ABSTRACT

AROMATASE PROTEIN CONTENT IN GLUTEAL AND ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE IN PREMENOPAUSAL CAUCASIAN AND AFRICAN AMERICAN WOMEN

by Elizabeth Cooper

December, 2016

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OBJECTIVE: To determine if aromatase protein content differs in abdominal and gluteal adipose tissue and to determine if there are racial differences in aromatase protein content that might influence racial differences in regional body fat distribution in overweight to obese premenopausal women.

MATERIALS/METHODS: Biopsies of the subcutaneous abdominal and gluteal adipose tissue were performed in 15 premenopausal women (7 C/8 AA, 25.1 $\pm$ 1.8 years, BMI 29.5 $\pm$ 0.5kg/m(2)). Adipose tissue protein content was measure by western blot analysis. Independent sample t-tests were used to determine racial differences in subject characteristics and sex hormones. Two-way repeated measure ANOVA (race x region) was used to determine racial differences (C versus AA) in estrogen receptor expression in the abdominal and gluteal SAT.

RESULTS: Aromatase protein content was not different between races or regions: C abdominal 0.25±0.06, C gluteal 0.25±0.12, AA abdominal 0.35±0.08, AA gluteal 0.32±0.09 arbitrary units (2-way ANOVA; race x region $p=0.73$, race $p=0.46$, region $p=0.71$).

CONCLUSIONS: This study showed no significant differences in aromatase protein content between regions or races in the observed group. No regional or racial differences in aromatase protein content were detected in this study.

AROMATASE PROTEIN CONTENT IN GLUTEAL AND ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE IN PREMENOPAUSAL CAUCASIAN AND AFRICAN AMERICAN WOMEN

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Elizabeth Cooper
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CHAPTER 1

INTRODUCTION

Obesity is one of the leading health issues in the world today, and incidence in the United States has risen dramatically over the past decades. [1, 2] Today, about one third of adult Americans are obese (33.8%), and no single state has a prevalence of obesity of less than 20%. Obesity carries with it the increased risk of many diseases, including coronary heart disease, Type 2 diabetes, many cancers, dyslipidemia and stroke.[1]

The distribution of subcutaneous adipose tissue (SAT), fat deposits beneath the skin, may have an effect on the severity of disease associated with obesity. Lower body adipose distribution, or gynoid distribution, is associated with a lower risk of metabolic disease than upper body distribution, or android distribution. Gynoid distribution could even have a protective effect against disease, as opposed to simply being less harmful as compared to android distribution.[4, 5] Gynoid obesity is generally of highest prevalence in women, whereas android obesity is generally of highest prevalence in men. However, women's transition through menopause, during which women experience a decrease in estrogen, is characterized by a shift in fat mass from gynoid to android distribution.[4,5] In order to advance efforts to prevent obesity and obesity-related chronic disease, it is important to understand the physiological mechanisms behind adipose tissue accumulation and reduction in each region of the body.

There is great interest in the regulation of SAT since excess adiposity is associated with a higher risk of metabolic abnormalities, such as diabetes and atherosclerosis.[6] Research suggests that estrogen has a significant influence on the distribution of SAT.[7–9] Specifically, as circulating estrogen levels decrease, especially during menopause, there is an increase in android distribution and higher chronic disease risk.[5,10,11] Estrogen has been shown to directly influence

the metabolism and the location of adipose tissue by altering lipogenesis and lipolysis.[12] Lipogenesis is the synthesis of fatty acids and triglycerides and lipolysis is the reverse, or the breakdown of fatty acids and triglycerides. In lipolysis, adipose triglyceride lipase (ATGL) initiates the hydrolysis of triacylglycerol (TAG) into diacylglycerols (DG) and free fatty acids (FFA). Hormone sensitive lipase (HSL) hydrolyses triacylglycerols, diacylglycerols and monoacylglycerols. Monoglyceride lipase hydrolyses monacylglycerol to form glycerol and fatty acids. [13]

Estrogen influences the metabolism and regional distribution of SAT via lipogenesis and lipolysis.[14–17,13] Aromatase is one of the enzymes that has a great influence on the regulation of these estrogens (Figure 1).[18,19,20,21]

Schematic of Tissue Steroid Metabolism[2]

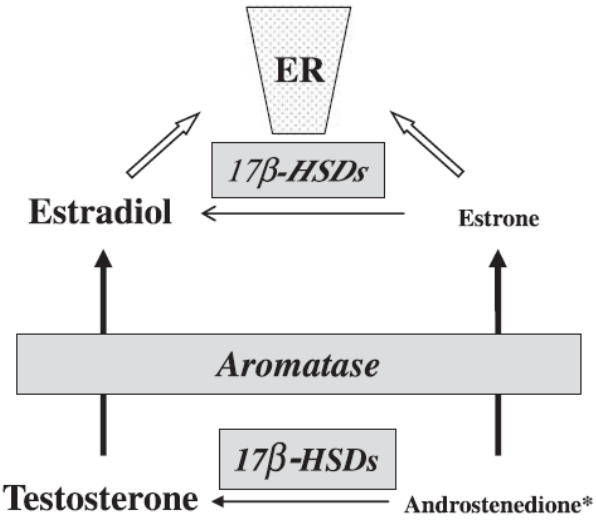

Figure 1. Aromatase is an enzyme that converts testosterone and androstenedione into estradiol and aromatase, respectively. HSD= hydroxysteroid dehydrogenase; ER = Estrogen receptor

Aromatase is a key enzyme that regulates steroid levels locally in adipose tissue by converting androstenedione into estrone (E1) and testosterone into estradiol (E2).[2] Aromatase deficiency in humans results in a predominance of androgens over estrogens and abdominal obesity, or an android distribution.[21] It has also been shown that aromatase mRNA expression differs between depots, with higher expression in the gluteal than the abdominal regions in both pre- and postmenopausal women.[22,23] Therefore, it is logical to assume that the conversion of androgens into estrogens (aromatase content) could indicate local concentrations of E2. This could have an influence on SAT and distribution via local estrogen exposure.[24] For this reason, the primary focus of this study was to determine if aromatase protein content differs in abdominal and gluteal SAT.

African Americans (AA) have the highest rates of obesity (44.1%) compared to Caucasians (C) who have a prevalence of 32.6%.[25] AA tend to gain weight at an earlier age than their C counterparts, and maintain a higher weight at comparable ages.[26] AAs also tend to have different body fat distributions, specifically less visceral adipose tissue for similar age and BMI[27–29], and even greater amounts of SAT when adjusted for total body fat.[27,30,31] It is important to understand the mechanisms behind these differences in order to develop and research individual treatment methods for obesity and obesity-related diseases. Therefore, a second focus of this study was to determine if there are racial differences in aromatase protein content that might influence racial differences in regional body fat distribution in overweight/obese premenopausal women.

Statement of the Problem

Is there a difference in aromatase protein content between gluteal and abdominal adipose tissue in premenopausal overweight and obese African American and Caucasian women?

Research Hypothesis

Aromatase protein content will be higher in the gluteal region than the abdominal region in premenopausal overweight and obese women.

Aromatase protein content will be higher in abdominal and gluteal subcutaneous adipose tissue of African American than Caucasian premenopausal overweight and obese women.

Delimitations

Sixteen healthy premenopausal women, 18-45 years old, overweight or obese [BMI 28-33 kg/m^2], not currently taking hormonal contraceptives or any other form of hormone replacement were studied. Women who had irregular menstrual cycles, a history of hormone sensitive cancer, diabetes or insulin resistance, cardiovascular disease including hypertension, thyroid dysfunction, abnormal liver or renal function, or woman taking any medications know to affect lipid metabolism were excluded from this study. All women were sedentary, exercised no more than 20 minutes twice a week, were weight stable (< 2.0 kg weight change in the past 6 months), were non-smokers and did not regularly consume alcohol.

List of Acronyms

AA: African American

C: Caucasian

SAT: Subcutaneous Adipose Tissue

FFA: Free fatty acids

TAG: Triacylglycerols

ATGL: Adipose Triglyceride Lipase

HSL: Hormone Sensitive Lipase

E1: Estrone

E2: Estradiol

CHAPTER II

REVIEW OF LITERATURE

It is well known that obesity, characterized by excess white adipose tissue, is associated with elevated risk of a wide array of morbidities including, but not limited to, heart disease, diabetes and certain types of cancer. It is also known that the type of obesity, or the localization of the fat mass, influences the risk associated with developing said morbidities. Gynoid obesity, or obesity with fat mass centered in the hips and thighs, is generally associated with women, whereas android obesity, or obesity centered in the abdomen, is generally associated with men. However, women's transition through menopause, during which women experience a decrease in estrogen, is characterized by a shift in fat mass from gynoid to android distribution.[4,5] Estrogen treatment has been shown to prevent the shift from gynoid to android obesity in post-menopausal women.[16,32] These results suggest that sex steroids may play a significant role in obesity patterns.

Regional Differences in Lipolysis

Lipolysis is the process of converting TAG from adipocytes into FFA and glycerol[13] using the ATGL[33] and HSL enzymes to hydrolyze intracellular triglycerides.[34] Lipolysis, therefore, is an important process in the reduction of fat mass in any region. By measuring FFA or glycerol in vivo it is possible to estimate local lipolytic activity. Published data demonstrate that there are differences in the lipolytic activity of adipocytes by body region. By measuring FFA in vivo researchers were able to show that lower body adipose tissue lipolytic rates were lower than that of the upper body in non-obese and obese premenopausal women.[35] Interestingly, this study also showed that FFA levels were lower in the lower body of obese women despite

higher upper body obesity, which may suggest a mechanism for premenopausal women to maintain their gynoid distribution. In contrast, in a study by Hickner, Fisher and Kohrt (1997), glycerol concentrations, an indication of lipolytic rates, were reported to be higher in femoral adipose tissue of nonobese premenopausal women as compared to abdominal adipose tissue measured via microdialysis recoveries.[36] Because of conflicting evidence, it is of great importance to pursue further research into the different mechanisms that regulate regional lipolysis.

Racial Differences in Lipolysis

African American (AA) women become obese at an earlier age than Caucasian (C) women and maintain a higher weight at comparable ages across the lifespan.[26] Albu et al., (1999) showed that AA women have lower basal rates of lipolysis than C women, but did not investigate the mechanisms behind this difference.[37] Barakat et al., (2002), also found that basal lipolytic rates in AA women were lower than those in C women as indicated by fasting FFA and glycerol concentrations.[38] Researchers did find lower hormone sensitive lipase (HSL) mass in AA women despite equal HSL mRNA, suggesting the HSL may be more biologically active in AA than C women.

Preliminary Data:

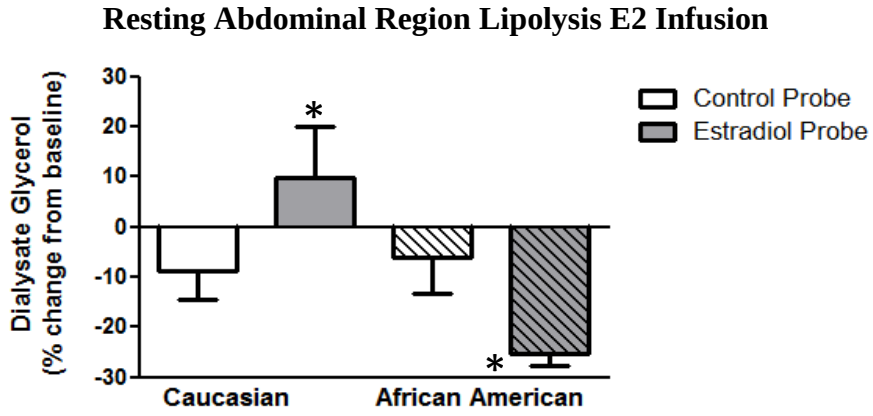

Figure 2. Regional and racial differences between premenopausal overweight to obese C (n=5; age 31.2 ± 10.03 years; BMI 29.62 ± 2.22 kg/m^2) and AA (n=5; age 29.00 ± 8.67 years; BMI 29.39 ± 2.20 kg/m^2) women show that when estradiol is infused into abdominal SAT in vivo at rest, there is a significant difference (p= 0.005*) in percent change of lipolytic response: an increase in lipolysis for C and a decrease for AA.

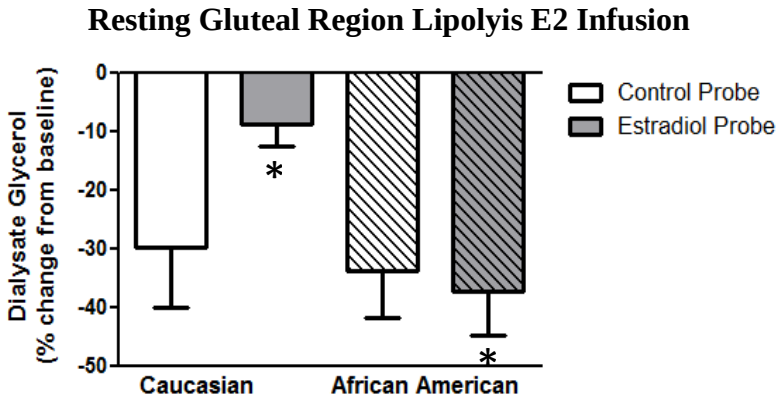

Figure 3. When estradiol is infused into gluteal SAT at rest, the difference between AA and C response is trending toward significance (p=0.058); a greater decrease in AA than in C.

Unpublished pilot data on regional and racial differences between premenopausal overweight to obese C (n=5; age 31.2 ± 10.03 years; BMI 29.62 ± 2.22 kg/m^2) and AA (n=5; age 29.00 ± 8.67 years; BMI 29.39 ± 2.20 kg/m^2) women show that when estradiol is infused into abdominal SAT in vivo at rest, there is a significant difference (p= 0.005) in percent change of lipolytic response: an increase in lipolysis for C and a decrease for AA (Figure 2). When

estradiol is infused into gluteal SAT at rest, the difference between AA and C response is trending toward significance (p=0.058); a greater decrease in AA than in C (Figure 3). The mechanism(s) behind this difference are not clear.

Estrogens and Lipolysis

White adipose tissue is an endocrine organ because of it's ability to store, metabolize and export sex hormones and other metabolic hormones affecting metabolism and energy balance (e.g. leptin which increases skeletal muscle fatty acid oxidation)[39]. Pre-menopausal women produce estrogens primarily from the ovaries and several extragonadal sites including mesenchymal cells of the adipose tissue, osteoblasts in bone, and vascular endothelial cells. Post-menopausal women primarily derive their estrogen from mesenchymal cells of adipose tissue.[17,23] Since estrogen produced from extragonadal sites is primarily active at the local tissue level, the tissue concentrations in these areas may be very high and can be of great significance locally.[23]

It has been well established that there is an association between the loss of circulating estrogen and a shift in body fat distribution in postmenopausal women and premenopausal women undergoing sex hormone suppression.[4,40,41] Evidence, such as estrogen therapy to treat low levels of circulating estrogen, suggests that sex steroids aid in regulating adipose distribution as indicated by reversing the shift from android back to gynoid distribution.[42,43,44] A study by Yükse (2007) showed that hormone replacement therapy (HRT) in postmenopausal women supplementing E2 resulted in a significant decrease in both waist circumference and SAT[42] indicating the positive influence of circulating estrogens on the favorable gynoid distribution. Davis et al. (2000) showed that HRT with E2 in postmenopausal women resulted in reduced hip

and abdominal circumferences as well as fat mass to fat-free mass ratio over the abdomen.[44] These studies support earlier evidence that varying levels of E2 in adipose tissue have been shown to play a role in patterns of body fat distribution.

Aromatase

Aromatase is an enzyme that catalyzes the rate-limiting irreversible aromatization of androgens to estrogens.[45] After menopause, when women are more inclined to an android distribution, there is an increase in SAT aromatase mRNA expression despite lower circulating estrogen levels, indicating that postmenopausal women might have increased estrogen in these tissues than premenopausal women.[46] In premenopausal and postmenopausal women, regional mRNA levels were found to be different between adipose tissue depots. Aromatase mRNA expression was higher in the thigh, buttock and flank regions as compared to the abdominal region in both pre- and postmenopausal women and was also found to increase with age.[22,23] Similarly, Misso et al. 2005 observed that aromatase mRNA expression was higher in the gluteal region than in the abdominal region and that aromatase mRNA expression was significantly higher in postmenopasal women.[47] This increase in aromatase mRNA expression as women age appears to be a mechanism to increase estrogen production in extragonadal sites as circulating levels of estrogens decrease. Therefore, adipose depot differences in aromatase mRNA expression and aromatase protein content, which are responsible for the conversion of testosterone to E2, may be influential in regulating adipose distribution and assist in providing potential mechanisms to account for the shift toward a more android distribution observed in the studies noted above.

Estrogen subtypes

Estradiol (E2) is the most biologically active estrogen in mammals[48] and is produced from the aromatization of testosterone. E2 has been shown to decrease adiposity, alter the release and production of fat hormones, and regulate the growth and localization of adipocytes. However, the mechanisms behind this process are still poorly understood.[9] Estrone (E1) is produced from the aromatization of circulating androstenedione[49] and appears to be the primary estrogen formed in postmenopausal women.[50]

Summary

The purpose of this study is to investigate differences in aromatase protein content between abdominal and gluteal SAT and between AA and C premenopausal overweight and obese women. Obesity is a growing health concern in the United States. With the knowledge that estrogens play a role in the distribution of SAT and regulation of lipolysis, and have different effects according to region and race, it is significant to find the mechanisms behind this process so that effective interventions, such as estrogen therapies after menopause, can be developed. Differences in regional and racial lipolytic responses provide rationale to find the mechanisms behind this process in order that researchers and medical professionals can develop more effective and appropriate individual strategies based on each person's unique biology. Because estrogens are known to be regulated through specific enzymes, namely aromatase, and because little is known about their protein expression between region and particularly between races, it is important to develop a more complete understanding in this area.

METHODS

Participants in this study included healthy premenopausal overweight to obese Caucasian (C) and African American (AA) women (N=15; C=7; AA=8; BMI=29.5 $\pm$ 0.5 kg/m^2) who were sedentary (exercising no more than 20 min/day twice a week) and weight stable (< 2.0 kg weight change in past 6 months). Participants were excluded from this study if they are taking hormonal contraceptives, had a history of hormone sensitive cancer, diabetes or insulin resistance, cardiovascular disease including hypertension, thyroid dysfunction, abnormal liver or renal function, took any medications known to affect lipid metabolism, smoke or regularly consume alcohol.

All participants underwent preliminary testing to ensure all enrollment criteria were met. Screening tests included: medical history, pregnancy test, DXA for body composition analysis, waist and hip circumferences and completion of menstrual cycle tracking calendar.

Following acceptance into the study all women were scheduled for two adipose tissue biopsies that took place during the early follicular phase of the menstrual cycle, during which circulating estrogen and progesterone levels are at their lowest[51]. A fasting blood sample was collected from an antecubital vein of each participant and analyzed for serum concentrations of glycerol, free fatty acids and circulating sex hormones (estradiol, estrone, progesterone, testosterone) to confirm menstrual cycle phase. This study was approved by the Medical Center Institutional Review Board at East Carolina University. (UMCIRB #11-077) (Appendix A)

Body Composition

Participants were weighed on an electronic scale with weight recorded to the nearest 0.1 kg. Height was measured with a standard stadiometer to the nearest centimeter (cm). Waist-to-hip ratio (WHR) was calculated from measures of minimal waist and hip circumferences performed according to previously published guidelines.[52] Total percent body fat, fat-free mass (lean mass + bone mineral content), and total and regional (android and gynoid) fat mass were determined using dual-energy x-ray absorptiometry (DXA; GE Lunar Prodigy Advance, Madison, WI).

Adipose Tissue Biopsy

Participants arrived at the East Carolina Diabetes and Obesity Institute at the East Carolina Heart Institute between 0700-0800 h after an overnight fast (≥10 hr) during the follicular phase of the menstrual cycle (day 2-8 after the start of menses). On average the biopsy visit occurred on day 5 ± 2 of the cycle. Approximately 1 gram of subcutaneous adipose tissue was removed under sterile conditions using the needle aspiration technique (14 gauge needle and 30cc syringe) after administration of local anesthesia (5cc of 1% lidocaine) and 5cc normal saline. Biopsies were taken from the abdominal (AB) subcutaneous adipose tissue, approximately 5cm lateral to the umbilicus, and the upper gluteal (GL) subcutaneous adipose tissue, approximately 5-10 cm from the medial line in the upper quadrant of the buttock. Fat tissue was immediately rinsed with Krebs Ringer Bicarbonate buffer containing 1% bovine serum albumin. Clots and blood vessels were then removed; tissue was immediately frozen in liquid nitrogen and stored at -80 °C for later analysis.

Western Blot

Adipose tissue samples were homogenized glass on glass in a 2:1 (volume-to-weight) ratio of cold homogenization buffer (50 mmol/L HEPES [pH 7.4], 1% sodium dodecyl sulfate, 2% Triton X-100, 2 mmol/L Ethylenediaminetetraacetic acid, 1 mmol/L sodium orthovanadate, 50 mmol/L sodium fluoride, 1 mmol/L benzamidine, 2 mmol/L dithiothreitol and protease inhibitor cocktail [Sigma P8340]). Homogenized samples were rotated for 60 min and centrifuged for 30 min at 10,000 x g, both at 4 °C. The supernatant was then removed and protein concentration determined by the Pierce BCA protein assay (Thermo Scientific, Rockford, IL). Samples were prepared in laemmli sample buffer (Bio-Rad, Hercules, CA) with 5% β-mercaptoethanol and heated in a water bath at 90 °C for 5 min.

Seventy micrograms of protein was loaded and separated on a 10% SDS-polyacrylamide gel (Criterion precast gels; Bio-Rad) and electrotransferred to reduced-fluorescence polyvinylidene difluoride membranes (Immobilon-FL; Millipore, Bedford, MA). Membranes were blocked for 1 hour at room temperature with Odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE) and then probed overnight at 4 °C for aromatase (1:200; sc-14245; Santa Cruz Biotechnology, Santa Cruz, CA), and β-actin (1:1000; 926-42212; LI-COR). Primary antibodies were diluted in Odyssey blocking buffer containing 0.2% tween-20. Blots were then washed with phosphate buffered saline containing 1% tween-20 (PBS-T) and incubated for 1 h at room temperature in fluorescent secondary detection antibodies (IRDye 800 CW-Donkey anti-Goat and IRDye 680LT Donkey anti-Mouse; LI-COR) diluted in Odyssey Blocking Buffer containing 0.2% tween-20 and 1% SDS. Fluorescence intensity data were quantified using the Odyssey infrared imaging system (LI-COR). All samples were normalized to β-actin protein as

well an internal control sample run on each gel and presented in arbitrary units. Myometrium served as negative control.[53,54] (Figure 4)

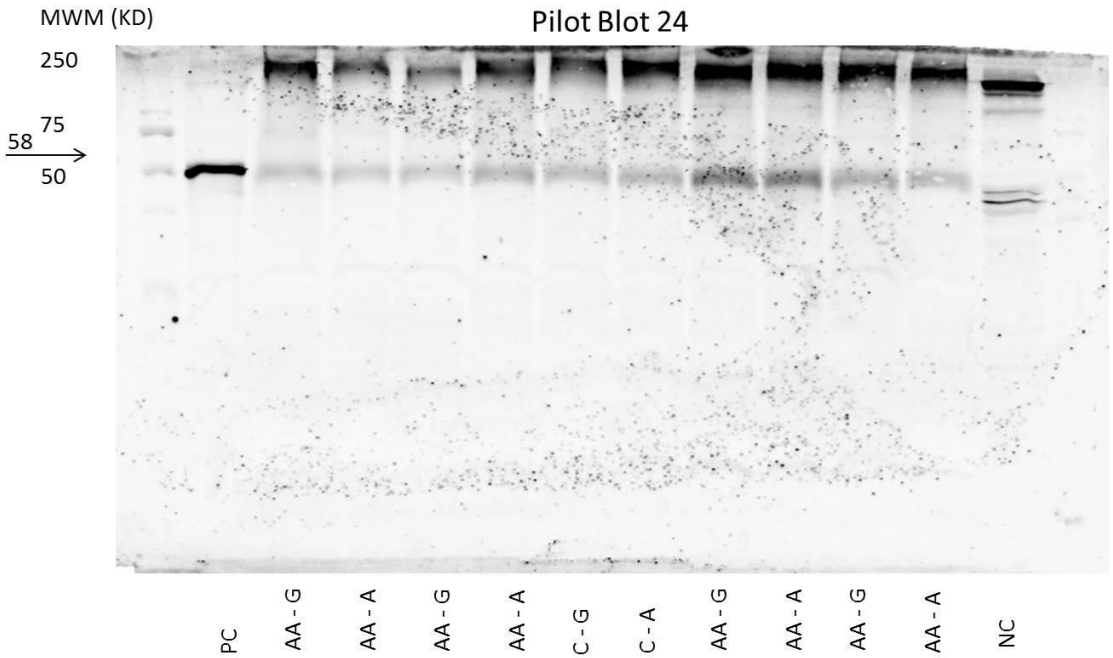

Figure 4.
MWM – Molecular Weight Marker
PC – Positive Control (rat ovary extract)
NC – Negative Control (Myometrium)
AA – African American
C – Caucasian
A – Abdominal
G - Gluteal

Figure 4. Demonstrates the use of the positive and negative controls to verify the results from study samples. The positive control for aromatase content used was rat ovary extract. The negative control was myometrium. Samples were paired by participant (AA or C) and region (A or G). This blot shows the positive control at the correct weight of 58 kilodaltons and the absence of the negative control at 58 kilodaltons.

Blood Samples

A fasting blood sample was drawn in the morning one day immediately preceding or after the biopsy visit. Serum estradiol, progesterone and testosterone were determined by Electrochemiluminescence Immunoassay (UniCel® DxC 600i Synchron® Access® Clinical System, Beckman Coulter, Inc., Brea, CA). The University of Colorado Denver Clinical and

Translational Research Center (CTRC) Core laboratory conducted the following analyses: Estrone by conventional radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX; 200 intra- and inter-assay CV's respectively 8.7% and 11.7%), dehydroepiandrosterone sulfate (DHEA-S) and sex hormone-binding 201 globulin (SHBG) by Electrochemiluminescence Immunoassay (Beckman coulter, DHEA-S 2.3% and 3.4%, SHBG 3.6% and 5.7%).

Statistics

Independent sample t-tests were used to determine racial differences in subject characteristics and sex hormones. Triglyceride, estradiol, estrone, progesterone, and SHBG data were log transformed before analysis due to its skewed distribution. Paired t-tests were used to determine regional differences in aromatase protein content. Two-way repeated measure ANOVA (race x region) was used to determine racial differences (Caucasian versus African American) in estrogen receptor expression in the abdominal and gluteal SAT. Significance was set at a value of $p < 0.05$. Data are presented as mean $\pm$ SEM.

RESULTS

Baseline characteristics of participants in the study are included in Table 1 and circulating sex hormone levels within one day of the biopsy in Table 2. African American women had elevated android fat mass compared to Caucasian women; all other participant characteristics were similar between the racial subgroups. There were no group differences in circulating sex hormones.

Table 1. Participant characteristcs

Variable	Pooled	Caucasian	African American
N	15	7	8
Age, (y)	25.1 ± 1.8	25.0 ± 2.7	25.1 ± 2.6
Weight, (kg)	81.3 ± 2.5	78.6 ± 1.5	83.8 ± 4.5
BMI, (kg/m^2)	29.5 ± 0.6	28.4 ± 0.3	30.5 ± 0.9
Waist Circumference, (cm)	88.4 ± 1.3	87.6 ± 1.5	89.2 ± 2.0
Waist-to-Hip Ratio	0.79 ± 0.01	0.79 ± 0.02	0.79 ± 0.02
Body fat, (%)	44.3 ± 1.2	43.7 ± 0.8	44.8 ± 2.2
Android Fat Mass, (kg)	3.1 ± 0.2	2.7 ± 0.2	3.4 ± 0.2*
Gynoid Fat Mass, (kg)	7.0 ± 0.2	6.9 ± 0.3	7.1 ± 0.3

Data are means ± SE unless otherwise indicated. *p<0.05 vs Caucasian

Table 2. Sex hormones

Variable	Pooled	Caucasian	African American
N	15	7	8
Past HC use, (yes/no)	7/8	5/2	2/6
Time since HC use, (y)	5.6 ± 2.5	6.4 ± 3.5	3.5 ± 0.5
Estradiol, (pg/mL)[a,b]	67 (38-115)	50 (27-95)	92 (37-231)
Estrone (ng/mL)[a,b]	0.064 (0.047-0.087)	0.055 (0.038-0.082)	0.072 (0.042-0.013)
Progesterone, (ng/mL)[a,b]	0.35 (0.21-0.60)	0.32 (0.15-0.67)	0.39 (0.16-0.94)
Testosterone, (ng,mL)[b]	0.29 ± 0.04	0.33 ± 0.052	0.29 ± 0.066
SHBG, (ug/mL)[a,b]	4.6 (3.63-5.82)	4.41 (2.65-7.28)	4.77 (3.5-6.5)
DEA-S (ug/mL)[b]	1.67 ± 0.22	2.07 ± 0.22	1.33 ± 0.27

Values are mean ± unless otherwise indicated
[a]Skewed variable, re-exponentiated to mean 95% confidence interval
[b]N=14 (African American N=7), one African American did not have a blood draw
HC, hormonal contraceptives; SHBG, Sex Hormone-Binding Globulin; DHEA-S, dehydroepiandosterone sulfate

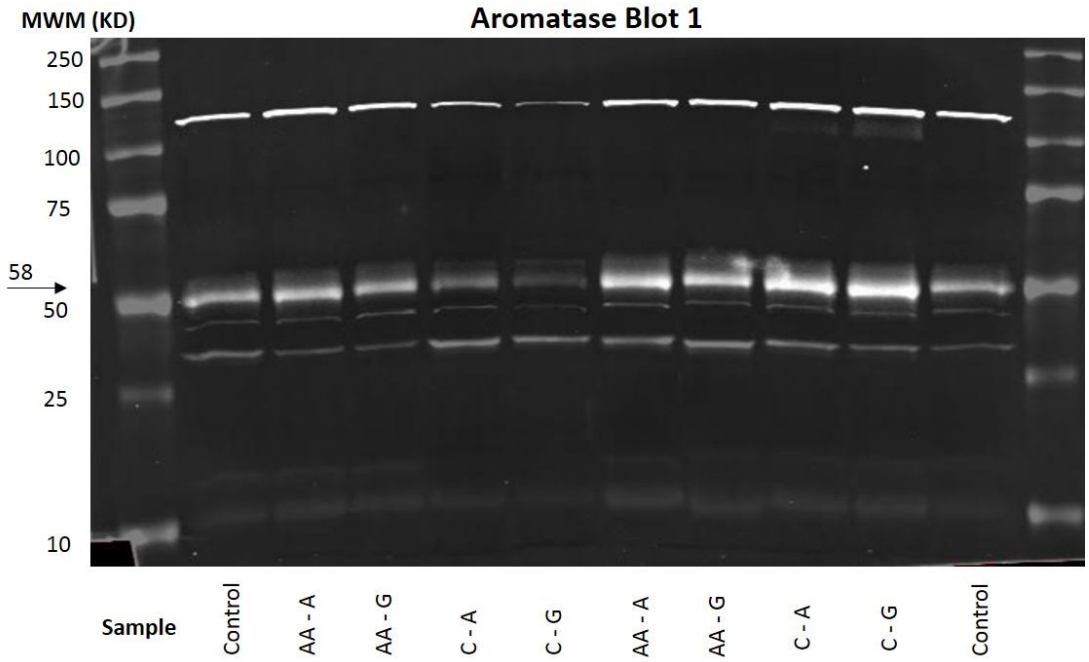

Figure 5.
MWM – Molecular weight marker
AA – African American
C – Caucasian
A – Abdominal
G – Gluteal

Figure 5. Presents evidence of correct band detection for aromatase at the expected level of 58 kDa. Samples were paired by participant according to race (AA or C) and region (A or G). Controls used in this figure are an average of aromatase content across all participants.

Aromatase Protein Content

Aromatase protein content was not different between races or regions: C abdominal 0.25±0.06, C gluteal 0.25±0.12, AA abdominal 0.35±0.08, AA gluteal 0.32±0.09 arbitrary units (2-way ANOVA; race x region p=0.73, race p=0.46, region p=0.71). The aromatase protein data are depicted in Figure 6.

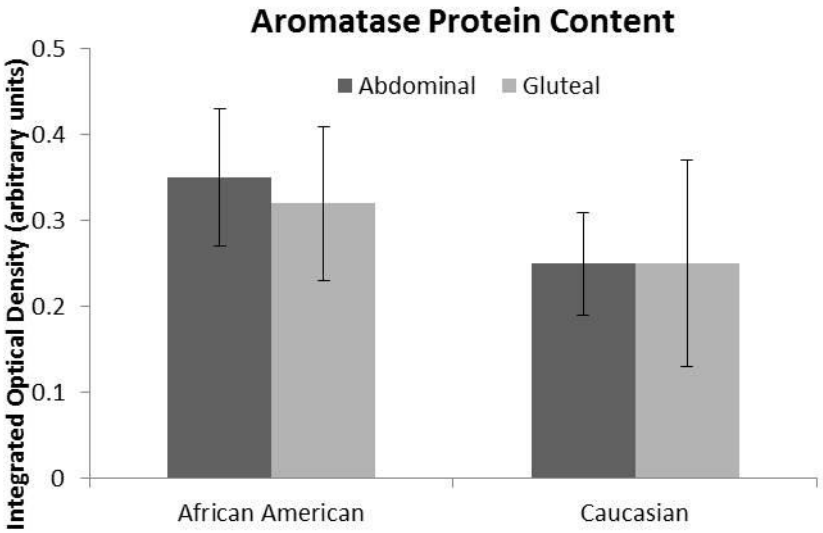

Figure 6. Subcutaneous adipose tissue biopsies were obtained in the fasting state from premenopausal overweight to obese (BMI =29.5 ± 0.5 kg/m²) African American (N=8) and Caucasian (N=7) women and analyzed using Western Blot for purpose of determining differences in protein content by race and region. Significance is set at $p \leq 0.05$. No significance was found in this analysis.

DISCUSSION

The aim of this study was to examine the potential for differences in aromatase protein content between gluteal and abdominal adipose tissue in premenopausal overweight and obese African American and Caucasian women. It was hypothesized that aromatase protein content would be higher in the gluteal region than the abdominal region in premenopausal overweight and obese women and that aromatase protein content would be higher in abdominal and gluteal subcutaneous adipose tissue of African American than Caucasian premenopausal overweight and obese women. This study showed no significant differences in aromatase protein content between regions or races in the observed group.

Estrogens, produced by enzymes in adipose tissue such as aromatase, have been shown to directly influence lipolysis. Estrogen influences the regulation of subcutaneous adipose tissue and could thereby help determine body fat distribution. Unpublished pilot data from our lab show significant regional and racial differences in in-vivo lipolysis between overweight-to-obese (BMI 29.4 $\pm$ 2.2) premenopausal women in response to local estradiol perfusion (Figure 1, 2). Even though previous studies in premenopausal women show regional differences in aromatase mRNA levels[22,23], no regional or racial differences in aromatase protein content were detected in this study.

Aromatase protein deficiency has been shown to cause a dominance in androgens over estrogens and an android body fat distribution[21]; however, several studies examining aromatase enzyme expression, as measured by mRNA, have observed increased aromatase expression as women age along with a shift towards android body fat distribution. The same studies also show that aromatase enzyme expression was higher in the gluteal regions than abdominal regions in both pre- and postmenopausal women despite the shift in body fat distribution.

However, Kendrick, 2014 showed that agreement between measurements of mRNA and protein content occur only about 40% of the time.[55] Consequently, it is not an exercise in redundancy to measure both mRNA and protein comparatively or independently. It is important to measure mRNA and protein content in order to fully understand the function of the cell. [56] While it could be assumed that mRNA would directly translate into protein and therefore the two would be correlated, that is often not the case. One explanation for this difference could involve the many steps between transcription and translation and how much mRNA actually results in protein due to difference in transcription rates, efficiency and degredation.[57] We measured only protein in the present study, as we believe protein content is the relevant measure for our study. A measure of aromatase activity would have been preferred to investigate the best functional outcome of both transcription and translation, but we were unable to develop an aromatase activity for the current study. However, to our knowledge, this is the first study to report aromatase protein content at the fat depot regional level and between AA and C women.

For this study, participants were asked to complete a menstrual cycle tracking calendar, and adipose tissue biopsies were performed during the early follicular phase of the menstrual cycle. This phase was selected because progesterone and estrogen levels are known to be at their lowest and most stable. Since progesterone has been shown to increases aromatase expression and estrogen levels[53,58] studying the participants during the time of the menstrual cycle with lowest estrogen and progesterone likely provided low variance in the hormonal milieu across participants. This process could have been further improved by verifying consistency in the menstrual cycle across several months, with no missed or late periods and little variation in time between menstrual periods. However, there were no variations in circulating sex hormones in this group (Table 2), which is ideal for this study in order that these hormones not influence

aromatase activity or content.

One limitation of this study was the small sample size. Recruitment could have been limited due to the discomfort of the procedures which included the adipose tissue biopsy that could lead to bruising. Previous studies have also further limited African American participants with the requirement that both their parents and grandparents be of African American descent. No distinction was made in this case and variation in racial descent could have confounded results.

In this study, African American women had significantly higher android mass (kg) (3.4 ± 0.2) than Caucasian women (2.7 ± 0.2) ($p<0.05$) yet no differences in aromatase protein content were observed. While aromatase protein concentration was not different, total protein content could be higher due to higher overall fat mass. It appears, in the group studied, that aromatase protein content is not a major contributor of body fat distribution. It is possible that aromatase activity, as well as other estrogen producing enzymes such as 17BHSD, may be different between depots or races, thereby influencing subcutaneous adipose tissue estrogen exposure.

Men were not considered in this study for several reasons. First, most previous studies did not use men in their methodology. Men tend to have more of an android distribution as opposed to a gynoid distribution and do not experience a change in said distribution as they age. We were also limited to the size of sample in this study. It would be of interest to explore the differences in aromatase content between regions and across age in future studies.

A final limitation of this study may be that there were not lean participants to compare our overweight/obese participants to. This would have been a beneficial comparison to determine if the concentration of aromatase and rate of lipolysis is different between lean and obese participants. A possible difficulty would have been finding lean participants who maintain a

similar body fat distribution to the overweight and obese participants.

In conclusion, we report no significant differences between region or race, contrary to the hypothesis. It would benefit future researchers to consider a long-term observation of participants to see how aromatase content changes throughout a lifespan and changes in body weight and distribution thereof. The addition of a lean group and a male group would add further depth the knowledge base on the subject as well. Further research into this area and its influence on body composition changes with age and racial discrepancies in disease and body composition could lead to evolved treatments of hormone-sensitive disease as well as individualized approaches based on age and race.

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Appendix A

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board Office
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Office **252-744-2914** · Fax **252-744-2284** · **www.ecu.edu/irb**

Notification of Continuing Review Approval

From: Biomedical IRB
To: Kathleen Gavin
CC: Robert Hickner
Date: 4/16/2012
Re: CR00000284
UMCIRB 10-0245
[IMPORTED] Pilot study investigating regional differences in aromatase and lipolytic activity in women.

I am pleased to inform you that at the convened meeting of the Biomedical IRB on 4/11/2012, this research study underwent a continuing review and the committee voted to approve the study. Approval of the study and the consent form(s) is for the period of 4/11/2012 to 4/10/2013.

The Biomedical IRB deemed this study Greater than Minimal Risk.

Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The investigator must adhere to all reporting requirements for this study.

The approval includes the following items:

Name	Description	Modified	Version
Gavin Flyer with Tabs Revisions 071510.doc	Recruitment Documents/Scripts	8/11/2011 10:15 AM	0.01
gavin Medical Form 071510.doc	Surveys and Questionnaires	8/11/2011 10:16 AM	0.01
GavinPilot_InformedConsent_Revisions_4.12.12_Clean.doc.pdf	Consent Forms	4/12/2012 12:28 PM	0.02

The following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study:

None

The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting: R. Hickner