3.2. Regression Analysis of the Adipokines with Breast Density Measurements

5.3.3.2.1. Adipokines and Breast Density Measurements in the Overall Study Cohort

We conducted regression analysis to evaluate the relationship between circulating adipokines and the breast density measurements in the overall study cohort. We included overall adiposity (BMI) in the partially adjusted model. We adjusted for other variables such as BMI, waist circumference, age at baseline visit, age at menarche, menstrual phase, and age at first live birth of a child in the fully adjusted model [Table 44].

Percent Density and Adipokines

The percent density was inversely related to the fasting serum leptin levels in the unadjusted model (β = -0.2741, p-value = 0.0106). This relationship though, was not statistically significant in the partially adjusted (β = -0.0798, p-value = 0.4800) nor in the fully adjusted models (β = 0.0735, p-value = 0.5387). In addition, the percent density was positively related to the HMW adiponectin in the unadjusted model (β = 0.1976, p-value = 0.0268) but not in the partially adjusted (β = 0.0648, p-value = 0.4687) nor in the fully adjusted models (β = 0.1296, p-value = 0.1507) [Table 44].

Absolute Dense Volume and Adipokines

The absolute dense volume showed a positive relationship with the fasting serum leptin levels in the fully adjusted model (β = 0.2406, p-value = 0.0451). This relationship was not significant in the unadjusted (β = -0.0184, p-value = 0.8505) or in the partially adjusted model (β = 0.1001, p-value = 0.3559). Additionally, the absolute dense volume was borderline related to HMW adiponectin in the unadjusted model (β = 0.1377, p-value = 0.0869). HMW adiponectin was not related to the absolute dense volume in the partially adjusted model (β = 0.0850, p-value = 0.3218) or in the fully adjusted model (β = 0.1252, p-value = 0.1727) [Table 44].

Non-Dense Volume and Adipokines

The non-dense volume showed a positive and statistically significant relationship with the fasting serum leptin levels in the unadjusted (β = 0.3004, p-value < 0.00001), partially adjusted (β = 0.1979, p-value = 0.0018), and fully adjusted models (β = 0.1615, p-value = 0.0255). The non-dense volume showed no significant relationship with the fasting serum HMW adiponectin levels in the unadjusted (β = -0.0814, p-value = 0.1271), partially adjusted (β = 0.0192, p- value = 0.7085), and fully adjusted models (β = -0.0078, p-value = 0.8888) [Table 44].

Total Breast Volume and Adipokines

Similar to that observed for the non-dense volume, the total breast volume was related to the fasting serum leptin levels in unadjusted (β = 0.2557, p-value < 0.00001), partially adjusted (β = 0.1799, p-value = 0.0008) and fully adjusted models (β = 0.1671, p-value = 0.0077). The total breast volume was not related to HMW adiponectin in the unadjusted (β = -0.0599, p-value = 0.1813), partially adjusted (β = 0.0202, p-value = 0.6435) or fully adjusted models (β = -0.0044, p-value = 0.9284) [Table 44].

Table 45. Linear regression analysis comparing the adipokines [a] with the breast density measurements [a] in the study cohort (N = 106).

Models of Explicative Variables [a]	Breast Density Measurements [a] (β, 95% CI, p-value)			
	Percent Density	**Absolute Dense Volume**	**Non-Dense Volume**	**Total Breast Volume**
Leptin				
Unadjusted model				
β	-0.2741	-0.0184	0.3004	0.2557
95% CI	(-0.4831, -0.0652)	(-0.2117, 0.1749)	(0.1864, 0.4144)	(0.1604, 0.3510)
p-value	0.0106	0.8505	<0.00001	<0.00001
Partially adjusted model [b]				
β	-0.0798	0.1001	0.1979	0.1799
95% CI	(-0.3030, 0.1435)	(-0.1140, 0.3143)	(0.0755, 0.3202)	(0.0763, 0.2835)
p-value	0.4800	0.3559	0.0018	0.0008
Fully adjusted model [c]				
β	0.0735	0.2406	0.1615	0.1671
95% CI	(-0.1635, 0.3104)	(0.0054, 0.4758)	(0.0204, 0.3026)	(0.0456, 0.2886)
p-value	0.5387	0.0451	0.0255	0.0077
HMW Adiponectin				
Unadjusted model				
β	0.1976	0.1377	-0.0814	-0.0599
95% CI	(0.0231, 0.3721)	(-0.0203, 0.2957)	(-0.1864, 0.0235)	(-0.1482, 0.0284)
p-value	0.0268	0.0869	0.1271	0.1813
Partially adjusted model [b]				
β	0.0648	0.0850	0.0192	0.0202
95% CI	(-0.1119, 0.2414)	(-0.0843, 0.2544)	(-0.0823, 0.1206)	(-0.0663, 0.1067)
p-value	0.4687	0.3218	0.7085	0.6435
Fully adjusted model [c]				
β	0.1296	0.1252	-0.0078	-0.0044
95% CI	(-0.0482, 0.3074)	(-0.0560, 0.3064)	(-0.1186, 0.1030)	(-0.1012, 0.0924)
p-value	0.1507	0.1727	0.8888	0.9284

(a) Variables followed a non-normal distribution and were base 10 log-transformed.
(b) The partially adjusted model included BMI.
(c) The fully adjusted model included anthropometric measures (BMI, waist circumference, waist-hip ratio), ethnicity, age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

5.3.3.2.2. Regression Analysis of Adipokines with Breast Density Measurements by Ethnicity

5.3.3.2.2.1. Adipokines and Breast Density Measurements in Hispanics

We used regression to estimate the relationship between the circulating adipokines and the breast density measurements in the Hispanic cohort (n = 41). The regression analysis was partially adjusted for overall adiposity (BMI) and fully adjusted for the waist circumference, age at baseline visit, age at menarche, menstrual phases, and the age at first live birth of a child [Table 45].

Percent Density and Adipokines in Hispanics

The percent density showed no relationships with the fasting circulating levels of leptin in the Hispanic cohort (n = 41) in the unadjusted (β = -0.2503, p-value = 0.1742), the partially adjusted (β = -0.0731, p-value = 0.7269), or in the fully adjusted models (β = 0.1527, p-value = 0.5946) [Table 44]. In addition, no relationships were found for the HMW adiponectin in the unadjusted (β = 0.1236, p-value = 0.4757), the partially adjusted (β = -0.0182, p-value = 0.9200), or in the fully adjusted models (β = 0.0726, p-value = 0.7433) [Table 45].

Absolute Dense Volume and Adipokines in Hispanics

Similar to the percent density, the absolute dense volume showed no relationships with the circulating levels of leptin in the Hispanics in the unadjusted (β = 0.0072, p-value = 0.9654), the partially adjusted (β = 0.0965, p-value = 0.6222), or in the fully adjusted models (β = 0.1808, p-value = 0.5261) [Table 45]. Likewise, the absolute dense volume was not related to HMW adiponectin in the unadjusted (β = 0.0891, p-value = 0.5642), the partially adjusted (β = 0.0532, p-value = 0.7538), or in the fully adjusted models (β = 0.1576, p-value = 0.4727) [Table 45].

Non-Dense Volume and Adipokines in Hispanics

The non-dense volume had a positive relationship with the fasting circulating levels of leptin in the unadjusted (β = 0.3090, p-value = 0.0020) and in the partially adjusted model (β = 0.2002, p-value = 0.0658). However, this relationship was not significant in the fully adjusted model (β = 0.0208, p-value = 0.8901). In addition, the non-dense volume was not related to HMW adiponectin in the unadjusted (β = -0.0548, p-value = 0.5794), the partially adjusted (β = 0.0718, p-value = 0.4541), or in the fully adjusted models (β = 0.0978, p-value = 0.3953) [Table 45].

Total Breast Volume and Adipokines in Hispanics

The total breast volume was positively related to the circulating levels of leptin in Hispanics for the unadjusted model (β = 0.2575, p-value = 0.0014). Leptin was borderline related to the total breast volume in the partially adjusted model (β = 0.1696, p-value = 0.0533). This relationship, though, was not significant in the fully adjusted model (β = 0.0282, p-value = 0.8218). Additionally, the total breast volume was not related to HMW adiponectin in the unadjusted (β = -0.0345, p-value = 0.6676), partially adjusted (β = 0.0714, p-value = 0.3563), or in the fully adjusted model (β = 0.0851, p- value = 0.3731). This is very similar to that observed for the non-dense volume and the circulating adipokines [Table 45].

Table 46. Linear regression analysis comparing the adipokines [a] with the breast density measurements [a] in Hispanics (n = 41).

Models of Explicative Variables [a]	Breast Density Measurements [a] (β, 95% CI, p-value)			
	Percent Density	**Absolute Dense Volume**	**Non-Dense Volume**	**Total Breast Volume**
Leptin				
Unadjusted model				
β	-0.2503	0.0072	0.3090	0.2575
95% CI	(-0.6159, 0.1154)	(-0.3264, 0.3408)	(0.1204, 0.4976)	(0.1056, 0.4093)
p-value	0.1742	0.9654	0.0020	0.0014
Partially adjusted model [b]				
β	-0.0731	0.0965	0.2002	0.1696
95% CI	(-0.4941, 0.3478)	(-0.2967, 0.4897)	(-0.0137, 0.4142)	(-0.0025, 0.3418)
p-value	0.7269	0.6222	0.0658	0.0533
Fully adjusted model [c]				
β	0.1527	0.1808	0.0208	0.0282
95% CI	(-0.4336, 0.7390)	(-0.4013, 0.7629)	(-0.2877, 0.3293)	(-0.2280, 0.2843)
p-value	0.5946	0.5261	0.8901	0.8218
HMW Adiponectin				
Unadjusted model				
β	0.1236	0.0891	-0.0548	-0.0345
95% CI	(-0.2235, 0.4707)	(-0.2208, 0.3990)	(-0.2533, 0.1436)	(-0.1957, 0.1267)
p-value	0.4757	0.5642	0.5794	0.6676
Partially adjusted model [b]				
β	-0.0182	0.0532	0.0718	0.0714
95% CI	(-0.3830, 0.3465)	(-0.2877, 0.3940)	(-0.1204, 0.2640)	(-0.0834, 0.2262)
p-value	0.9200	0.7538	0.4541	0.3563
Fully adjusted model [c]				
β	0.0726	0.1576	0.0978	0.0851
95% CI	(-0.3811, 0.5262)	(-0.2898, 0.6050)	(-0.1361, 0.3317)	(-0.1090, 0.2791)
p-value	0.7433	0.4727	0.3953	0.3731

(a) Variables followed a non-normal distribution and were base 10 log-transformed.
(b) The partially adjusted model included BMI.
(c) The fully adjusted model included anthropometric measures (BMI, waist circumference, waist-hip ratio), ethnicity, age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

5.3.3.2.2.2. Adipokines and Breast Density Measurements in Non-Hispanics

We used regression models to investigate the relationship between the circulating adipokines and the breast density measurements in the Non-Hispanic cohort (n=64). We included overall adiposity (BMI) for a partial adjustment in the regression analysis. Fully adjusted models included anthropometric measures (BMI and waist circumference), age (age at baseline visit), and reproductive factors (age at menarche, menstrual phases, age at first live birth of a child) [Table 46].

Percent Density and Adipokines in Non-Hispanics

The percent density was inversely related to serum leptin in the unadjusted model (β = -0.2847, p-value = 0.0297). However, this relationship lost significance after adjusting for covariates. In addition, the percent density was positively related to the HMW adiponectin in the unadjusted model (β = 0.2509, p-value = 0.0146). The percent density, though, was no longer related to HMW adiponectin after adjusting for covariates [Table 46].

Absolute Dense Volume and Adipokines in Non-Hispanics

The absolute dense volume was not related to the circulating levels of leptin in the unadjusted model (β = -0.0226, p-value = 0.8491) or in the partially adjusted model (β = 0.1226, p-value = 0.332). However, the absolute dense volume was related to leptin in the fully adjusted model (β = 0.3132, p-value = 0.0319). Additionally, the absolute dense volume showed no relationships with the HMW adiponectin [Table 46].

Non-Dense Volume and Adipokines in Non-Hispanics

The non-dense volume was related to the circulating levels of leptin in the unadjusted model (β = 0.3031, p-value = 0.0001). This relationship remained significant for the partially adjusted model (β = 0.2039, p-value = 0.0101) and for the fully adjusted model (β = 0.2627, p-value = 0.0062). In addition, the non-dense volume was not related to the HMW adiponectin [Table 46].

Total Breast Volume and Adipokines in Non-Hispanics

The total breast volume was positively related to the circulating levels of leptin in the unadjusted model (β = 0.2622, p-value = 0.0001). This relationship remained significant for the partially adjusted model (β = 0.1929, p-value = 0.0053) and for the fully adjusted model (β = 0.2578, p-value = 0.0023). The total breast volume was not related to the fasting circulating levels of HMW adiponectin in the Non-Hispanic cohort [Table 46].

Table 47. Linear regression analysis comparing the adipokines [a] with the breast density measurements [a] in Non-Hispanics (n = 64).

Models of Explicative Variables [a]	Breast Density Measurements [a] (β, 95% CI, p-value)			
	Percent Density	**Absolute Dense Volume**	**Non-Dense Volume**	**Total Breast Volume**
Leptin				
Unadjusted model				
β	-0.2847	-0.0226	0.3031	0.2622
95% CI	(-0.5405, -0.0289)	(-0.2585, 0.2134)	(0.1567, 0.4494)	(0.1379, 0.3864)
p-value	0.0297	0.849	0.0001	0.0001
Partially adjusted model [b]				
β	-0.0703	0.1226	0.2039	0.1929
95% CI	(-0.3283, 0.1877)	(-0.1281, 0.3732)	(0.0503, 0.3574)	(0.0593, 0.3262)
p-value	0.588	0.332	0.0101	0.0053
Fully adjusted model [c]				
β	0.0554	0.3132	0.2627	0.2578
95% CI	(-0.2324, 0.3432)	(0.0286, 0.5978)	(0.0788, 0.4466)	(0.0977, 0.4178)
p-value	0.6993	0.0319	0.0062	0.0023
HMW Adiponectin				
Unadjusted model				
β	0.2509	0.1719	-0.1034	-0.0790
95% CI	(0.0514, 0.4505)	(-0.009, 0.3527)	(-0.231, 0.0242)	(-0.1883, 0.0302)
p-value	0.0146	0.0621	0.1104	0.1533
Partially adjusted model [b]				
β	0.1151	0.1054	-0.0133	-0.0098
95% CI	(-0.0799, 0.3101)	(-0.0854, 0.2961)	(-0.1369, 0.1103)	(-0.1182, 0.0987)
p-value	0.2424	0.2737	0.8303	0.8579
Fully adjusted model [c]				
β	0.1537	0.1199	-0.0381	-0.0337
95% CI	(-0.0513, 0.3586)	(-0.0968, 0.3367)	(-0.1849, 0.1087)	(-0.1644, 0.0969)
p-value	0.1376	0.2702	0.6033	0.6050

(a) Variables followed a non-normal distribution and were base 10 log-transformed.
(b) The partially adjusted model included BMI.
(c) The fully adjusted model included anthropometric measures (BMI, waist circumference, waist-hip ratio), ethnicity, age at baseline visit, age at menarche, menstrual phases, and age at first live birth of a child.

5.4. Discussion

Leptin and adiponectin can contribute to breast cancer burden through promoting breast cell proliferation and apoptosis [Hu 2002, Dossus 2017, Barb 2007]. The present cross-sectional analysis aimed to determine the association between circulating adipokines and breast density parameters, acquired by fat-water MRI, in a cohort of premenopausal women with elements of metabolic syndrome. Leptin was related to the non-dense volume and the total breast volume after adjusting for covariates. In addition, we showed an association between leptin and the absolute dense volume after adjustment of potential confounders. Leptin was not related to the percent density. Furthermore, the HMW adiponectin was not related to the breast parameters following adjustment of potential confounders.

When we conducted the analysis by ethnicity, leptin was positively related to the non-dense volume and the total breast volume. However, those associations lost significance after adjusting for covariates in Hispanics. Leptin was related to the absolute dense volume, after full adjustment, in Non-Hispanics. In addition, Non-Hispanics showed a relationship between leptin and non-dense volume after adjusting for covariates. Similar to this, Non-Hispanics showed a relationship between leptin and total breast volume after full adjustment. The HMW adiponectin was not related to the breast parameters for any of the ethnic groups. More studies with larger multi-ethnic populations are needed to confirm our findings.

Two cross-sectional studies described an inverse association between leptin and the mammographic percent density [Maskarinec 2010, Dossus 2017]. This relationship was lost after adjusting for covariates in one study [Dossus 2017]. Leptin was positively related to the non-dense volume ($r = 0.49$) and inversely related to the absolute dense areas ($r = -0.19$) [Woolcott 2013]. We reported an association between leptin and non-dense volume. Similarly, we observed an inverse association between leptin and percent density and absolute dense volume. Following adjustment for covariates such as anthropometric measures, age, and reproductive factors, leptin was not related to percent density and was positively related to the absolute dense volume. These observations suggest the importance of consideration of confounders in evaluating the relationships between leptin and breast density.

Limited studies examined the relationship between adiponectin and breast density. After adjustment for overall adiposity, the majority of those studies report no association between total adiponectin and breast density [Maskarinec 2010, Woolcott 2013, Dossus 2017]. Similarly, our data are consistent with those findings, as the associations between HMW adiponectin and breast density measurements were attenuated or lost after adjusting for overall adiposity. In addition, the association between HMW adiponectin and breast parameters lost significance after adjusting for covariates.

In this work, we suggest that HMW adiponectin may not exert an effect on breast density. The higher levels of leptin were related to increased absolute dense volume, non-dense volume, and total breast volume after adjusting for potential confounders. We need more studies to determine the underlying biological mechanisms responsible for those associations.

Our study has several strengths, such as the usage of fat-water MRI as the imaging modality to assess breast density on non-compressed breasts. In addition, data collected from the diet, reproductive factors, and the demographics of our study cohort allowed for adjustment for potential confounding effects. We acknowledge that our study population is small, and the translation of our data may require further research with larger populations of premenopausal

women with high adiposity and metabolic disturbances.

In conclusion, we showed that leptin is positively related to the absolute dense volume and non-dense volume in a cohort of premenopausal women with elements of metabolic syndrome. The HMW adiponectin was not related to breast density measurements in our study cohort. We suggest that higher levels of leptin were related to a larger absolute dense volume and non-dense volume, while HMW adiponectin was not related to breast density. Further studies are needed to confirm our findings and to determine the underlying biological mechanisms for those associations.

CONCLUSIONS

Distinct risk factors contribute to the development of breast cancer. Among those, breast density is an established breast cancer risk factor. Metabolic disturbances and high adiposity also increase breast cancer burden. However, the relationship between metabolic disturbances and breast density is inconsistent in the literature. Partial attribution is due to the potential bias in breast density assessment by mammography, where this imaging modality requires breast compression. We performed cross-sectional analysis on the baseline participant data collected from premenopausal women with high adiposity and elements of metabolic syndrome recruited to a Phase II clinical trial. The analysis aimed to determine the associations between metabolic disturbances and breast density parameters acquired by fat-water MRI on non-compressed breasts.

This cross-sectional analysis shows a heterogeneous pattern for the breast density parameters acquired by fat-water MRI for the study cohort. Each breast parameter followed a non-normal distribution comprised of a mix of normally distributed subpopulations.

Our work showed that percent density and absolute density are not related to anthropometric measurements of adiposity. The non-dense volume and total breast volume were only positively related to waist circumference following adjustment of potential confounders of anthropometric measures, age, and reproductive factors. Having elevated blood pressure in women with a large waist was related to a larger non-dense volume and total breast volume in Non-Hispanics. Having elevated fasting glucose in women with a large waist was related to a lower percent and absolute densities.

We showed that the insulin/HOMA-IR was inversely related to the absolute dense volume, while the bioavailable IGF-1 was positively related to the percent density. The findings of the inverse associations between insulin/HOMA-IR and absolute dense volume are consistent with the observed inverse associations between elevated fasting glucose and absolute dense volume and percent density. These data suggest that the insulin/IGF axis may have a differential relationship with breast density. Furthermore, we showed that leptin is positively related to both the absolute dense volume and non-dense volume after adjustment of confounders of anthropometric measures, age, and reproductive factors. The HMW adiponectin is not related to breast density measurements in our study cohort. These data suggest that higher leptin levels are linked to larger absolute dense volume and non-dense volume while HMW adiponectin is not related to breast density.

This cross-sectional analysis provides additional evidence of the associations between metabolic risk factors and breast density parameters in a cohort of overweight/obese premenopausal women with metabolic dysregulation. To our knowledge, this is the first study to evaluate these associations by using breast density parameters derived from fat-water MRI. We acknowledge that our sample size is small. Additional studies are needed to confirm the observed associations. Future studies are required to determine the causal relationships of those associations affecting breast density and to develop strategies for reducing breast cancer risk.

APPENDIX A: Additional Figures

Figure A1. Consort Flowchart of Phase II Trial of Metformin for Obesity-Associated Breast Cancer Risk (as of February, 2017).

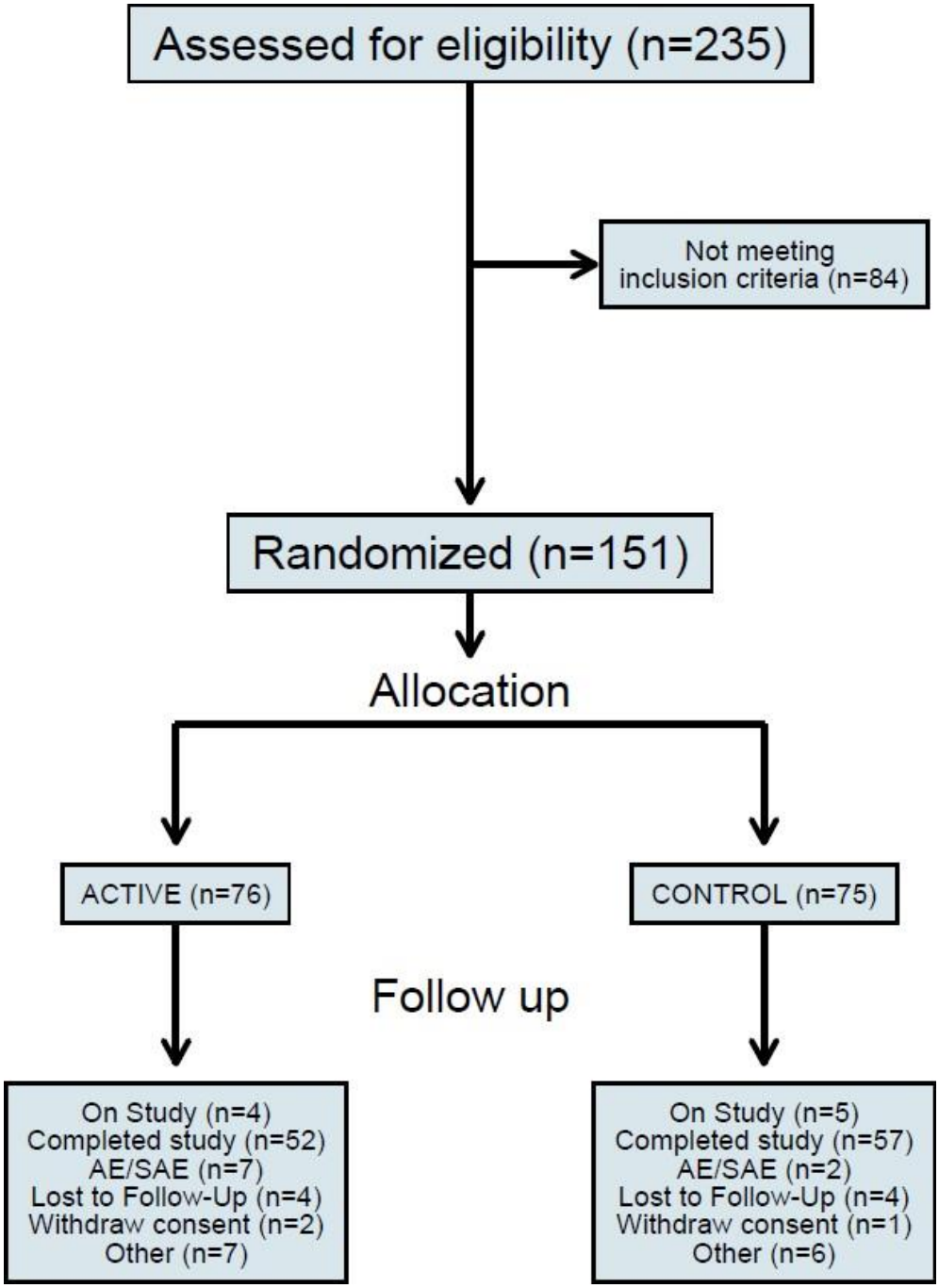


Figure A2. Percent Density and Anthropometric Measurements of Adiposity by Ethnicity.

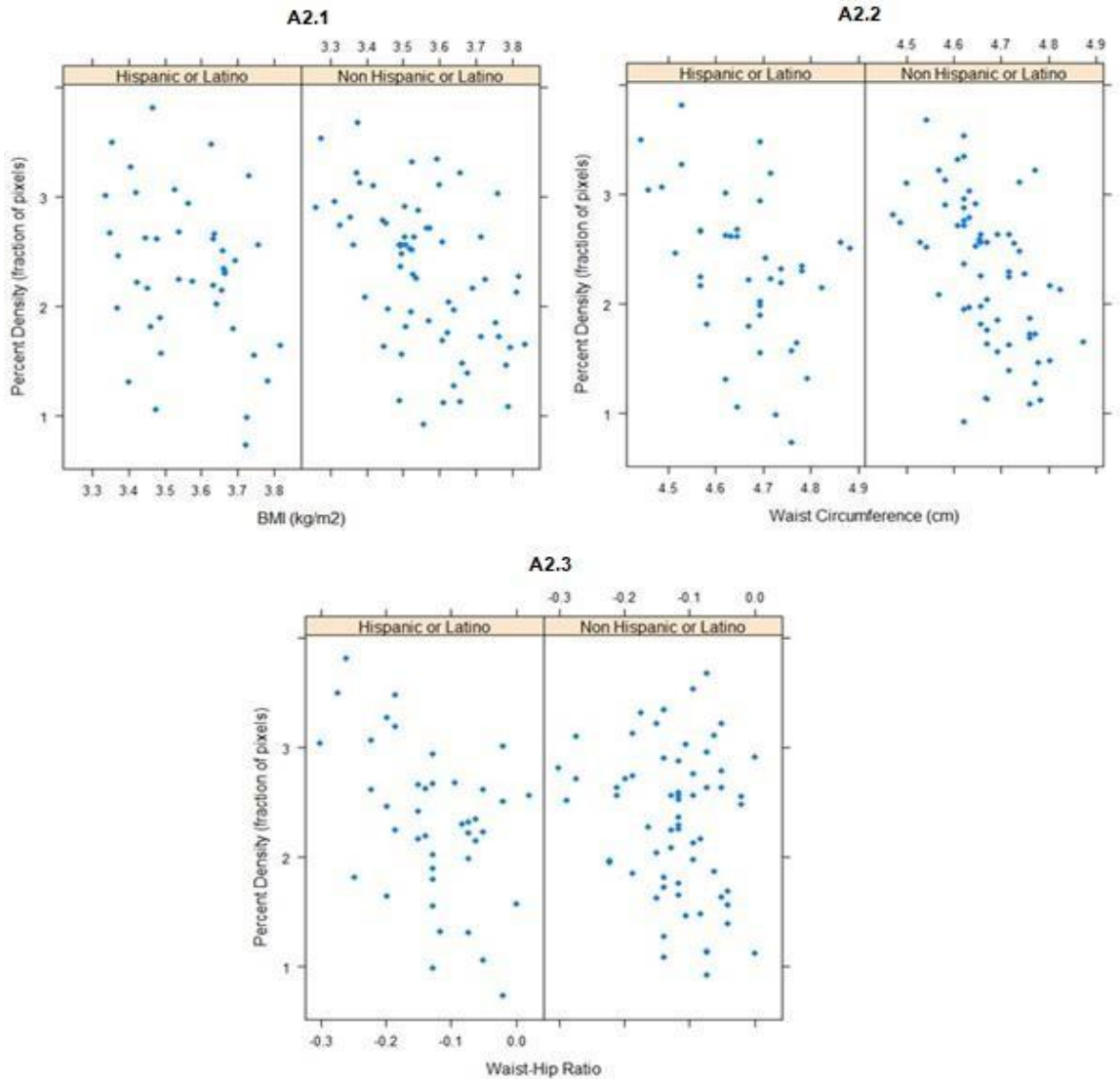


Figure A2.1 Percent density and BMI stratified by ethnicity. Hispanics present a statistically significant correlation of the percent density with the BMI (r= -0.41, p< 0.00001). Non-Hispanics present a statistically significant correlation of the percent density with the BMI (r= -0.50, p< 0.00001).

Figure A2.2. Percent density and waist circumference stratified by ethnicity. Hispanics present a statistically significant correlation of the percent density with the waist circumference (r= -0.48, p< 0.00001). Non-Hispanics present a statistically significant correlation of the percent density with the waist circumference (r= -0.52, p< 0.00001).

Figure A2.3. Percent density and waist-hip ratio stratified by ethnicity. Hispanics present a statistically significant correlation of the percent density with the waist-hip ratio (r= -0.33, p= 0.0006). Non-Hispanics do not present a statistically significant correlation.

The percent density and the anthropometric measures presented a non-normal distribution and were log base 10 transformed.

Figure A3. Absolute Dense Volume and Anthropometric Measurements of Adiposity by Ethnicity.

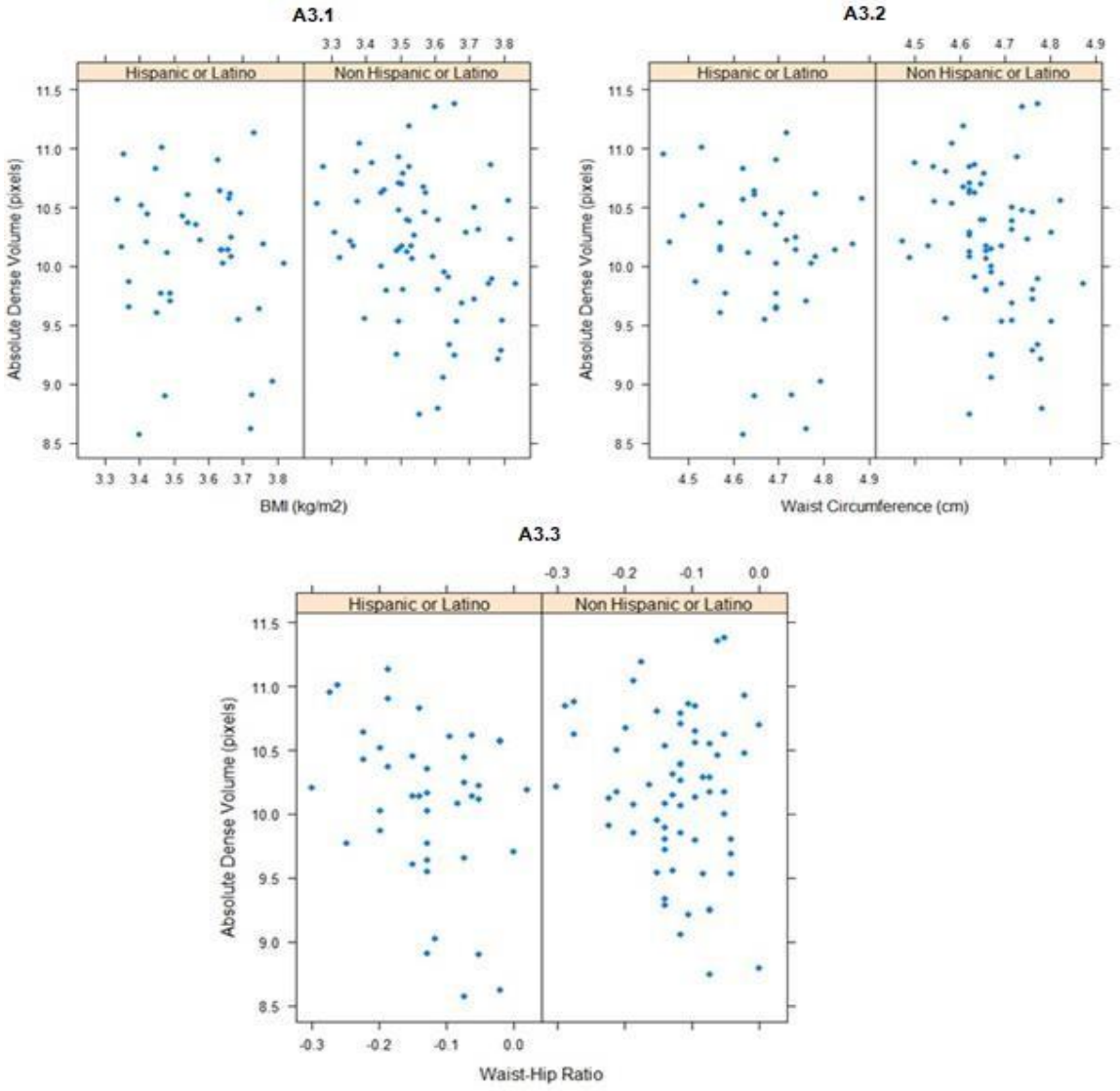


Figure A3.1. Absolute dense volume and BMI stratified by ethnicity. Hispanics (r= -0.21, p= 0.0325) and Non-Hispanics (r= -0.30, p= 0.0164) present an inverse correlation of the absolute dense volume with the BMI.

Figure A3.2. Absolute dense volume and waist circumference stratified by ethnicity. Hispanics (r= -0.24, p= 0.135) and Non-Hispanics (r= -0.28, p= 0.0252) present an inverse correlation of the absolute dense volume with the waist circumference.

Figure A3.3. Absolute dense volume and waist-hip ratio stratified by ethnicity. Hispanics present a statistically significant correlation of the absolute dense volume with the waist-hip ratio (r= -0.26, p= 0.0075). Non-Hispanics do not present a statistically significant correlation.

The absolute dense volume and the anthropometric measures presented a non-normal distribution and were log base 10 transformed.

Figure A4. Non-Dense Volume and Anthropometric Measurements of Adiposity by Ethnicity.

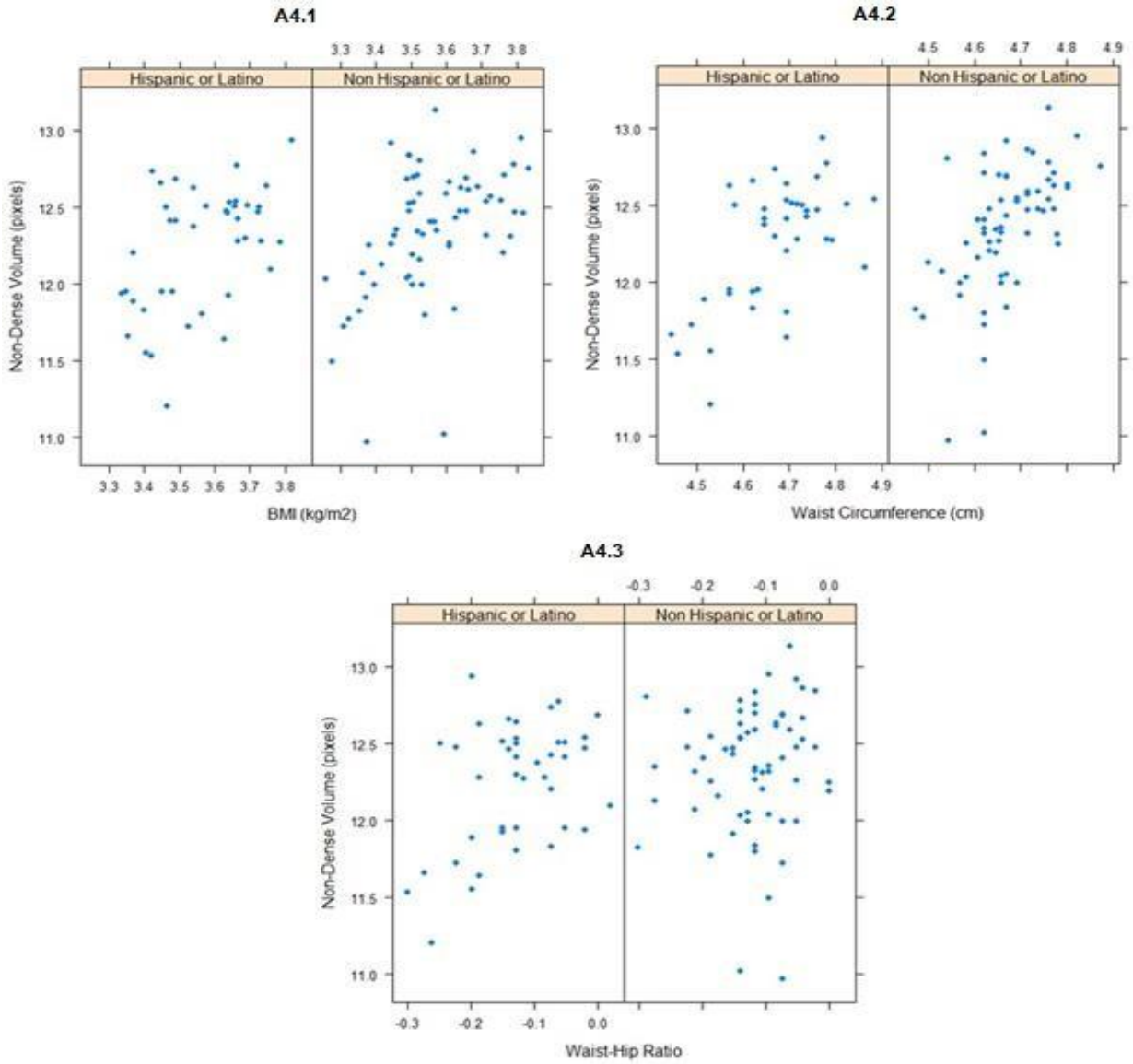


Figure A4.1. Non-dense volume and BMI stratified by ethnicity. Hispanics present a statistically significant correlation of the non-dense volume with the BMI (r= 0.47, p< 0.00001). Non-Hispanics present a statistically significant correlation of the non-dense volume with the BMI (r= 0.49, p< 0.00001).

Figure A4.2. Non-dense volume and waist circumference stratified by ethnicity. Hispanics present a statistically significant correlation of the non-dense volume with the waist circumference (r= 0.58, p< 0.00001). Non-Hispanics present a statistically significant correlation of the non-dense volume with the waist circumference (r= 0.56, p<0.00001).

Figure A4.3. Non-dense volume and waist-hip ratio stratified by ethnicity. Hispanics present a statistically significant correlation of the non-dense volume with the waist-hip ratio (r= 0.24, p= 0.0143). Non-Hispanics do not present a statistically significant correlation.

The non-dense volume and the anthropometric measures followed a non-normal distribution and were log base 10 transformed.

Figure A5. Total Breast Volume and Anthropometric Measurements of Adiposity by Ethnicity.

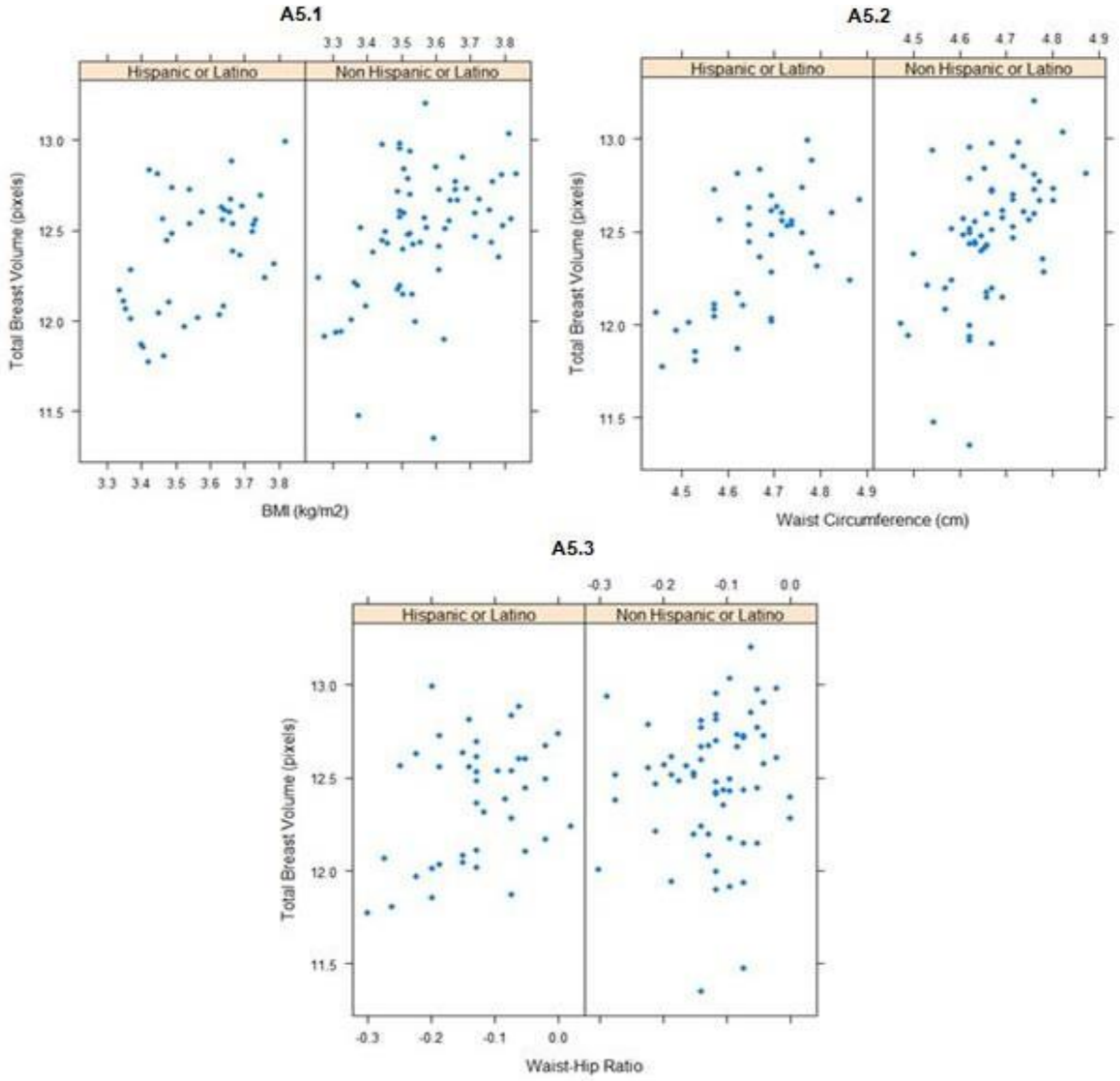


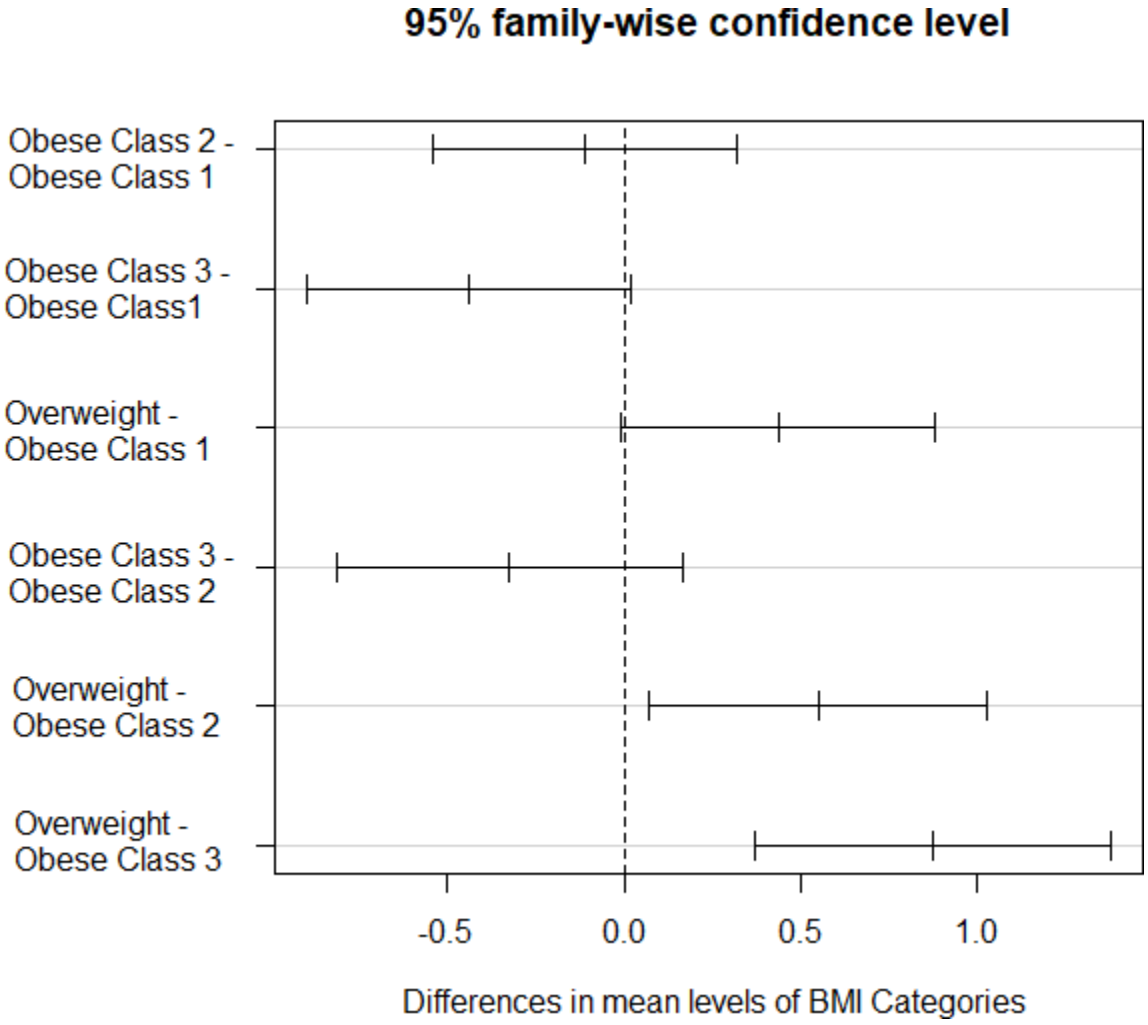
Figure A5.1. Total breast volume and BMI stratified by ethnicity. Hispanics present a statistically significant correlation of the total breast volume with the BMI (r= 0.45, p< 0.00001). Non-Hispanics present a statistically significant correlation of the total breast volume with the BMI (r= 0.44, p= 0.0003).

Figure A5.2. Total breast volume and waist circumference stratified by ethnicity. Hispanics present a statistically significant correlation of the total breast volume with the waist circumference (r= 0.54, p< 0.00001). Non-Hispanics present a statistically significant correlation of the total breast volume with the waist circumference (r= 0.52, p<0.00001).

Figure A5.3. Total breast volume and waist-hip ratio stratified by ethnicity. Hispanics present a borderline statistically significant correlation of the total breast volume with the waist-hip ratio (r= 0.19, p= 0.0504). Non-Hispanics do not present statistically significant correlations.

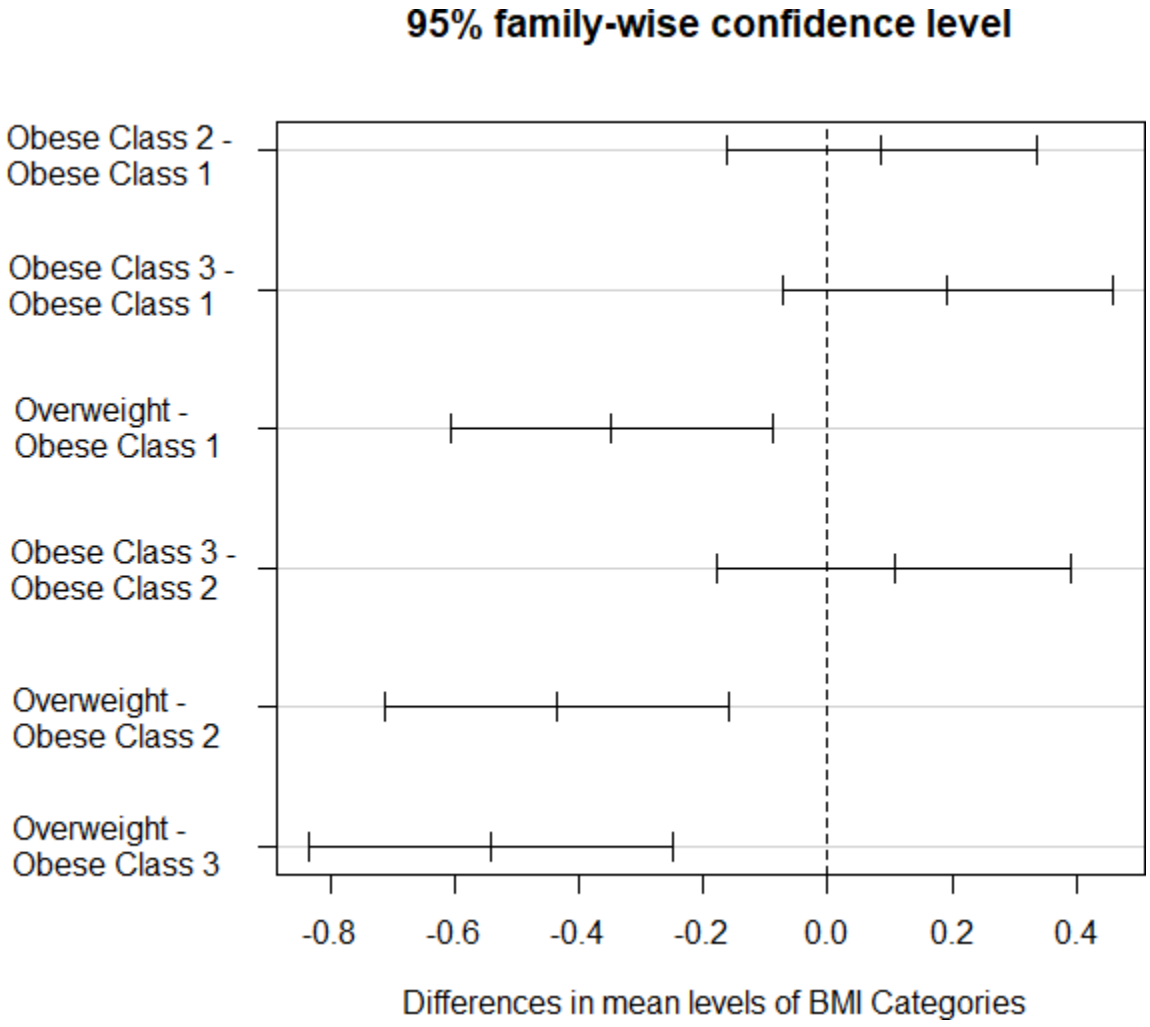
The total breast volume and the anthropometric measures were non-normally distributed and were log base 10 transformed.

Figure A6. Pairwise comparisons of the BMI categories in terms of the means of the percent density.



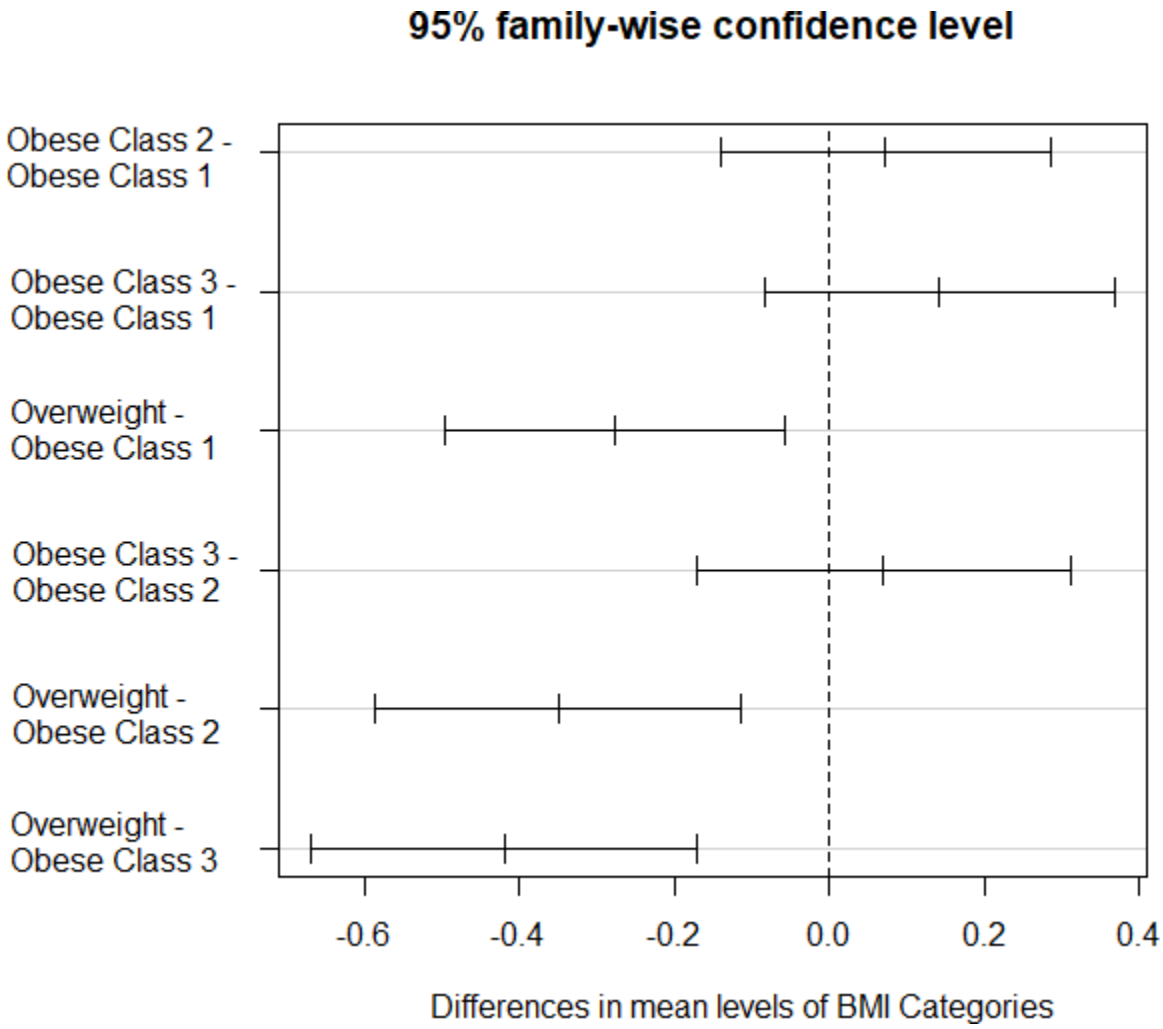
Pairwise comparisons of means were conducted by Tukey's test in terms of the percent density. According to the multiple pairwise comparison, the overweight class (25.0 – 29.9 kg/m^2) was considered as different from the obese class 1 (30.0 – 34.9 kg/m^2), the obese class 2 (35.0 – 39.9 kg/m^2), and the obese class 3 (≥ 40.0 kg/m^2). No significant differences were observed for other pairwise comparisons.

Figure A7. Pairwise comparisons of the BMI categories in terms of the means of the non-dense volume.



Pairwise comparisons of means were conducted by Tukey's test in terms of the non-dense volume. According to the multiple pairwise comparisons, the overweight class (25.0 – 29.9 kg/m^2) was considered as different from the obese class 1 (30.0 – 34.9 kg/m^2), the obese class 2 (35.0 – 39.9 kg/m^2), and the obese class 3 (≥ 40.0 kg/m^2). No significant differences were observed for other pairwise comparisons.

Figure A8. Pairwise comparisons of the BMI categories in terms of the means of the total breast volume.



Pairwise comparisons of means were conducted by Tukey's test in terms of the total breast volume. According to the multiple pairwise comparisons, the overweight class (25.0 – 29.9 kg/m^2) was considered as different from the obese class 1 (30.0 – 34.9 kg/m^2), the obese class 2 (35.0 – 39.9 kg/m^2), and the obese class 3 (≥ 40.0 kg/m^2). No significant differences were observed for other pairwise comparisons.

Figure A9. The Circulating Levels of Fasting Insulin [a] and Breast Density Measurements [a] by Ethnicity.

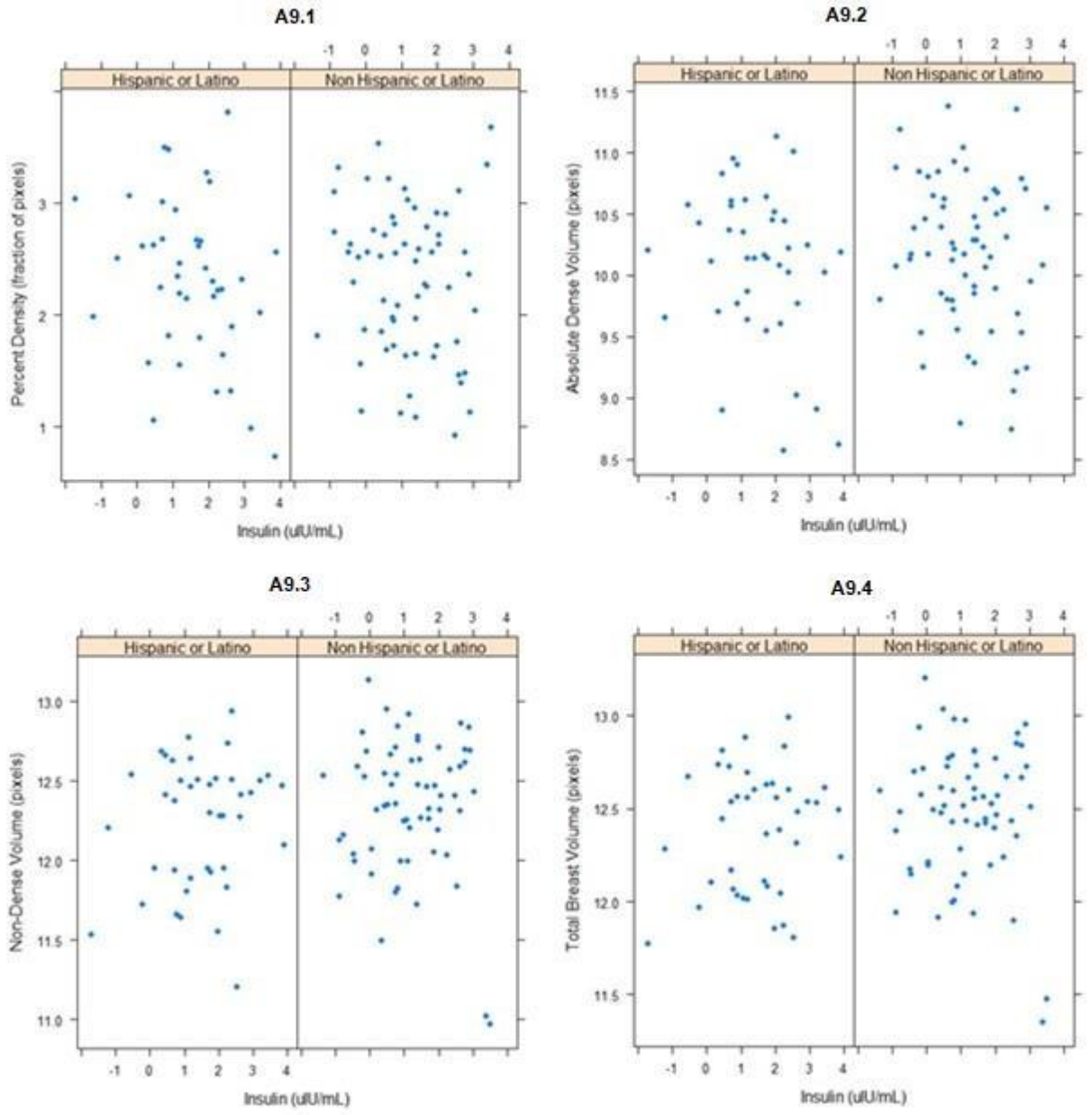


Figure A9.1. The circulating fasting serum insulin (uIU/ml) and the percent density (fraction) by ethnicity.
Figure A9.2. The circulating fasting serum insulin (uIU/ml) and the absolute dense volume (pixel units) by ethnicity.
Figure A9.3. The circulating fasting serum insulin (uIU/ml) and the non-dense volume (pixel units) by ethnicity.
Figure A9.4. The circulating fasting serum insulin (uIU/ml) and the total breast volume (pixel units) by ethnicity.
(a) Variables followed a non-normal distribution and were log base 10 transformed.

Figure A10. The HOMA-IR[a] and Breast Density Measurements[a] by Ethnicity.

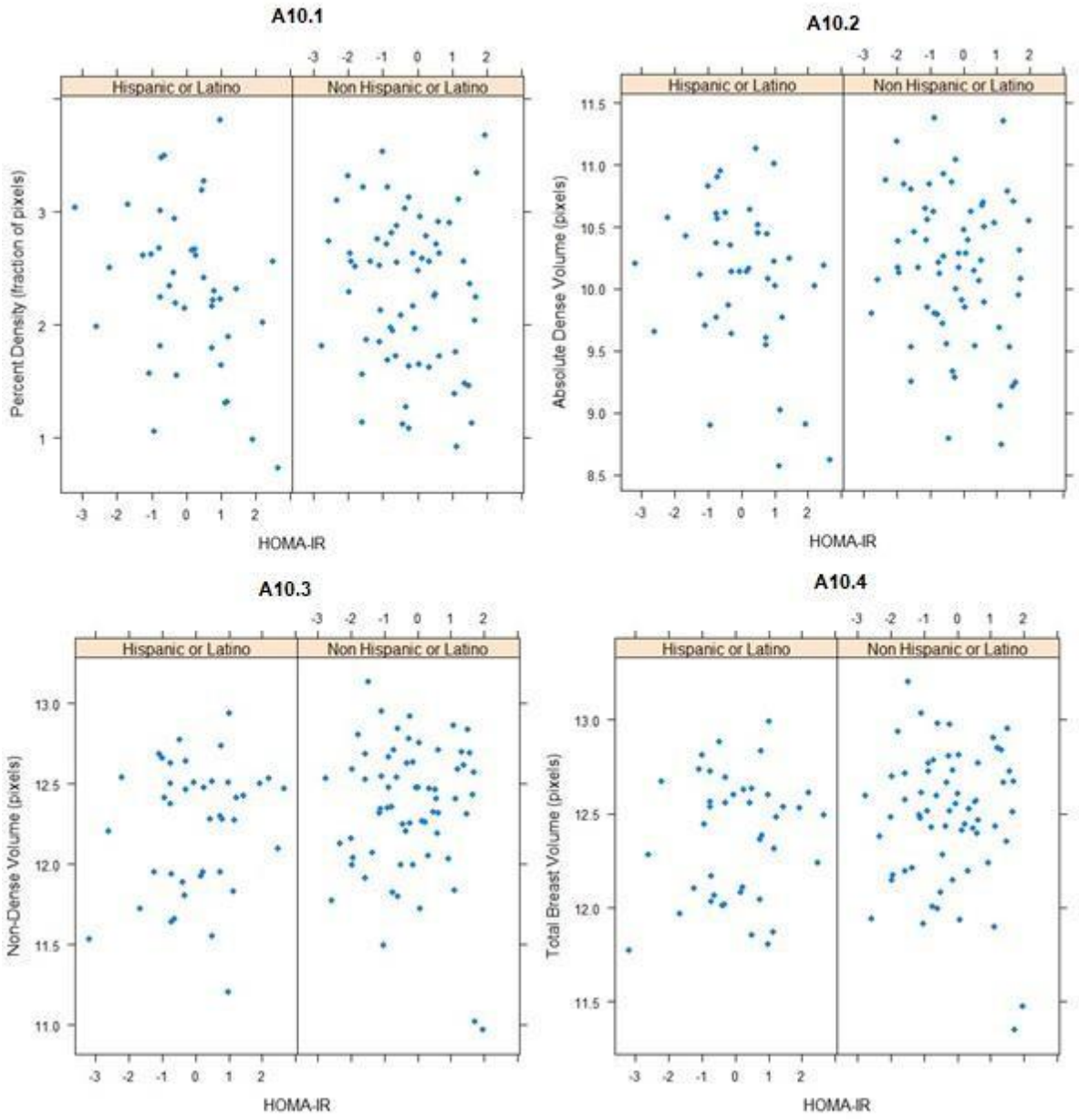


Figure A10.1. The HOMA-IR and the percent density (fraction) by ethnicity.
Figure A10.2. The HOMA-IR and the absolute dense volume (pixel units) by ethnicity.
Figure A10.3. The HOMA-IR and the non-dense volume (pixel units) by ethnicity.
Figure A10.4. The HOMA-IR and the total breast volume (pixel units) by ethnicity.
(a) Variables followed a non-normal distribution and were log base 10 transformed.

Figure A11. The Circulating Levels of IGF-1[a] and Breast Density Measurements[a] by Ethnicity.

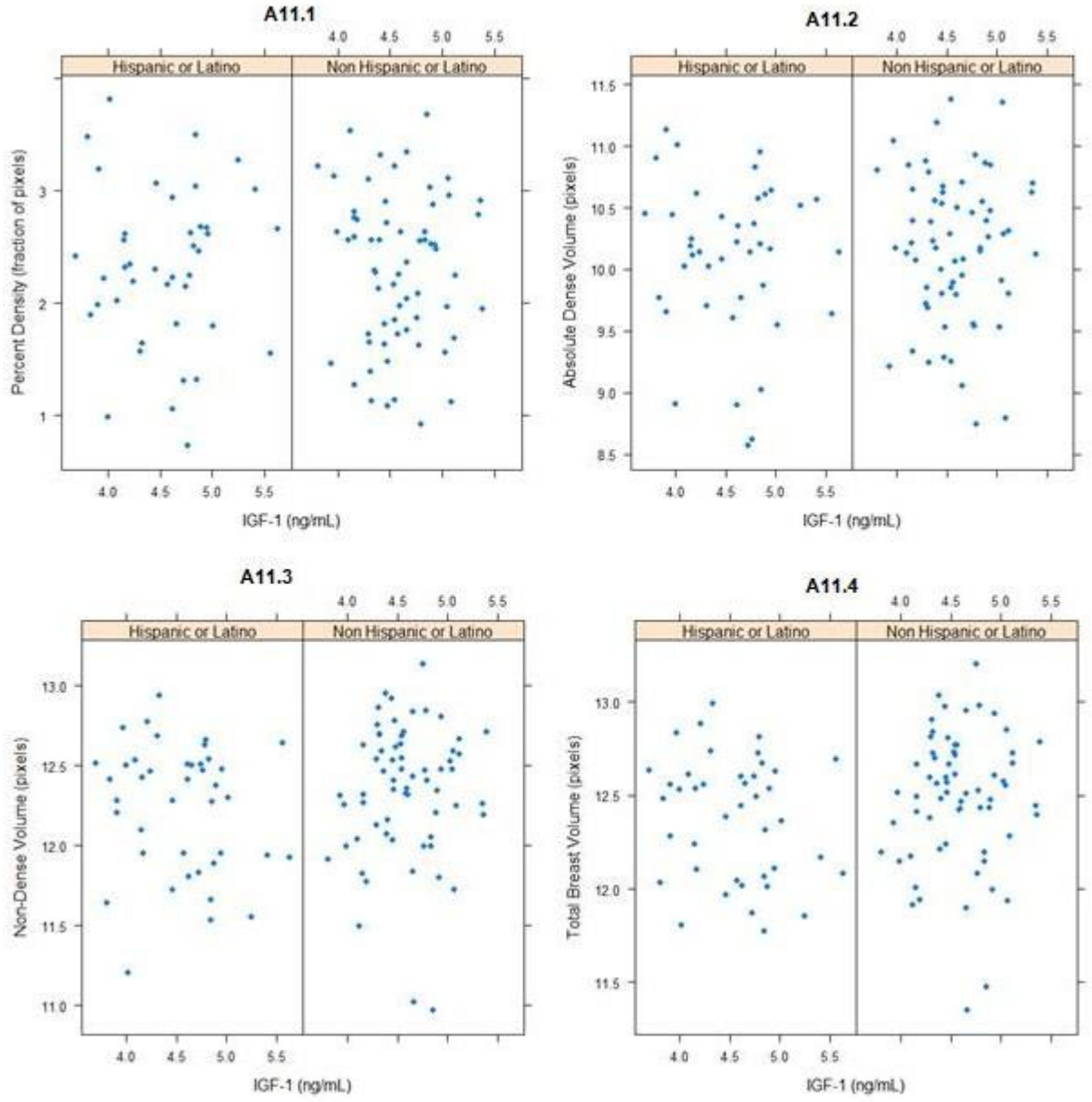


Figure A11.1. The circulating fasting serum IGF-1 (ng/ml) and the percent density (fraction) by ethnicity.
Figure A11.2. The circulating fasting serum IGF-1 (ng/ml) and the absolute dense volume (pixel units) by ethnicity.
Figure A11.3. The circulating fasting serum IGF-1 (ng/ml) and the non-dense volume (pixel units) by ethnicity.
Figure A11.4. The circulating fasting serum IGF-1 (ng/ml) and the total breast volume (pixel units) by ethnicity.
(a) Variables followed a non-normal distribution and were log base 10 transformed.

Figure A12. The Circulating Levels of IGFBP-3[a] and Breast Density Measurements[a] by Ethnicity.

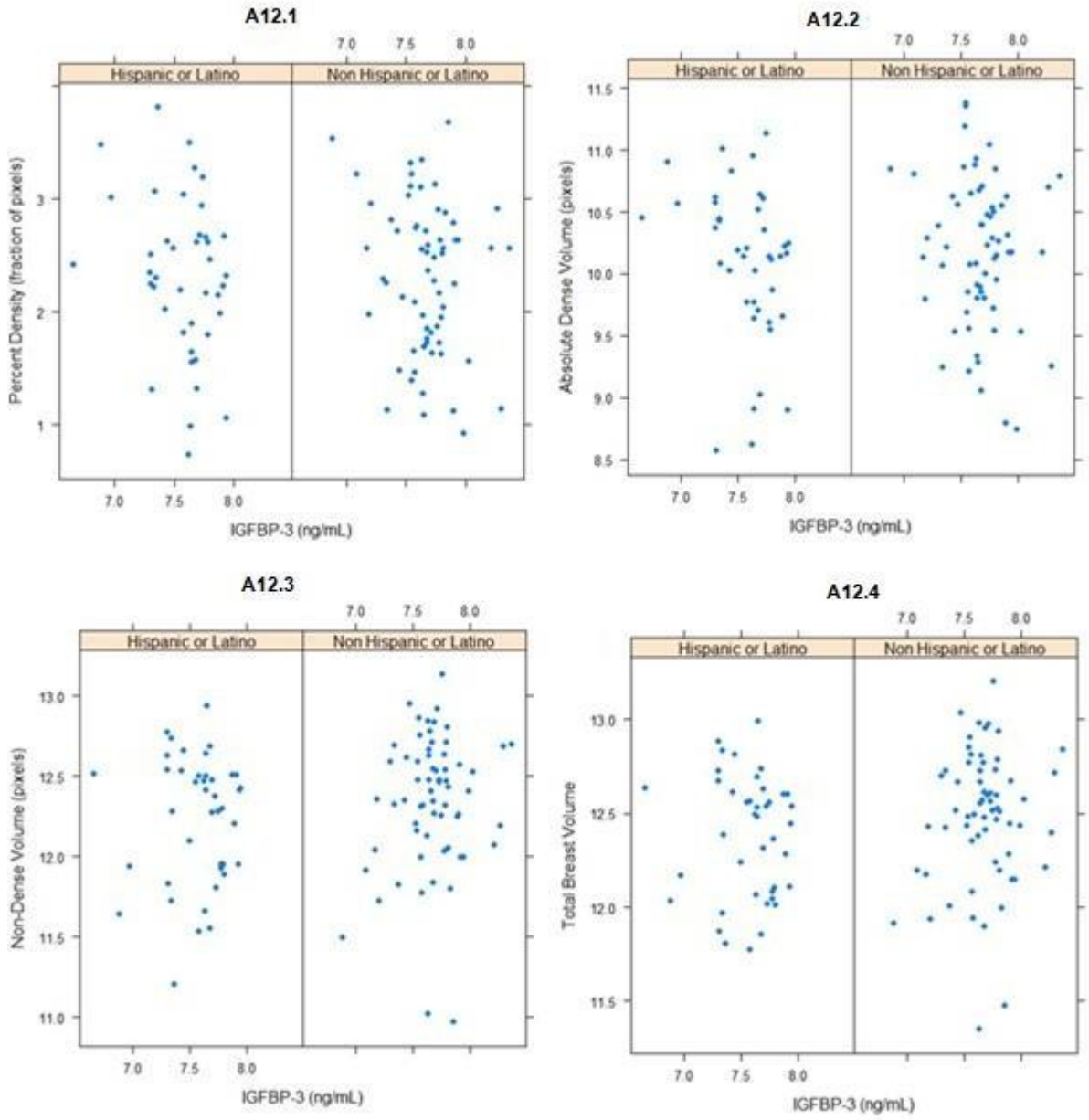


Figure A12.1. The circulating fasting serum IGFBP-3 (ng/ml) and the percent density (fraction) by ethnicity.
Figure A12.2. The circulating fasting serum IGFBP-3 (ng/ml) and the absolute dense volume (pixel units) by ethnicity.
Figure A12.3. The circulating fasting serum IGFBP-3 (ng/ml) and the non-dense volume (pixel units) by ethnicity.
Figure A12.4. The circulating fasting serum IGFBP-3 (ng/ml) and the total breast volume (pixel units) by ethnicity.
(a) Variables followed a non-normal distribution and were log base 10 transformed.

Figure A13. The Molar Ratio IGF-1/IGFBP-3[a] and Breast Density Measurements[a] by Ethnicity.

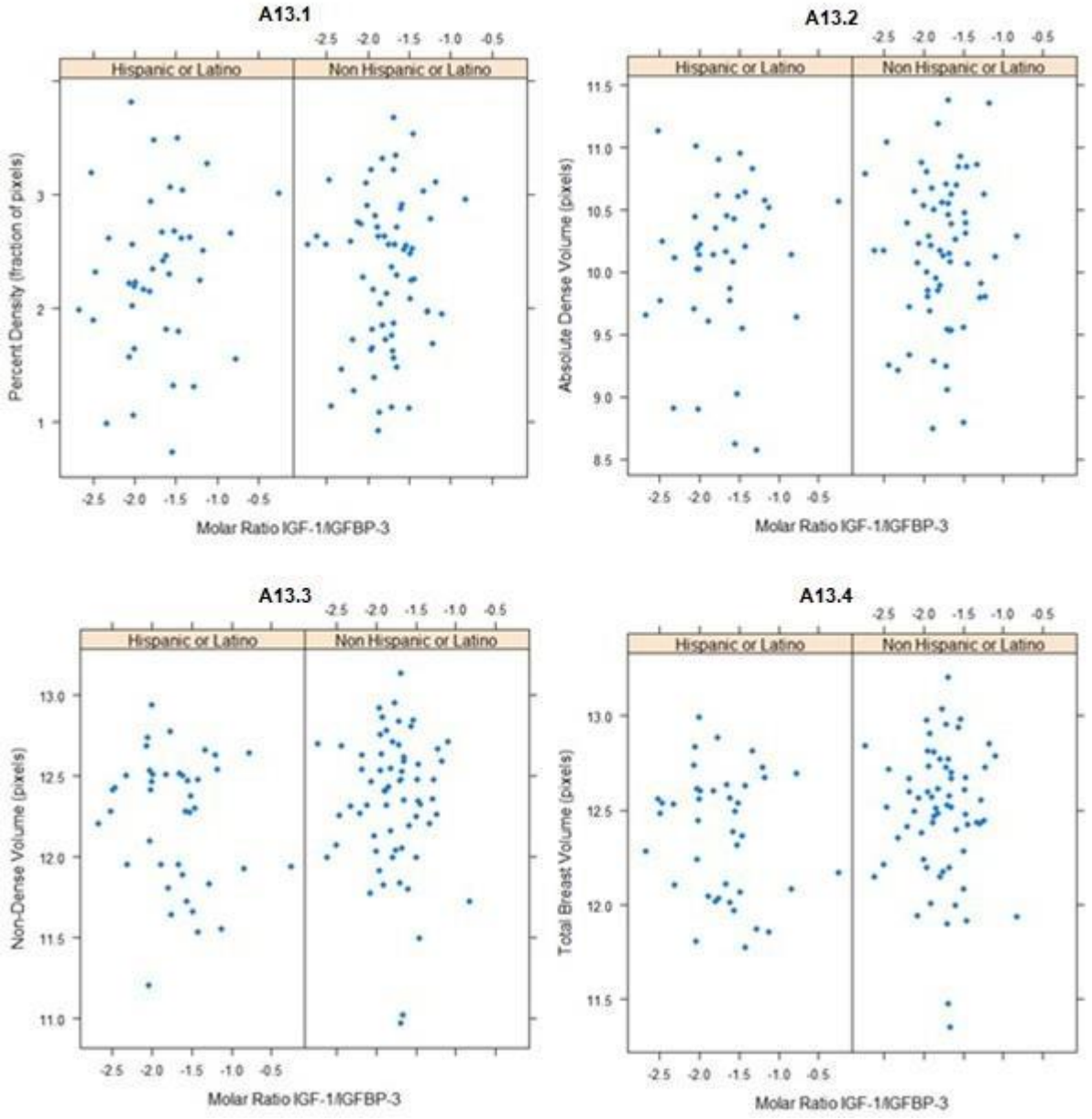


Figure A13.1. The molar ratio IGF-1/IGFBP-3 and the percent density (fraction) by ethnicity.
Figure A13.2 The molar ratio IGF-1/IGFBP-3 and the absolute dense volume (pixel units) by ethnicity.
Figure A13.3 The molar ratio IGF-1/IGFBP-3 and the non-dense volume (pixel units) by ethnicity.
Figure A13.4 The molar ratio IGF-1/IGFBP-3 and the total breast volume (pixel units) by ethnicity.
(a) Variables followed a non-normal distribution and were log base 10 transformed.

Figure A14. The Fasting Circulating Levels of Leptin [a] and Breast Density Measurements [a] by Ethnicity.

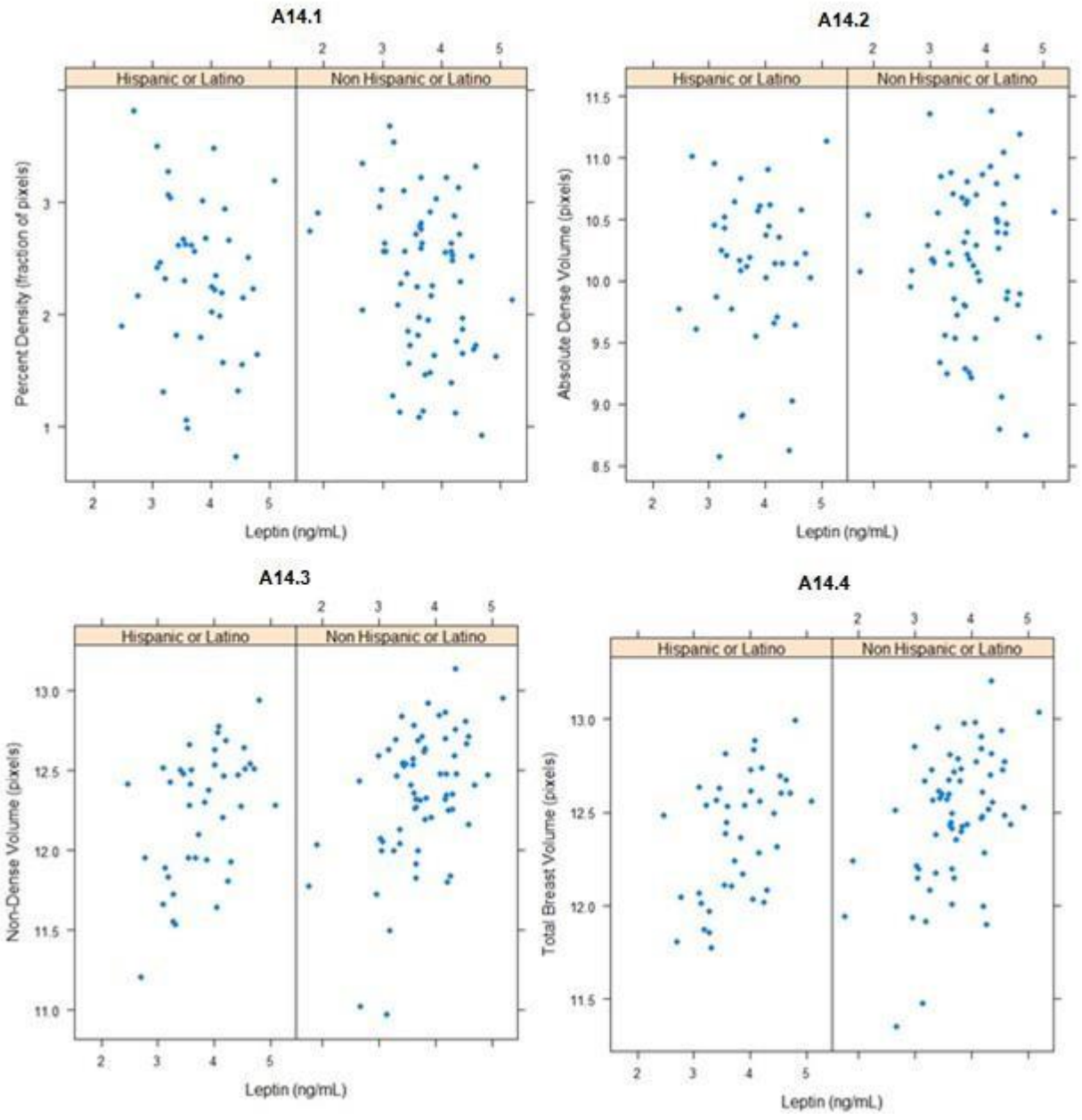


Figure A14.1. The fasting circulating levels of leptin and the percent density (fraction of pixels) by ethnicity.
Figure A14.2. The fasting circulating levels of leptin and the absolute dense volume (pixel units) by ethnicity.
Figure A14.3. The fasting circulating levels of leptin and the non-dense volume (pixel units) by ethnicity.
Figure A14.4. The fasting circulating levels of leptin and the total breast volume (pixel units) by ethnicity.
(a) Variables presented a non-normal distribution and were log base 10 transformed.

Figure A15. The Fasting Circulating Levels of HMW Adiponectin [a] and Breast Density Measurements [a] by Ethnicity.

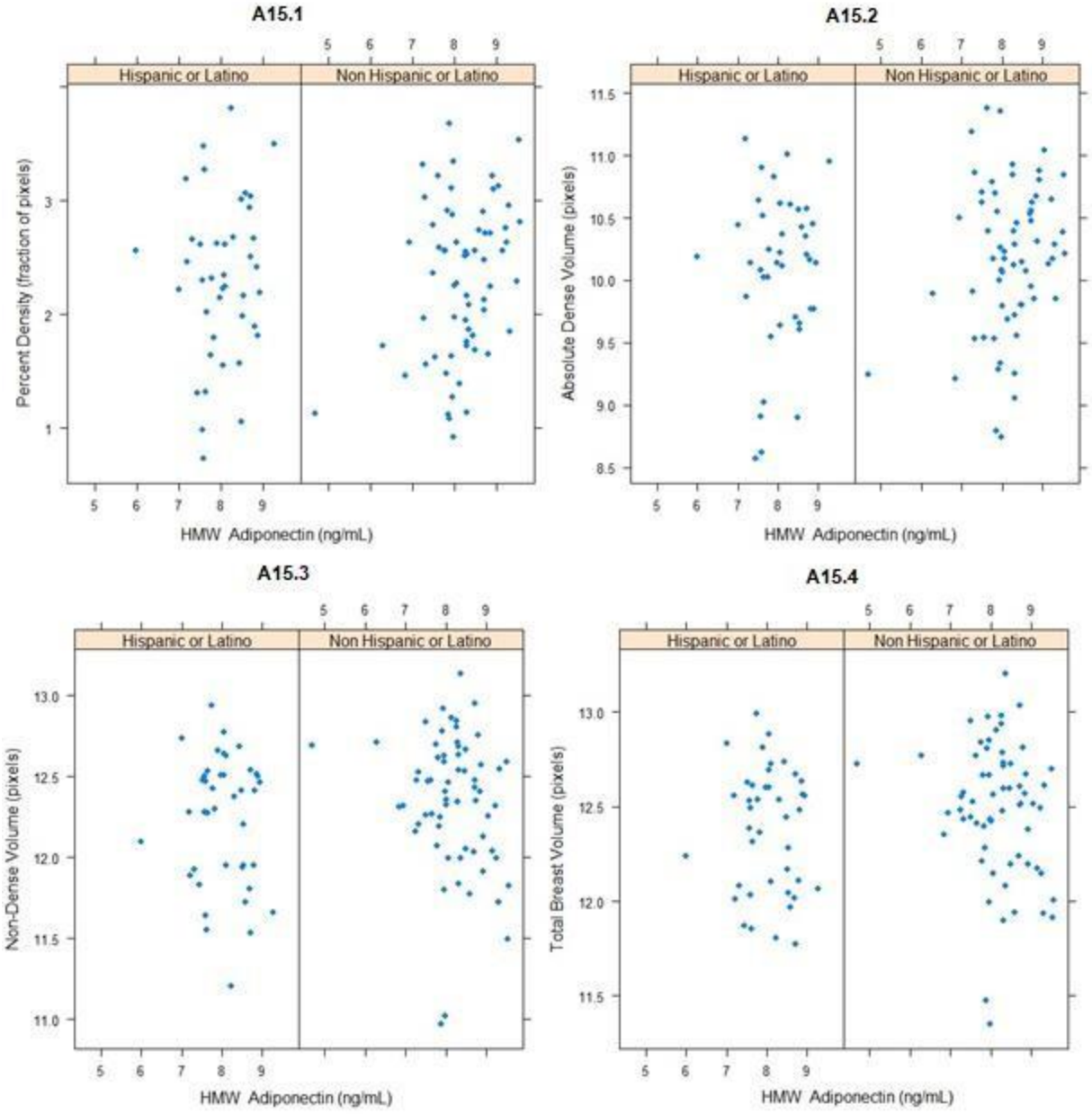


Figure A15.1. The fasting circulating levels of HMW adiponectin and the percent density (fraction) by ethnicity.
Figure A15.2. The fasting circulating levels of HMW adiponectin and the absolute dense volume (pixel units) by ethnicity.
Figure A15.3. The fasting circulating levels of HMW adiponectin and the non-dense volume (pixel units) by ethnicity.
Figure A15.4. The fasting circulating levels of HMW adiponectin and the total breast volume (pixel units) by ethnicity.
(a) Variables presented a non-normal distribution and were log base 10 transformed.

APPENDIX B: Additional Tables

Table B1. Pairwise comparisons of the BMI categories in terms of the means of the percent density.

Pairwise Comparison	Mean Difference	Lower limit	Upper limit	Adjusted p-value
Obese Class 2 - Obese Class 1	-0.113	-0.543	0.315	0.89
Obese Class 3 - Obese Class 1	-0.439	-0.897	0.018	0.06
Overweight - Obese Class 1	0.434	-0.010	0.879	0.05*
Obese Class 3 – Obese Class 2	-0.325	-0.814	0.164	0.31
Overweight - Obese Class 2	0.548	0.070	1.025	0.01*
Overweight - Obese Class 3	0.873	0.370	1.376	0.00*

*P-value ≤ 0.05 and considered as statistically significant.

A Tukey's test was conducted to evaluate pairwise comparisons of means in terms of the percent density. The overweight class is shown as different from the obese class 1 (p-value= 0.05), the obese class 2 (p-value= 0.01), and the obese class 3 (p-value< 0.01) in terms of the means of the percent density. Overweight class cutoff is from 25.0 – 29.9 kg/m^2, obese class 1 cutoff is from 30.0 – 34.9 kg/m^2, obese class 2 cutoff is from 35.0 – 39.9 kg/m^2, and obese class 3 cutoff is ≥40.0 kg/m^2.

Table B2. Pairwise comparisons of the BMI categories in terms of the means of the non-dense volume.

Pairwise Comparison	Mean Difference	Lower Limit	Upper Limit	Adjusted p-value
Obese Class 2 – Obese Class 1	0.086	-0.162	0.335	0.801
Obese Class 3 – Obese Class 1	0.192	-0.073	0.458	0.237
Overweight – Obese Class 1	-0.348	-0.606	-0.089	0.003*
Obese Class 3 – Obese Class 2	0.106	-0.177	0.390	0.762
Overweight – Obese Class 2	-0.434	-0.711	-0.157	0.000*
Overweight – Obese Class 3	-0.540	-0.833	-0.248	0.000*

*P-value ≤ 0.05 and considered as statistically significant.

A Tukey's test was conducted to evaluate pairwise comparisons of means in terms of the non-dense volume. The overweight class is shown as different from the obese class 1 (p-value= 0.003), the obese class 2 (p-value< 0.001), and the obese class 3 (p-value< 0.001) in terms of the means of the non-dense volume. No significant differences were observed for other pairwise comparisons. The overweight class cutoff is from 25.0 – 29.9 kg/m^2, obese class 1 cutoff is from 30.0 - 34.9 kg/m^2, obese class 2 cutoff is from 35.0 – 39.9 kg/m^2, and obese class 3 cutoff is ≥40.0 kg/m^2.

Table B3. Pairwise comparisons of the BMI categories in terms of the means of the total breast volume.

Pairwise Comparison	Mean Difference	Lower Limit	Upper Limit	Adjusted p-value
Obese Class 2 – Obese Class 1	0.072	-0.139	0.285	0.807
Obese Class 3 – Obese Class 1	0.142	-0.084	0.369	0.359
Overweight – Obese Class 1	-0.277	-0.497	-0.057	0.007*
Obese Class 3 – Obese Class 2	0.069	-0.172	0.311	0.875
Overweight – Obese Class 2	-0.350	-0.586	-0.114	0.001*
Overweight – Obese Class 3	-0.420	-0.669	-0.170	0.000*

*P-value ≤ 0.05 and considered as statistically significant.

A Tukey's test was conducted to evaluate pairwise comparisons of means in terms of the total breast volume. The overweight class is shown as different from the obese class 1 (p-value= 0.003), the obese class 2 (p-value< 0.001), and the obese class 3 (p-value< 0.001) in terms of the means of the total breast volume. No significant differences were observed for other pairwise comparisons. The overweight class cutoff is from 25.0 – 29.9 kg/m^2, obese class 1 cutoff is from 30.0 - 34.9 kg/m^2, obese class 2 cutoff is from 35.0 – 39.9 kg/m^2, and obese class 3 cutoff is ≥40.0 kg/m^2.

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