

# REGULATION OF LIPOLYSIS BY BETA-ADRENERGIC ACTIVATION AND EXERCISE IN OBESE AFRICAN-AMERICAN AND CAUCASIAN WOMEN

By

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May, 2012

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African-American women (AAW) are twice as likely to be obese as Caucasian women (CW); however, previous in-vitro studies have shown that AAW have higher densities of beta-adrenergic receptors (B-AR) in the subcutaneous (SC) adipose tissue and an increased lipolytic response to B-AR stimulation when compared to CW. There are no in-vivo studies to help resolve this apparent contradiction between in-vitro lipolytic response and the incidence of obesity. The purpose of this study is therefore to determine if AAW, as compared to CW, have a larger lipolytic response to pharmacological (isoproterenol) and physiological (exercise) stimulation. Methods: 33 obese women (16 AAW; 17 CW) participated in the microdialysis (MD) study. MD consisted of two probes placed in SC abdominal adipose tissue and perfused with either isoproterenol (ISO) or a control solution. Dialysate glycerol (index of lipolysis) was measured from probes at rest and, from the control probe, during exercise. Results: Dialysate glycerol increased 384.9% in AAW and

191.2% in CW in response to ISO ( $p=0.046$  AAW vs CW). Dialysate glycerol increased  $66 \mu\text{mol/L}$  in AAW and  $84 \mu\text{mol/L}$  in CW in response to exercise ( $p=0.94$  AAW vs CW). Conclusion: AAW have a higher lipolytic response to pharmacological B-AR stimulation, but have a similar lipolytic response to exercise, as compared to CW. These findings suggest that lipolytic response to B-AR stimulation and exercise are likely not contributing factors to the higher incidence of obesity in AAW as compared to CW.

Regulation of lipolysis by  $\beta$ -adrenergic activation and exercise in obese  
African-American and Caucasian women

A Thesis

Presented to the Faculty of the Department of Kinesiology  
East Carolina University

In Partial Fulfillment of the Requirements for the Degree  
Master's of Science in Exercise Physiology

by

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## TABLE OF CONTENTS

LIST OF TABLES .....	xii
LIST OF FIGURES.....	xiii
CHAPTER I: INTRODUCTION .....	1
CHAPTER II: REVIEW OF LITERATURE .....	2
Adiposity and Racial Effects on Lipolysis.....	4
Alpha- and Beta-Adrenergic Receptors .....	5
Beta-receptor Activation with Isoproterenol.....	6
Exercise and Lipolysis .....	8
CHAPTER III: METHODS .....	9
Research Participants.....	9
Screening.....	9
V <sub>O<sub>2</sub>Max</sub> Test .....	10
Microdialysis .....	10
Exercise: Stage 5 .....	11
Statistical Analysis .....	11
CHAPTER IV: RESULTS.....	12
Subject Characteristics.....	12
Lipolysis in response to Isoproterenol Perfusion .....	12
Effects on Exercise on Lipolysis .....	12

CHAPTER V: DISCUSSION.....	18
Limitations.....	20
Conclusion.....	21
REFERENCES .....	22
APPENDIX A: IRB APPROVAL FORMS.....	25
APPENDIX B: DOCUMENTS PRESENTED .....	41
APPENDIX C: MEDICAL HISTORY FORM .....	49

## LIST OF TABLES AND FIGURES

MICRODIALYSIS PROTOCOL.....	13
SUBJECT CHARACTERISTICS.....	14
STIMULATION OF LIPOLYSIS BY ISOPROTERENOL.....	15
PERCENT CHANGE BY ISOPROTERENOL.....	16
STIMULATION OF LIPOLYSIS BY EXERCISE.....	17

## **CHAPTER I: *Introduction***

Obesity is prevalent in half as many Caucasian women (CW) as in African-American women (AAW) [20, 25]. One reason for the difference in obesity incidence between CW and AAW may be differences in fat breakdown (lipolysis), although differences in fat synthesis may also play a role. It is also known that obese individuals have a lower basal and exercise induced lipolytic rates than that of lean individuals when compared on a per cell basis [4, 6, 8, 20, 29]. The causes of this difference, however, are not known. Theories point in the direction of increases in alpha-adrenergic receptor (A-AR) density and activation, beta-adrenergic receptor (B-AR) insensitivity and other factors that have not been studied [1, 3, 4, 5, 9, 12, 13, 16, 17, 18, 23].

In human adipose tissue, free-fatty acids (FFA) and glycerol are stored as triglyceride molecules. Triglycerides are hydrolyzed in a process called lipolysis that can be regulated by altering receptor density and activation. FFA and glycerol are the products of lipolysis, with glycerol being the product that can be measured using in-vivo microdialysis [3, 4].

In the current study, microdialysis (MD) was used to perfuse a pharmacological agent, isoproterenol, through subcutaneous adipose tissue. Isoproterenol affected lipolysis, and changes in dialysate glycerol were measured as the outcome [1, 3, 9, 12, 17, 23]. An increase in dialysate glycerol, in the absence of overt changes in blood flow, is understood to represent greater lipolytic rate.

Although in some studies AAW have lower lipolytic rates than CW at rest, in-vitro data and pilot data suggests the AAW may have an increased response to beta-adrenergic

activation. The purpose of this study was to determine if AAW were more responsive than CW to stimulation of lipolysis by the B-AR agonist isoproterenol administered locally to subcutaneous abdominal adipose tissue via MD at rest. A second aim of the current study was to determine if AAW have a higher lipolytic response to exercise than CW. Based on previous in-vitro data and pilot data, it was hypothesized that under beta-adrenergic stimulation by isoproterenol, AAW will show a larger increase in lipolysis compared to CW. It was also hypothesized that exercise-stimulated lipolysis will be higher in AAW than CW.

## CHAPTER II: *Review of Literature*

Lipolysis is the catabolism of triglycerides into free-fatty acids and glycerol. [3, 30]. Lipolytic rates are increased during exercise by sympathetic release of catecholamine hormones, which attach to adrenergic receptors on the cell membrane. These receptors are divided into sub-categories, alpha-adrenergic receptors (A-AR) and beta-adrenergic receptors (B-AR), based on their relative sensitivities to adrenergic agonists and antagonists. Lipolytic regulation is determined by receptor sensitivity or density, insulin levels or insulin sensitivity, adenosine and adenosine receptor densities and by the intensity of exercise [30]. Each regulator of lipolysis can have an effect on other regulators. An example of this would be acute exercise, which has been shown to stimulate B-ARs so much as to overcome decreases in lipolytic rates that A-AR agonists create.

It is not clear from previous microdialysis studies whether or not there are differences in glycerol production between AAW and CW at rest, with pharmacological alterations or with exercise. Some studies measuring plasma glycerol show a higher basal lipolytic rate in AAW than CW [16], while others using *in-vitro* techniques have confounding results, reporting a higher lipolytic rate in each race [6, 8]. However, pilot data for the current study shows trends that AAW are more responsive to isoproterenol than their CW counterparts.

While there is not one substrate or process that controls lipolysis, it has been observed that sensitivity to certain hormones, such as insulin, is varied across race [3]. Basal lipolytic rates are determined also by a person's natural sensitivity to A-AR and B-AR

stimulation. By increasing B-AR and/or decreasing A-AR density or sensitivity, lipolysis can be increased [1, 9, 18, 23].

### ***Adiposity and Racial Effects on Lipolysis***

It has been shown that obese individuals have lower rates of lipolysis than their age and race matched lean counterparts under both basal and exercise conditions [8]. The reason for this difference is not yet fully understood. Elizalde *et al.* (2000) observed that obese individuals have less hormone-sensitive lipase (HSL) in their adipocytes compared to lean individuals. HSL is a rate-limiting enzyme for lipolysis in the fat cells.

Obesity is not always associated with low lipolytic rates. Race has also been targeted as a contributing factor determining lipolytic rates [3, 6, 8, 15, 16]. Obesity incidence is nearly twice as high in AAW as CW. Racial differences also include less weight loss from similar weight-loss programs and weight gain commencing at earlier ages in AAW than in CW [20]. It has been shown that AAW have higher B-AR densities than CW [15], but do these higher densities translate to increased B-AR stimulation? Barakat *et al.* (2002), showed that *in-vitro* basal lipolytic rates for AAW ( $0.63\mu\text{mol/glycerol}/10^6\text{ cells/h}$ ) were half that of CW ( $1.20\mu\text{mol/glycerol}/10^6\text{ cells/h}$ ). After isoproterenol was introduced to the fat cells, glycerol release from cells of AAW increased to  $1.60\mu\text{mol/glycerol}/10^6\text{ cells/h}$  whereas glycerol release from cells of CW only increased to  $2.20\mu\text{mol/glycerol}/10^6\text{ cells/h}$ . Our *in-vivo* pilot data also indicated basal lipolysis is higher in AAW than CW, so what is causing this disconnect? If there are differences between *in-vitro* and *in-vivo* stimulated lipolysis when comparing races, further research may be needed to investigate this disparity.

When BMI matched, AAW have lower mortality rates despite having higher rates of obesity than CW. AAW also have fewer incidences of impaired glucose tolerance and dyslipidemia when compared to CW, when matched by BMI. Theories suggest that increased fatty acid and glycerol production from higher lipolytic rates may cause health problems in CW [21]. However, the extent to which lipolytic rates are different in AAW and CW is in question. The increased health risks associated with obesity are not the focus of this thesis, but the tie between lipolysis, obesity and health risks provides a global rationale for study of racial differences in lipolysis.

Differences in lipolytic rates between AAW and CW may not be due to differences in circulating catecholamine concentrations [8]. Since catecholamines bind to the B-AR sites [3], B-AR agonists would markedly increase lipolysis in both AAW and CW. However, the extent of stimulation may be different in these groups even at the same prevailing catecholamine concentrations in each group due to differences in adrenergic receptor density. Adrenergic receptor density was not measured in this study, but may be one mechanism responsible for any between-group differences in lipolysis.

### ***Alpha- and Beta-Adrenergic Receptors***

One of these major contributors in the process of lipolysis is the activity of B-ARs. B-ARs control activity of lipolysis through catecholamine concentrations. B-AR responses to catecholamine potency is in the order of isoproterenol > epinephrine ≥ norepinephrine, with isoproterenol being the strongest agonist [30]. Increases in B-AR density or activation results in an increase in lipolysis under basal and exercising conditions [5, 13, 16, 18].

The other adrenergic receptors are the A-ARs [1, 9, 17, 18, 23, 30]. A-ARs, specifically, have been known to respond to administered catecholamines in the order of potency; norepinephrine > epinephrine > isoproterenol [30]. This means that norepinephrine has the greatest effect at increasing A-AR site activity and isoproterenol has the least effect. Epinephrine and norepinephrine both would decrease lipolysis since they both are agonists of the A-ARs, the stimulation of which suppresses lipolysis. Increases in A-AR activity have a negative influence on dietary weight loss, causing individuals to become obese [1]. A-AR activity predominates in subcutaneous abdominal adipose tissue, meaning that A-AR influences have been shown to “out compete” B-AR influences on lipolysis [18]. The study also reported that by using phentolamine to block A-ARs in obese subjects, the suppressive effect of alpha-receptor activity on lipolysis during exercise was eliminated.

Pathways, other than through activation of the A-AR and B-AR, can also increase or decrease lipolysis. Insulin was also found to impair B-AR's effects on increasing lipolysis, but insulin has a lesser effect on lipolysis than catecholamine activity [13]. Gliszinski *et al.* (2009) and Moro *et al.* (2004) found that, during exercise, increased catecholamine levels alter A-AR and B-AR activity. During exercise, B-AR and A-AR activities are increased, which increases and decreases lipolysis respectively. In the current study, B-AR stimulation of lipolysis by isoproterenol was monitored only at rest.

### ***Beta-receptor Activation with Isoproterenol***

B-AR pathways are easy to manipulate. There are pharmacological agents that, when administered directly into adipose tissue with MD, have a strong effect on the

receptors. The drug used for the current study was isoproterenol and has been shown to directly increase lipolysis [3, 9, 12, 23, 30]. Agonist and antagonists are described by potency, affinity of the compound for the receptor site, and intrinsic activity, which is the maximal response the compound can elicit. Koppo *et al.* (2010) states that B-AR stimulation has larger effects on lipolysis than that of A-AR inhibition. B-AR antagonists are known to decrease lipolysis, and we see this in individuals on the well-known heart and blood pressure medication, “beta-blockers”. In the present study, a B-AR agonist was used to increase lipolysis. The B-AR agonist that was used is isoproterenol. In-vitro studies have shown isoproterenol can increase lipolysis in both CW and AAW equally, but since AAW have lower basal rates of lipolysis, their percent change is much higher than CW [3]. Some AAW subjects increased lipolytic rates by nearly ten-fold when isoproterenol was used. In another study by Jordan *et al.* (2001), isoproterenol was compared with epinephrine to evaluate the effects each had on lipolysis at concentrations of 0 $\mu$ M, 0.01 $\mu$ M, 0.1  $\mu$ M and 1.0  $\mu$ M. Both isoproterenol and epinephrine increased glycerol production by 6 times normal rates, with epinephrine having a slightly larger effect. It was found that even with blood flow increasing, glycerol concentration increased when isoproterenol was administered, meaning that B-AR activation does increase lipolysis. The main question that was to be answered in this study was following: Does isoproterenol increase *in-vivo* lipolytic rates to a greater extent in AAW than CW? A higher isoproterenol-stimulated lipolysis in AAW than CW women is expected due to the higher B-AR densities in AAW reported in *in-vitro* studies [15].

## ***Exercise and Lipolysis***

We measured lipolysis during exercise with perfusion of the microdialysis probe with a Ringer solution. Previous studies have shown that exercise does increase lipolysis in obese and lean subjects [3, 10, 14, 17, 26,]. One such study noted a 300% increase in dialysate glycerol ( $\sim 20 \mu\text{mol/liter}$  at rest to  $\sim 60 \mu\text{mol/liter}$ ) during the final minutes of a 30- minute bout of aerobic exercise at 66%  $\text{VO}_{2\text{Peak}}$  [2]. The current study monitored the differences in lipolysis between AAW and CW during exercise.

Exercise intensity of 60%  $\text{VO}_{2\text{peak}}$  was found to elicit maximal fat metabolism by Friedlander *et al.* (1998). Lipolytic rates are low at rest, and increase with an increase in  $\text{VO}_2$  up to 65% of  $\text{VO}_{2\text{peak}}$ , where lipolysis increases less dramatically. To maintain exercise for an hour, 60% of  $\text{VO}_{2\text{peak}}$  will be used, which was determined from an incremental  $\text{VO}_2$  stationary bike test 7 days prior to the day of the microdialysis procedure.

Based on these previous data, it is understood that exercise does increase lipolysis. Drugs, such as isoproterenol, are sometimes used to mimic exercise's effects on lipolysis. In consideration of previous *in-vitro* findings regarding AAW having higher B-AR densities and responses to B-AR stimulation than CW, the current study will have 2 aims. The purpose of this study was to determine if AAW are more responsive than CW to stimulation of lipolysis by the B-AR agonist isopreterenol administered locally to subcutaneous abdominal adipose tissue via MD at rest. A second aim of the current study was to determine if AAW have a higher lipolytic response to exercise than CW.

Based on previous data, it was hypothesized that AAW will have a greater response to B-AR stimulation by isoproterenol than CW, and that AAW will have a greater increase in lipolysis during exercise than CW.

## **CHAPTER III: *Methods***

### *Research Participants*

Thirty-three overweight or obese (BMI 27-63 kg/m<sup>2</sup>), but otherwise healthy premenopausal women were studied. Participants were not currently taking hormonal contraceptives or any other hormone replacement drugs. Potential participants were excluded from the study if they 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 take any medications known to affect lipid metabolism. All women were sedentary, exercising no more than 20 minutes twice a week, weight stable (< 2.0 kg weight change in the past 6 months), non-smokers and did not regularly consume alcohol.

### *Screening*

Participants reported to the Fitness Instruction Testing and Training (FITT) Building once for a screening visit to ensure all enrollment criteria were met. Screening tests included: medical history, pregnancy test (if necessary), DXA (General Electric, Lunar Prodigy) for body composition analysis, waist and hip circumferences, completion of menstrual cycle tracking calendar and a VO<sub>2</sub> peak exercise test on a stationary exercise bike. If all criteria were met, women accepted into the study were scheduled for a microdialysis visit according to menstrual cycle. All microdialysis visits occurred between day 2 and 6 of the menstrual cycle, which corresponds to the early follicular (EF) phase. It

was vital to be sure the phase of menstrual cycle was consistent between subject visits. The EF phase was selected because it is the phase during which circulating estrogen and progesterone levels are at their lowest. Therefore, subcutaneous adipose tissue (SCAT) exposure to circulating estrogens was at a minimum during this phase, not disrupting glycerol production levels.

### *VO<sub>2Max</sub> Test*

Participants performed a bike (LODE Corival Ergometer) VO<sub>2Max</sub> test that consisted of 2-minute stages with an increase of 25 watts at each stage (50, 75, 100, 125, 150...). Prior to each test, calibration was performed to the manufacturer's instructions (Polar, Lake Success, NY). Gas calibration uses a tank consisting of 16% oxygen and 4% carbon dioxide with a nitrogen balance. A 3-liter syringe was used for calibration of flow rate. Heart rate monitors (Polar, Lake Success, NY) measured heart rates throughout the test. Headgear stabilized the mouthpiece that collects the expired gases, which were collected throughout the test and recovery period. Nose clips were used to prevent any expired gases from escaping through the nose.

### *Microdialysis*

All participants fasted for 12 hours prior to the microdialysis. Two microdialysis probes were inserted into the abdominal adipose tissue, 2-3 cm apart. The insertion sites were first sprayed with ethyl chloride prior to insertion of the 20-gauge needle through the skin, into the subcutaneous tissue. In the current study, each probe perfused a Ringer solution (5mM ethanol & saline) for 40 minutes at 2.0  $\mu$ l/min with a CMA/102 infusion

pump during stage 1 to obtain baseline numbers. Probe 1 remained perfused with the Ringer solution to provide a control for basal lipolysis. The remaining probe and stage components can be found on **Table 1**. Samples were collected in intervals of 15-30 minutes. An automatic Microdialysis Analyzer (CMA 600, CMA/Microdialysis, Stockholm Sweden) was used to measure glycerol. Probe 2 was specifically monitored for the effects of isoproterenol on lipolysis.

#### *Exercise: Stage 5*

Stage 5 consisted of 60 minutes of stationary bicycle exercise performed at 60% VO<sub>2</sub>peak as determined by the preliminary screening incremental peak oxygen consumption test on a stationary bike 7 days prior to the microdialysis experiment. Approximately 60% VO<sub>2</sub>peak is the point of maximal lipolytic response to exercise. The control probe was used to monitor exercise's effect on lipolysis in each race.

#### *Statistical Analysis*

A One-way ANOVA was used to compare glycerol response to isoproterenol or exercise between race. A Newman Keuls' post hoc analysis was used where significant differences are detected using ANOVA. The level of significance was set at  $P < 0.05$

## **CHAPTER IV: Results**

Tables and Figures referred to in this section can be found at the end of the results.

Data are presented as “mean  $\pm$  standard error”.

### *Subject Characteristics*

Participant characteristics (race, age, BMI, percent body-fat and waist to hip ratio) are reported in **Table 2**.

### *Lipolysis in response to Isoproterenol Perfusion*

There was no significant difference in basal dialysate glycerol between races (CW  $74.06 \pm 9.92 \mu\text{mol/L}$ ; AAW  $56.76 \pm 8.42 \mu\text{mol/L}$ ; N.S.; See Figure 1). With the perfusion of isoproterenol ( $10^{-6}$  M), dialysate glycerol increased in probes from CW and AAW by  $133.30 \mu\text{mol/L}$  and  $189.44 \mu\text{mol/L}$ , respectively (N.S.; see Figure 1). In terms of a percent change from basal, dialysate glycerol increased more in probes from AAW than CW (CW  $191.18 \pm 22.26\%$ ; AAW  $384.94 \pm 93.49\%$ ; See Figure 3;  $P=0.046$ ).

### *Effects of Exercise on Lipolysis*

Each race's lipolytic rates are affected by exercise similarly. Dialysate glycerol from probes in SCAT of CW increased from  $74.1 \pm 9.9 \mu\text{mol/L}$  to  $158.1 \pm 17.6 \mu\text{mol/L}$ , while dialysate glycerol from probes in SCAT of AAW increased from  $56.8 \pm 8.4 \mu\text{mol/L}$  to  $122.0 \pm 16.0 \mu\text{mol/L}$  (N.S.; See Figure 2). With exercise as the stimulus, dialysate glycerol from probes in CW increased by  $84.0 \mu\text{mol/L}$ , while dialysate glycerol from probes in AAW increased by  $65.2 \mu\text{mol/L}$  (Figure 2).

Probe	Stage 1			Stage 2		Stage 3 (exercise)		
1	Con1	Con2	Con3	Con4	Con5	Con6	Con7	Con8
2	Con1	Con2	Con3	Iso1	Iso2			

Table 1. The current study used this microdialysis probe configuration. Each cell signifies a collection time.

			CW (N=17)	CW	AAW (N=16)	AAW
	Mean	StdDev	Mean	StdDev	Mean	StdDev
Age	28.2	6.6	29.3	7.3	27.2	5.9
BMI	34.8	8.4	34.9	8.9	34.7	8.3
BF%	48.7	4.1	48.4	3.8	49.0	4.4
W:H	0.80	0.05	0.79	0.06	0.81	0.04

Table 2. Participant characteristics. All presented statistical analysis were conducted on this subject base.

## Stimulation of Lipolysis by Isoproterenol in CW vs AAW

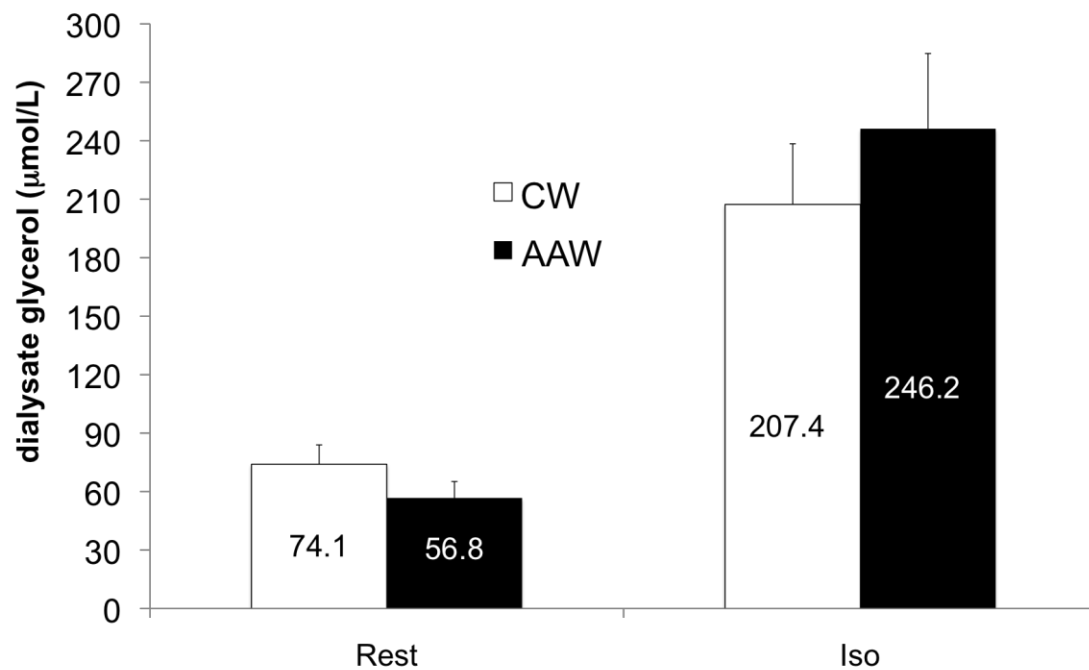


Figure 1. : 17 CW and 16 AAW were studied at rest and in response to local SCAT perfusion of 10<sup>-6</sup> M isoproterenol (Iso). Data are mean±SEM. P=not significant.

## Exercise Stimulated Lipolysis: CW vs AAW

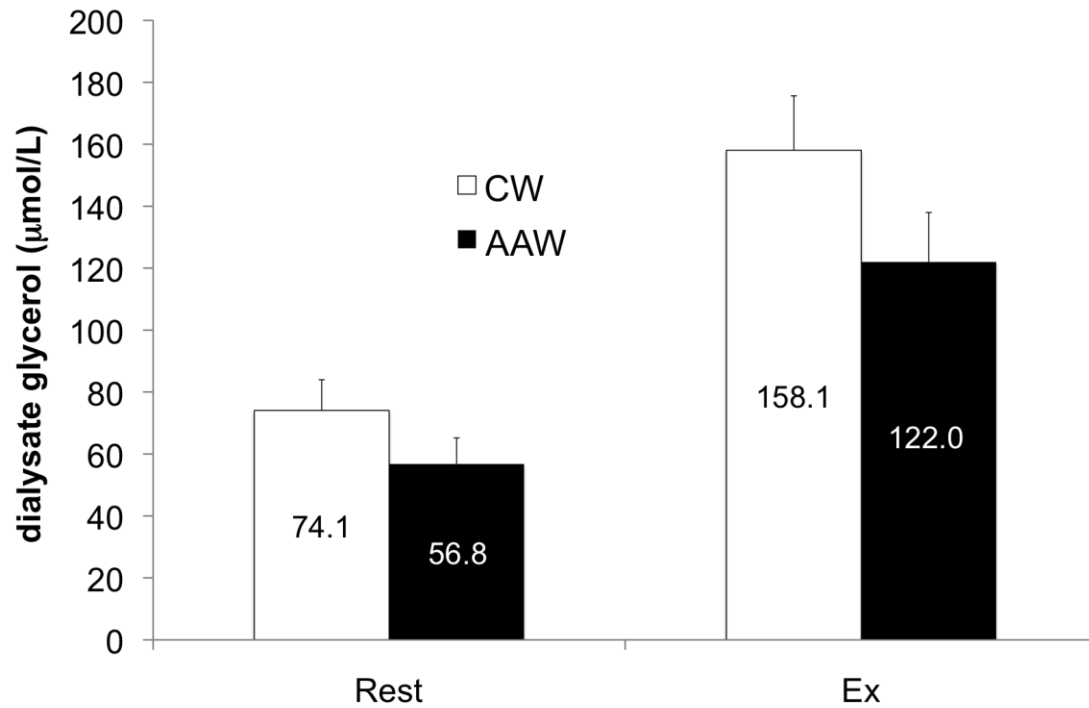


Figure 2. 17 CW and 16 AAW were studied at rest and in response to exercise at 60% $VO_{2peak}$  on a LODE bike. Data are mean $\pm$ SEM. P=not significant.

### Percent Increase of Lipolysis by Isoproterenol CW vs AAW

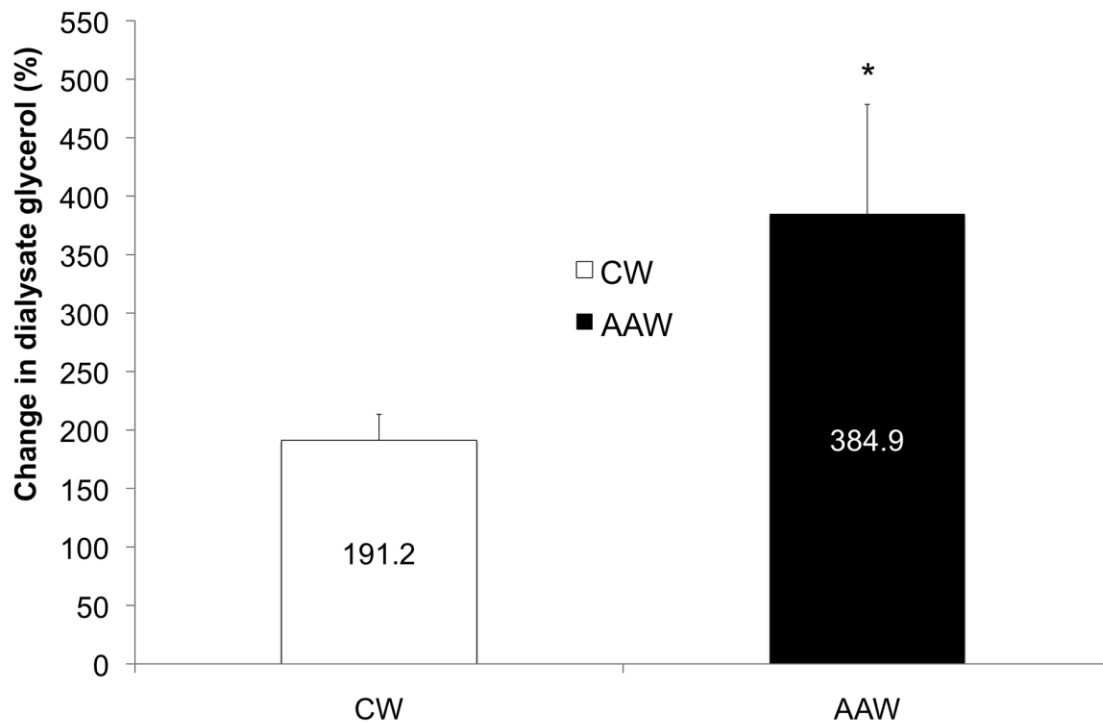


Figure 3. 17 CW and 16 AAW were studied at rest and in response to local perfusion of  $10^{-6}$  M isoproterenol. Data are presented as percent change from basal and are mean $\pm$ SEM. P=0.046.

## **CHAPTER V: Discussion**

The purpose of this study was to determine if AAW were more responsive than CW to stimulation of lipolysis by the B-AR agonist isoproterenol administered locally to subcutaneous abdominal adipose tissue via MD at rest. A second aim of the current study was to determine if AAW have a higher lipolytic response to exercise than CW.

Lipolytic responses in this study were represented by dialysate glycerol production in units of “ $\mu\text{mol/L}$ ”. Based on previous *in-vitro* and *in-vivo* studies, our hypotheses were that AAW would have a higher lipolytic stimulation response to isoproterenol than CW, and that AAW would increase lipolysis more in response to an exercise stimulus than CW. The findings of our study supported the first hypothesis; however, our data did not support the second hypothesis.

### *Isoproterenol stimulated lipolysis*

Numerous *in-vitro* studies have investigated the effects isoproterenol has on lipolysis [3, 15, 17]. The basis for our hypothesis was structured around the McConnaughey *et al.* (2004) study that found AAW have higher B-AR densities than CW. This used *in-vitro* techniques to show that AAW do have higher B-AR densities than CW in the SCAT.

Prior to that 2004 study, Barakat *et al.* (2002) used *in-vitro* techniques to demonstrate that AAW do have a higher fold stimulation than CW in response to isoproterenol. This was due to the AAW having lower basal rates of lipolysis in combination with the increased response to isoproterenol, a finding also observed in the present study. These previous studies have investigated how isoproterenol increased

lipolysis in both AAW and CW, but these researchers only used *in-vitro* methods. These *in-vitro* findings prompted us to determine if these *in-vitro* results could be duplicated using the *in-vivo* technique of microdialysis.

Our findings support the hypothesis that AAW have more of an increase in lipolysis in response to isoproterenol perfusion than CW. Dialysate glycerol increased in the SCAT of AAW by  $385\% \pm 94\%$  and in SCAT of CW by  $191\% \pm 22\%$ . To our knowledge, this is the first time that these two races have been compared with respect to their *in-vivo* response to isoproterenol perfusion.

#### *Exercise stimulated lipolysis*

There have been many studies that show how exercise increases lipolysis in every race and in both sexes. Our participants performed an acute bout of exercise (60 minutes) at the end of the microdialysis procedure. Subjects biked at  $60\% \text{VO}_{2\text{Peak}}$  for the final stage, during which dialysate samples were collected and analyzed post-test in the CMA-600 analyzer.

Johnson *et al.* (2010) demonstrated that with 60 minutes of exercise at  $65\% \text{VO}_{2\text{PEAK}}$ , dialysate glycerol concentrations from microdialysis probes placed in SCAT of premenopausal women nearly tripled. Friedlander *et al.* (1998) also used exercise to increase glycerol production. They also used a cycle ergometer for one hour at  $65\% \text{VO}_{2\text{PEAK}}$  to elicit an increase in plasma glycerol from 0.03 mM to 0.2 mM. We saw a two- to three-fold increase from basal in dialysate glycerol in response to exercise. Both races responded similarly to the exercise bout in terms of increases in dialysate glycerol concentrations from basal. The lack of difference in response to exercise in AAW and CW despite a greater fold-increase in dialysate glycerol in AAW than CW in response to

isoproterenol could mean that exercise mediated pathways of lipolysis work independently from the B-AR pathways. However, others have shown that lipolysis during exercise is in large part due to stimulation of the B-AR pathway. It is more likely that CW have more inhibition or less stimulation of the B-AR in response to isoproterenol, and that this difference between the races is overcome during exercise. Further research investigating microdialysis perfusion of multiple drug combinations could reveal the mechanism underlying the differences between CW and AAW in response to isoproterenol perfusion, as well as why differences are not present in response to exercise.

Our study did reproduce results of previous studies, in that exercise does increase lipolysis. However, our hypothesis that AAW are more responsive to exercise's stimulation of lipolysis than CW was incorrect. There was no racial difference indicated in lipolytic response to exercise stimulation in this study.

### *Limitations*

Studies that use *in-vitro* techniques allow better control of the experimental conditions than do *in-vivo* studies such as those using microdialysis to monitor changes in lipolysis. Many factors can change the rate of lipolysis *in-vivo*, and it is difficult to regulate and measure these numerous variables. One of the variables that should be considered is insulin, which has been shown to have an antilipolytic effect. AAW have been shown to be insensitive to the effects of insulin, from stimulation of glucose uptake to the antilipolytic effects. Based on this insensitivity to insulin alone, AAW should have higher lipolytic rates than CW if matched by insulin concentrations. Knowing plasma, and interstitial, insulin concentrations during the experiments would allow for some knowledge of how insulin affected the results. Fasting and exercise both can change how much insulin is secreted and

how effective it is in the body. Other variables to consider that were not controlled in the current study are hormone sensitive lipase, A-AR agonists, A-AR antagonists, nitric-oxide, adenosine and B-AR antagonists. Further investigations should use a combination of methods to control for most, if not all, of these other variables to determine how isoproterenol and exercise differentially affect lipolytic rates in AAW and CW.

### *Conclusion*

We based our hypothesis on the theory that because B-AR densities are higher in AAW than CW, AAW should have a greater capacity to increase lipolysis than CW. AAW displayed a higher stimulation of lipolysis, as determined by dialysate glycerol concentration, than CW: our theory was therefore supported in the isoproterenol stimulation experiments. However, the hypothesis that stimulation by exercise would also be higher in AAW due to the increased number of B-ARs was not supported.

There is evidence that AAW have more trouble losing weight than CW, some of which may be based on physiological differences between AAW and CW. Further research that can account for both A-AR and B-AR densities and responsiveness, along with insulin and adenosine control may help us understand the mechanisms responsible for this difference.

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## APPENDIX A: IRB APPROVAL FORM



### EAST CAROLINA UNIVERSITY

University & Medical Center Institutional Review Board Office  
1L-09 Brody Medical Sciences Building • 600 Moye Boulevard • Greenville, NC 27834  
Office 252-744-2914 • Fax 252-744-2284 • [www.ecu.edu/irb](http://www.ecu.edu/irb)

TO: Kathleen Gavin, MS, Department of EXSS, ECU, 363 Ward Sports Medicine Building, Mailstop #158  
FROM: UMCIRB JTC  
DATE: May 2, 2011  
RE: Full Committee Approval of a Study  
TITLE: "Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women"

#### UMCIRB #11-077

The above referenced research study was initially reviewed by the convened University and Medical Center Institutional Review Board (UMCIRB) on 2/9/11. The research study underwent a review and approval of requested modifications on 2/14/11 by expedited review. The UMCIRB deemed this **unfunded** study **more than minimal risk** requiring a continuing review in **12 months**. 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 above referenced research study has been given approval for the period of 2/9/11 to 2/8/12. The approval includes the following items:

- Internal Processing Form (revised, dated 2/14/11)
- Protocol (dated 1/19/11)
- Protocol summary
- Informed consent (revised, dated 2/14/11)
- COI disclosure form (dated 1/25/11)
- Recruitment flyer
- 3 day food record/instructions
- Medical history questionnaire

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

NOTE: The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting:  
None

**The UMCIRB applies 45 CFR 46, Subparts A-D, to all research reviewed by the UMCIRB regardless of the funding source. 21 CFR 50 and 21 CFR 56 are applied to all research studies under the Food and Drug Administration regulation. The UMCIRB follows applicable International Conference on Harmonisation Good Clinical Practice guidelines.**



**Consent to Participate in Research that is Greater than Minimal Risk**  
**Information to Consider Before Taking Part in This Research**

Title of Research Study: Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women

Principal Investigator: Kathleen M. Gavin, MS  
Institution/Department or Division: Department of Exercise and Sport Science, College of Health and Human Performance  
Address: Mailstop 158, 363 Ward Sports Medicine Building, Greenville, NC 27858  
Telephone #: 716-713-4226 (cell), 252-744-5104 (lab)

Researchers at East Carolina University (ECU) study diseases, health problems, environmental problems, behavior problems and the human condition. Our goal is to try to find better ways to improve the lives of you and others. To do this, we need the help of people who are willing to take part in research.

The person who is in charge of this research is called the Principal Investigator. The Principal Investigator may have other research staff members who will perform some of the procedures.

The person explaining the research to you may be someone other than the Principal Investigator. Robert Hickner, PhD may be asking you to take part in this study.

You may have questions that this form does not answer. If you do have questions, feel free to ask the person explaining the study, as you go along. You may have questions later and you should ask those questions, as you think of them. There is no time limit for asking about this research.

You do not have to take part in this research. Take your time and think about the information that is provided. If you want, have a friend or family member go over this form with you before you decide. It is up to you. If you choose to be in the study, then you should sign the form when you are comfortable that you understand the information provided below. If you do not want to take part in the study, you should not sign this form. That decision is yours and it is okay to decide not to volunteer.

This form explains why this research is being done, what will happen during the research, and what you will need to do if you decide to volunteer to take part in this research.

**Why is this research being done?**

The purpose of this research study is to understand the reasons why premenopausal women tend to carry their body fat in their hips and thighs. It is known that carrying body fat in the hips and thighs is not as highly associated with cardiovascular disease and diabetes risk as carrying it in the stomach area. We are asking you to take part in this research. However, the decision is yours to make. By doing this research, we hope to learn what role the female hormone, estrogen, plays in deciding where premenopausal women store their body fat and how their body fat is broken down.

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UMCIRB Number: 11-077

Consent Version # or Date: Revision 1 2/14/11  
UMCIRB Version 2011.1.10

Page 1 of 8

Participant's Initials

### **Why am I being invited to take part in this research?**

You are being invited to take part in this research because you are a healthy premenopausal woman, 18-45 years old, with a BMI between 26-34 kg/m<sup>2</sup>. If you volunteer to take part in this study, you will be one of about 20 people to do so.

### **Are there reasons I should not take part in this research?**

I understand I should not volunteer for this study if I am a smoker, under 18 or over 45 years of age, take part in regular physical activity (greater than 20 minutes per day, more than 2 days per week), I am pregnant, lactating or trying to get pregnant, taking any hormone therapy (including hormonal birth control), or are actively trying to lose weight.

### **What other choices do I have if I do not take part in this research?**

You have the choice of not taking part in this research study.

### **Where is the research going to take place and how long will it last?**

The research procedures will be conducted at both the East Carolina University's Human Performance Laboratory FITT building and at the East Carolina Heart Institute (ECHI). You will need to come to FITT building one time during the study. That visit will take about 2.5 hours. You will need to come to ECHI rooms 2377/2379 at least two times during the study. One visit will take 8 hours the other will take 1 hour. The total amount of time you will be asked to volunteer for this study is 11.5 hours over the next few months.

### **What will I be asked to do?**

The following procedures will be done strictly for research purposes.

#### **Screening Procedures (at Human Performance Laboratory's FITT Building)**

- **Medical History Questionnaire:** Before beginning the study, you will complete a medical history questionnaire to provide information on your medical status. Completion of the questionnaire will take approximately 15 minutes.
- **Determination of your body composition using dual energy x-ray absorptiometry (DEXA).** Your body composition (amount of lean and fat tissue) will be measured using an FDA-approved bone density machine (Prodigy, GE Lunar Corp., Madison, WI) known as DEXA. This procedure involves a minimal amount of radiation that is within an acceptable range as provided by "North Carolina Regulations for Protection Against Radiation". You have been informed any time an individual is exposed to radiation there is potential risk. The amount of radiation (1-3 microSieverts) that you would be exposed to is quite minimal. During this procedure, you will lay face up on a table for the duration of the test which will last for 8-10 minutes. The scanner will then pass over your body. You will be wearing typical workout clothing for the procedure but no metal can be worn. You have been informed that a person trained for the use of the DEXA will perform all testing. One benefit of this testing is that it provides the most accurate assessment of body composition available. You will undergo this test at the beginning of the study.
- **Waist to Hip ratio.** Waist and hip circumferences will be taken with a tape measure against the skin around the waist and hip regions. This test will last approximately 5 minutes.
- **Exercise testing.** You will undergo a VO<sub>2max</sub> test at the beginning of the study to determine your aerobic capacity using a stationary bicycle. You will engage in a brief warm-up period before start of the test. You will begin the test by pedaling against a low resistance. The difficulty of the exercise will increase (by increasing the resistance you are pedaling against) every two minutes until you are no longer able to continue further with the test. The air

UMCIRB Number: 11-077

Page 2 of 8

Consent Version # or Date: Revision 1 2/14/11

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UMCIRB Version 2011.1.10

you breathe out will be collected continuously, blood pressure will be assessed every three minutes and heart rate will be measured every minute throughout the test. Total exercise time for this test will be 10-15 minutes.

- **Menstrual cycle tracking.** Because several tests in the study are based on the level of hormones in your body at certain times during the month, we will ask you to try and remember the dates your previous 5 cycles started and well as keep track of your menstrual cycle for 1-2 months between your screening visit and your research visit. You will be asked to call the study coordinator on day one of your cycle each month. Using these records, the study coordinator will be able to schedule your next two study visits during the appropriate phase if your menstrual cycle.
- **3 day food record.** You will be asked to record all of the food that you eat and beverages that you consume in the 3 days prior to the microdialysis visit.

The following research procedures will be scheduled according to your menstrual cycle tracking, it is important for at least one of the microdialysis visits to take place during early follicular phase of your menstrual cycle (day 2-6 after the start of menses, or bleeding).

**Research Procedures (at the East Carolina Heart Institute):**

**Microdialysis Visit Procedures**

We will ask you report to ECHI at 8am after an overnight fast (no food or drink other than water after 10pm the night before).

- **Microdialysis.** For the microdialysis test 8 small flexible pieces of plastic tubing (about an inch long and the width of a small needle) will be inserted through your skin into the fat just under your skin around your stomach and upper buttock (4 into each region). A numbing solution (ethyl chloride) will be sprayed on your skin to reduce the pain experienced when the tubing is inserted. These pieces of tubing will be inserted about 1/8 to 1/4 inch below the skin by first inserting a needle surrounded by a plastic liner. The needle will be taken out of the fat and replaced with one of the small pieces of tubing. This tubing will not be located in a blood vessel, it will be between the fat cells. During the experiment a salt solution with a small amount of ethanol (alcohol) will be pumped through these pieces of tubing to monitor blood flow in the fat tissue. Since the pumped fluid is similar to the fluid already present in your body, this fluid is harmless to you. Approximately one small drop of this solution will be pumped through the tubing per hour. A very small amount of alcohol will be included in this solution, but the amount of alcohol going through the tubing over the entire experiment is less than that in one small drop (1/4 the size of the erasure on a pencil) of alcoholic beverage. Samples of the fluid from these tubes will be collected by a member of the research team every 15-30 minutes throughout the visit.

At certain times we will add some other compounds to the solution (isoproterenol, phentolamine and estrogen) to see if they change blood flow to your fat tissue or the level of fat breakdown in your body. You will not feel the presence or effects of any of these substances. These types of substances have been infused into people's veins many times with rarely harmful effects. Following insertion of the tubing, you will be asked to sit resting in a hospital bed for 6.5 hours after which you will be asked to exercise on a stationary bike at a moderate intensity for 1 hour. Following the exercise bout all probes will be removed and you will be free to go.

- **Blood Samples.** During the microdialysis procedure blood samples will be drawn from an intravenous (IV) catheter placed in a vein in your arm at six different times: 5 times during the resting portion of the visit and once 10 minutes before you stop exercising. Placement of the IV catheter allows you to have multiple blood samples taken from only one needle stick. These blood samples will be used to determine the blood concentration of certain substances related to fat breakdown as well as your sex hormones and how these are different at rest and

UMCIRB Number: 11-077

Page 3 of 8

Consent Version # or Date: Revision 1 2/14/11

UMCIRB  
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FROM 2-9-11  
TO 2-8-12

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your heart rate and blood pressure throughout the test and you will be able to stop the test at any time if you feel ill or uncomfortable for any reason. Wearing the mouthpiece needed to collect the air you breathe out may become uncomfortable. You may experience a dry mouth and throat while wearing the mouthpiece, water will be available to drink before and after the test.

- Overnight fasting may cause hunger and possibly dizziness.
- Resting energy expenditure test – Laying completely still for 30 minutes may prove to be difficult and wearing the mouthpiece needed to collect the air you breathe out may become uncomfortable. You may experience a dry mouth and throat while wearing the mouthpiece. Water will be available for you to drink before and after the test.
- Risks associated with an IV catheter and blood draw are small, and include hematoma (minor swelling and bruising) and infection. The insertion of the IV catheter will be performed by qualified personnel to reduce these risks.
- Fat Biopsies – Robert Hickner, Ph.D, will perform the fat biopsies with Dr. James deVente, MD providing medical coverage. There is a small risk of hematoma or infection around the biopsy site, as well as mild tenderness and bruising. The risk will be minimized by using sterile procedures and applying pressure to the biopsy site until bleeding has stopped. You will feel mild pain, similar to the pain you feel when receiving a shot in the arm, during injection of numbing medication (Lidocaine) under the skin over their abdominal and gluteal adipose tissue before the adipose tissue biopsy. Because there is an extremely remote risk of allergic reaction to the Lidocaine anesthesia, risk will be minimized by using subject's who have had prior exposure to Lidocaine or Novocaine anesthesia. To your knowledge, you are not allergic to "caine-type" anesthetics. For example, you have not had an allergic reaction to an injection at the dentist's office. You cannot participate in this research if you knowingly have heart disease or any condition that could result in excessive bleeding.

There is also the risk of potential embarrassment with adipose tissue biopsy of the upper buttock area. This risk will be minimized by the involvement of minimal staff associated with the procedure and conducting the procedure in a private room or behind a privacy curtain.

- Insertion of microdialysis probes (thin plastic tubing) - You will feel mild pain, similar to the pain you feel when you get a blood draw when the probes are being placed, but we will use a cold spray to diminish your discomfort. You should not feel pain from the microdialysis probe after insertion of the probe into your fat. You will feel no discomfort from the substances (for example, alcohol) pumped through the microdialysis probe. Risks associated with this procedure, as well as the blood draw, are small and include hematoma (minor swelling and bruising) and infection. To minimize the risk of bruising or infection, insertion of the microdialysis probes will be performed using sterile techniques. The microdialysis probes are made of materials that will not cause allergic reactions. The probes will be removed immediately post-exercise and pressure will be applied to the area to eliminate any potential bleeding. There is also the risk of potential embarrassment with microdialysis of the upper buttock area. This risk will be minimized by the presence of minimal staff necessary to insert the probes and conducting the procedure in a private room or behind a privacy curtain.

The risks from compounds added to the liquid that will be pumped through the probes are minor because the compounds in the small amounts used do not affect your whole-body circulation or metabolism. There is a risk of infection or allergic reaction from the chemicals, but this risk is minimized by using sterile techniques and filtering the solution the day of the experiment.

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Page 5 of 8

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*Title of Study:* Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women

There is always a chance that any medical intervention may cause you some discomfort or harm and the procedures in this study are no different. We will do everything possible to keep you from being harmed. There may be other risks or side effects that occur which we do not know about at this time.

It is important for you to tell us as quickly as possible if you experience a side effect.

**Are there any reasons you might take me out of the research?**

During the study, information about this research may become available that would be important to you. This includes information that, once learned, might cause you to change your mind about wanting to be in the study. We will tell you as soon as we can. This might include information about the side effects that are caused by taking part in this study. If that happens, we can tell you about these new side effects and let you decide whether you want to continue to take part in the research.

There may be reasons we will need to take you out of the study, even if you want to stay in. We may find out that it is not safe for you to stay in the study. It may be that the side effects are so severe that we need to stop the study or take you out of the study to reduce your risk of harm. If we find that the research might harm you or that it is not providing enough of a benefit to justify the risks you are taking, we will contact you immediately with an explanation of why you can no longer participate and give you copies of results from any screening or research tests that would be of interest to you or your primary care physician. We may also find that you cannot come for your study visits as scheduled. If that is found to be true, we will need to take you out of the study.

**What are the possible benefits I may experience from taking part in this research?**

We do not know if you will get any benefits by taking part in this study. That is why we are doing this research. This research should help us learn more about the female sex hormone, estrogen, and fat storage and breakdown in premenopausal women.

There may be no personal benefit from your participation but the information gained by doing this research may help others in the future.

**Will I be paid for taking part in this research?**

We will pay you for the time you volunteer while being in this study. Compensation will be provided according to the visits you complete as part of the study. \$25 will be provided for the screening visit, \$100 for the microdialysis visit and \$50 for the fat biopsy visit for a possible total of \$175. Appropriate compensation will be provided after completion of the study or after your participation in the study has stopped for any reason.

**What will it cost me to take part in this research?**

It will not cost you any money to be part of the research, it will only cost your personal time. You will be responsible for providing your own transportation to and from the FITT Building and ECHI for each study related visit.

**Who will know that I took part in this research and learn personal information about me?**

To do this research, ECU and the people and organizations listed below may know that you took part in this research and may see information about you that is normally kept private. With your permission, these people may use your private information to do this research:

- The research team, including the Principal Investigator, study coordinator, research nurses, and all other research staff or students involved in conducting the study.

UMCIRB Number: 11-077

Page 6 of 8

Consent Version # or Date: Revision 1 2/14/11

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**UMCIRB HIPAA Privacy Authorization**

The Brody School of Medicine (BSOM)/Pitt County Memorial Hospital (PCMH):  
**Research Participant Authorization to Use and Disclose Protected Health Information  
for Storing Tissues/Samples/Specimens for Future Research**

**For use only with the research consent form for: UMCIRB#: 11-077**

**PI: Kathleen M. Gavin, MS**

**Study Title: Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in  
Premenopausal Women.**

When taking part in research, protected health information (PHI) is collected, used, and shared with others who are involved in the research. Federal laws require that researchers and health care providers protect your PHI. Also, federal laws require that we get your permission to use collected PHI for the research. This permission is called authorization.

In order to complete the secondary research project in which you have decided to take part, we need to collect and use some of your PHI as described below.

**What types of protected health information (PHI) about me will be used or disclosed?**

- |                                                          |                                                                                                  |
|----------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| <input type="checkbox"/> BSOM/PCMH Billing records       | <input type="checkbox"/> PCMH medical records (in and out patient)                               |
| <input type="checkbox"/> BSOM/PCMH Mental Health records | <input type="checkbox"/> PCMH/BSOM lab, pathology and/or radiology results                       |
| <input type="checkbox"/> BSOM Physician/clinic records   | <input type="checkbox"/> PHI previously collected for research purposes                          |
| <input type="checkbox"/> Other:                          | <input checked="" type="checkbox"/> Samples/tissue/specimens collected as part of the main study |

**Who will use or disclose my PHI?**

- Principal Investigator
- Other members of the research team
- Other providers involved in your care during research procedures, outpatient/inpatient stays during which research is being performed, or physician office visits during which research is being performed.

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**Location where research will be conducted**

The members of the research team will conduct the research study at:  
 East Carolina University (ECU)    PCMH    ECU & PCMH    Other

**Who will receive my PHI?**

- Sponsor or other funding source to provide oversight for entire research project
- Research investigators to conduct and oversee the research project
- Research team members to participate in the various research activities
- FDA or other regulatory agencies to provide regulatory oversight
- UMCIRB to provide continuing review of the research project
- Institutional officials in connection with duties for monitoring research activity
- Researchers at other sites to participate in the research when more than one research site is involved
- Other

We will share only the PHI listed above with the individuals/agencies listed above. If we need to share other PHI or if we need to send PHI to other individuals/agencies not listed above, we will ask for your permission in writing again

**How my PHI may be released to others:**

The BSOM and PCMH are required under law to protect your PHI. However, those individuals or agencies who receive your PHI may not be required by the Federal privacy laws to protect it and may share your PHI with others without your permission, if permitted by the laws governing them.

**What if I do not sign this form?**

Your tissue/samples/specimens will not be stored for future research. You can still participate in the main part of the study without agreeing to this use of your tissues/samples/specimens without penalty.

**How may I revoke (take back or withdraw) my authorization?**

You have the right to stop sharing your PHI. To revoke (or take back) your authorization, you must give the investigator your request to revoke (or take back) your authorization in writing. If you want us to stop collecting your PHI for future research, your samples/tissue/specimens will be removed from storage. This will not affect your ability to receive standard medical care or any other benefits for which you are entitled to receive. PHI used for future research prior to revoking (or taking back) your Authorization will continue to be used for the purposes of that research study. Also, the FDA (if involved with your study) can look at your PHI related to the study even if you withdraw this authorization.

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 TO no expiration

**Restrictions on access to my PHI:**

You may not be able to see your PHI in your medical record related to this study until the study is complete. If it is necessary for your care, your PHI will be provided to you or your physician.

**How long may the PHI about me be used or disclosed for this study?**

Research information continues to be looked at after the study is finished so it is difficult to say when use of your PHI will stop. There is not an expiration date for this authorization to use and disclose your PHI for this study.

If you have questions about the sharing of PHI related to this research study, call the principal investigator, Kathleen Gavin at phone number 252-744-5104. Also, you may telephone the University and Medical Center Institutional Review Board at 252-744-2914. In addition, if you have concerns about confidentiality and privacy rights, you may phone the Privacy Officer at Pitt County Memorial Hospital at 252-847-6545 or the Privacy Officer at East Carolina University at 252-744-5200.

**Authorization**

To authorize the use and disclosure of your PHI for future research in the way that has been described in this form, please sign below and date when you signed this form. A signed copy of this Authorization will be given to you for your records.

Participant's Name (print)	Signature	Date
Authorized Representative Name (print) —Relationship	Signature	Date
Person Obtaining Authorization	Signature	Date

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TO no expiration

**UMCIRB HIPAA Privacy Authorization**

The Brody School of Medicine (BSOM)/Pitt County Memorial Hospital (PCMH):  
Research Participant Authorization to Use and Disclose Protected Health Information for  
Research

**For use only with the research consent form for: UMCIRB#: 11-077**

**PI: Kathleen M. Gavin, MS**

**Title: Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in  
Premenopausal Women.**

When taking part in research, protected health information (PHI) is collected, used, and shared with others who are involved in the research. Federal laws require that researchers and health care providers protect your PHI. Also, federal laws require that we get your permission to use collected PHI for the research. This permission is called authorization.

In order to complete the research project in which you have decided to take part, we need to collect and use some of your PHI as described below.

**What types of protected health information (PHI) about me will be used or disclosed?**

- |                                                                         |                                                                            |
|-------------------------------------------------------------------------|----------------------------------------------------------------------------|
| <input type="checkbox"/> BSOM/PCMH Billing records                      | <input type="checkbox"/> PCMH medical records (in and out patient)         |
| <input type="checkbox"/> BSOM/PCMH Mental Health records                | <input type="checkbox"/> PCMH/BSOM lab, pathology and/or radiology results |
| <input type="checkbox"/> BSOM Physician/clinic records                  | <input type="checkbox"/> PHI previously collected for research purposes    |
| <input checked="" type="checkbox"/> Other: Research related information |                                                                            |

**Who will use or disclose my PHI?**

- Principal Investigator
- Other members of the research team
- Other providers involved in your care during research procedures, outpatient/inpatient stays during which research is being performed, or physician office visits during which research is being performed.

**Location where research will be conducted**

The members of the research team will conduct the research study at:  
 East Carolina University (ECU)  PCMH  ECU & PCMH  Other

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FROM 4-28-11  
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Page 1 of 3

**Who will receive my PHI?**

- Sponsor or other funding source to provide oversight for entire research project
- Research investigators to conduct and oversee the research project
- Research team members to participate in the various research activities
- FDA or other regulatory agencies to provide regulatory oversight
- UMCIRB to provide continuing review of the research project
- Institutional officials in connection with duties for monitoring research activity
- Researchers at other sites to participate in the research when more than one research site is involved
- Other

We will share only the PHI listed above with the individuals/agencies listed above. If we need to share other PHI or if we need to send PHI to other individuals/agencies not listed above, we will ask for your permission in writing again

**How my PHI may be released to others:**

The BSOM and PCMH are required under law to protect your PHI. However, those individuals or agencies who receive your PHI may not be required by the Federal privacy laws to protect it and may share your PHI with others without your permission, if permitted by the laws governing them.

**What if I do not sign this form?**

You will not be eligible to participate in this study if you do not sign this Authorization form.

**How may I revoke (take back or withdraw) my authorization?**

You have the right to stop sharing your PHI. To revoke (or take back) your authorization, you must give the investigator your request to revoke (or take back) your authorization in writing. If you want us to stop collecting your PHI for the study, you may be removed from the study. If you are removed from the study it will not affect your ability to receive standard medical care or any other benefits for which you are entitled to receive. PHI collected for the research study prior to revoking (or taking back) your Authorization will continue to be used for the purposes of the research study. Also, the FDA (if involved with your study) can look at your PHI related to the study even if you withdraw this authorization.

**Restrictions on access to my PHI:**

You may not be able to see your PHI in your medical record related to this study until the study is complete. If it is necessary for your care, your PHI will be provided to you or your physician.

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Page 2 of 3

**How long may the PHI about me be used or disclosed for this study?**

Research information continues to be looked at after the study is finished so it is difficult to say when use of your PHI will stop. There is not an expiration date for this authorization to use and disclose your PHI for this study.

If you have questions about the sharing of PHI related to this research study, call the principal investigator Kathleen Gavin at phone number 716-713-4226 . Also, you may telephone the University and Medical Center Institutional Review Board at 252-744-2914. In addition, if you have concerns about confidentiality and privacy rights, you may phone the Privacy Officer at Pitt County Memorial Hospital at 252-847-6545 or the Privacy Officer at East Carolina University at 252-744-5200.

**Authorization**

To authorize the use and disclosure of your PHI for this study in the way that has been described in this form, please sign below and date when you signed this form. A signed copy of this Authorization will be given to you for your records.

Participant's Name (print)	Signature	Date
Authorized Representative Name (print)---Relationship	Signature	Date
Person Obtaining Authorization	Signature	Date

UMCIRB  
APPROVED  
FROM 4-28-11  
TO no expiration

\*\*\*\*\*IMPORTANT INFORMATION\*\*\*\*\*

**Continuing Review/Closure Obligation**

As a investigator you are required to submit a continuing review/closure form to the UMCIRB office in order to have your study renewed or closed before the date of expiration as noted on your approval letter. This information is required to outline the research activities since it was last approved. You must submit this research form even if you there has been no activity, no participant s enrolled, or you do not wish to continue the activity any longer. The regulations do not permit any research activity outside of the IRB approval period. Additionally, the regulations do not permit the UMCIRB to provide a retrospective approval during a period of lapse. Research studies that are allowed to be expired will be reported to the Vice Chancellor for Research and Graduate Studies, along with relevant other administration within the institution. The continuing review/closure form is located on our website at [www.ecu.edu/irb](http://www.ecu.edu/irb) under forms and documents. The meeting dates and submission deadlines are also posted on our web site under meeting information. Please contact the UMCIRB office at 252-744-2914 if you have any questions regarding your role or requirements with continuing review.  
<http://www.hhs.gov/ohrp/humansubjects/guidance/contrev0107.htm>

**Required Approval for Any Changes to the IRB Approved Research**

As a research investigator you are required to obtain IRB approval prior to making any changes in your research study. Changes may not be initiated without IRB review and approval, except when necessary to eliminate an immediate apparent hazard to the participant. In the case when changes must be immediately undertaken to prevent a hazard to the participant and there was no opportunity to obtain prior IRB approval, the IRB must be informed of the change as soon as possible via a protocol deviation form.  
<http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm#46.103>

**Reporting of Unanticipated Problems to Participants or Others**

As a research investigator you are required to report unanticipated problems to participants or others involving your research as soon as possible. Serious adverse events as defined by the FDA regulations may be a subset of unanticipated problems. The reporting times as specified within the research protocol, applicable regulations and policies should be followed.  
<http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm>

# APPENDIX B: CONSENT APPROVAL FORM

Study ID:UMCIRB 11-077 Date Approved: 1/25/2012 Expiration Date: 1/24/2013



## Consent to Participate in Research that is Greater than Minimal Risk Information to Consider Before Taking Part in This Research

Title of Research Study: Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women

Principal Investigator: Kathleen M. Gavin, MS  
Institution/Department or Division: Department of Exercise and Sport Science, College of Health and Human Performance  
Address: Mailstop 158, 363 Ward Sports Medicine Building, Greenville, NC 27858  
Telephone #: 716-713-4226 (cell), 252-744-5104 (lab)

Researchers at East Carolina University (ECU) study diseases, health problems, environmental problems, behavior problems and the human condition. Our goal is to try to find better ways to improve the lives of you and others. To do this, we need the help of people who are willing to take part in research.

The person who is in charge of this research is called the Principal Investigator. The Principal Investigator may have other research staff members who will perform some of the procedures.

The person explaining the research to you may be someone other than the Principal Investigator. Robert Hickner, PhD may be asking you to take part in this study.

You may have questions that this form does not answer. If you do have questions, feel free to ask the person explaining the study, as you go along. You may have questions later and you should ask those questions, as you think of them. There is no time limit for asking about this research.

You do not have to take part in this research. Take your time and think about the information that is provided. If you want, have a friend or family member go over this form with you before you decide. It is up to you. If you choose to be in the study, then you should sign the form when you are comfortable that you understand the information provided below. If you do not want to take part in the study, you should not sign this form. That decision is yours and it is okay to decide not to volunteer.

This form explains why this research is being done, what will happen during the research, and what you will need to do if you decide to volunteer to take part in this research.

### Why is this research being done?

The purpose of this research study is to understand the reasons why premenopausal women tend to carry their body fat in their hips and thighs. It is known that carrying body fat in the hips and thighs is not as highly associated with cardiovascular disease and diabetes risk as carrying it in the stomach area. We are asking you to take part in this research. However, the decision is yours to make. By doing this research, we hope to learn what role the female hormone, estrogen, plays in deciding where premenopausal women store their body fat and how their body fat is broken down.

UMCIRB Number: 11-077

Page 1 of 8

Consent Version # or Date: Revision 2 06/07/11  
UMCIRB Version 2011.1.10

Participant's Initials

*Title of Study:* Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women

### **Why am I being invited to take part in this research?**

You are being invited to take part in this research because you are a healthy premenopausal woman, 18-45 years old, with a BMI between 26-34 kg/m<sup>2</sup>. If you volunteer to take part in this study, you will be one of about 20 people to do so.

### **Are there reasons I should not take part in this research?**

I understand I should not volunteer for this study if I am a smoker, under 18 or over 45 years of age, take part in regular physical activity (greater than 20 minutes per day, more than 2 days per week), I am pregnant, lactating or trying to get pregnant, taking any hormone therapy (including hormonal birth control), or are actively trying to lose weight.

### **What other choices do I have if I do not take part in this research?**

You have the choice of not taking part in this research study.

### **Where is the research going to take place and how long will it last?**

The research procedures will be conducted at both the East Carolina University's Human Performance Laboratory FITT building and at the East Carolina Heart Institute (ECHI). You will need to come to FITT building one time during the study. That visit will take about 2.5 hours. You will need to come to ECHI rooms 2377/2379 at least two times during the study. One visit will take 8 hours the other will take 1 hour. The total amount of time you will be asked to volunteer for this study is 11.5 hours over the next few months.

### **What will I be asked to do?**

The following procedures will be done strictly for research purposes.

#### **Screening Procedures (at Human Performance Laboratory's FITT Building)**

- **Medical History Questionnaire:** Before beginning the study, you will complete a medical history questionnaire to provide information on your medical status. Completion of the questionnaire will take approximately 15 minutes.
- **Determination of your body composition using dual energy x-ray absorptiometry (DXA).** Your body composition (amount of lean and fat tissue) will be measured using an FDA-approved bone density machine (Prodigy, GE Lunar Corp., Madison, WI) known as DEXA. This procedure involves a minimal amount of radiation that is within an acceptable range as provided by "North Carolina Regulations for Protection Against Radiation". You have been informed any time an individual is exposed to radiation there is potential risk. The amount of radiation (1-3 microSieverts) that you would be exposed to is quite minimal. During this procedure, you will lay face up on a table for the duration of the test which will last for 8-10 minutes. The scanner will then pass over your body. You will be wearing typical workout clothing for the procedure but no metal can be worn. You have been informed that a person trained for the use of the DEXA will perform all testing. One benefit of this testing is that it provides the most accurate assessment of body composition available. You will undergo this test at the beginning of the study.
- **Waist to Hip ratio.** Waist and hip circumferences will be taken with a tape measure against the skin around the waist and hip regions. This test will last approximately 5 minutes.
- **Exercise testing.** You will undergo a VO<sub>2max</sub> test at the beginning of the study to determine your aerobic capacity using a stationary bicycle. You will engage in a brief warm-up period before start of the test. You will begin the test by pedaling against a low resistance. The difficulty of the exercise will increase (by increasing the resistance you are pedaling against) every two minutes until you are no longer able to continue further with the test. The air

UMCIRB Number: 11-077

Page 2 of 8

Consent Version # or Date: Revision 2 06/07/11

UMCIRB Version 2011.1.10

Participant's Initials

*Title of Study:* Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women

you breathe out will be collected continuously, blood pressure will be assessed every three minutes and heart rate will be measured every minute throughout the test. Total exercise time for this test will be 10-15 minutes.

- **Menstrual cycle tracking.** Because several tests in the study are based on the level of hormones in your body at certain times during the month, we will ask you to try and remember the dates your previous 5 cycles started and well as keep track of your menstrual cycle for 1-2 months between your screening visit and your research visit. You will be asked to call the study coordinator on day one of your cycle each month. Using these records, the study coordinator will be able to schedule your next two study visits during the appropriate phase of your menstrual cycle.
- **3 day food record.** You will be asked to record all of the food that you eat and beverages that you consume in the 3 days prior to the microdialysis visit.

The following **research procedures** will be scheduled according to your menstrual cycle tracking, it is important for at least one of the microdialysis visits to take place during early follicular phase of your menstrual cycle (day 2-6 after the start of menses, or bleeding).

**Research Procedures (at the East Carolina Heart Institute):**

**Microdialysis Visit Procedures**

We will ask you report to ECHI at 8am after an overnight fast (no food or drink other than water after 10pm the night before).

- **Microdialysis.** For the microdialysis test 8 small flexible pieces of plastic tubing (about an inch long and the width of a small needle) will be inserted through your skin into the fat just under your skin around your stomach and upper buttock (4 into each region). A numbing solution (ethyl chloride) will be sprayed on your skin to reduce the pain experienced when the tubing is inserted. These pieces of tubing will be inserted about 1/8 to 1/4 inch below the skin by first inserting a needle surrounded by a plastic liner. The needle will be taken out of the fat and replaced with one of the small pieces of tubing. This tubing will not be located in a blood vessel, it will be between the fat cells. During the experiment a salt solution with a small amount of ethanol (alcohol) will be pumped through these pieces of tubing to monitor blood flow in the fat tissue. Since the pumped fluid is similar to the fluid already present in your body, this fluid is harmless to you. Approximately one small drop of this solution will be pumped through the tubing per hour. A very small amount of alcohol will be included in this solution, but the amount of alcohol going through the tubing over the entire experiment is less than that in one small drop (1/4 the size of the erasure on a pencil) of alcoholic beverage. Samples of the fluid from these tubes will be collected by a member of the research team every 15-30 minutes throughout the visit.  
At certain times we will add some other compounds to the solution (isoproterenol, phenolamine and estrogen) to see if they change blood flow to your fat tissue or the level of fat breakdown in your body. You will not feel the presence or effects of any of these substances. These types of substances have been infused into people's veins many times with rarely harmful effects. Following insertion of the tubing, you will be asked to sit resting in a hospital bed for 6.5 hours after which you will be asked to exercise on a stationary bike at a moderate intensity for 1 hour. Following the exercise bout all probes will be removed and you will be free to go.
- **Blood Samples.** During the microdialysis procedure blood samples will be drawn from an intravenous (IV) catheter placed in a vein in your arm at six different times: 5 times during the resting portion of the visit and once 10 minutes before you stop exercising. Placement of the IV catheter allows you to have multiple blood samples taken from only one needle stick. These blood samples will be used to determine the blood concentration of certain substances related to fat breakdown as well as your sex hormones and how these are different at rest and

UMCIRB Number: 11-077

Page 3 of 8

Consent Version # or Date: Revision 2 06/07/11

UMCIRB Version 2011.1.10

Participant's Initials

*Title of Study:* Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women

during exercise. The total amount of blood that drawn as part of this study will be approximately 6 tablespoons, this amount is very small compared to the total amount (about a gallon) of blood that you have.

- **Determination of resting energy expenditure.** While you are resting quietly during the microdialysis visit you will undergo a resting energy expenditure test. This test will determine how many calories you burn at rest. During this test the air you exhale will be continuously collected for 30 minutes through a mouthpiece similar to what you will use while you are exercising. During this time you will lay quietly while remaining awake. The total time this test will be approximately 30-40 minutes.

#### **Fat Biopsy Visit Procedures**

We will ask you report to ECHI at 8am after an overnight fast (no food or drink other than water after 10pm the night before).

- **Adipose tissue (fat) biopsies.** You will undergo biopsies to determine the level of proteins related to lipolysis and sex hormone production in your fat. This will usually be conducted the day immediately preceding or following the microdialysis procedure. For this procedure, a small amount of anesthesia (less than 1 teaspoon of 1% Lidocaine) will be injected in a ½ inch area under the skin of the abdomen and buttock. About 1 teaspoon of saline (salt water) will also be injected under the skin. Adipose tissue will be aspirated (sucked out) using a needle with suction provided by a large syringe. Because there is an extremely remote risk of allergic reaction to the Lidocaine anesthesia, risk will be minimized by using subject's who have had prior exposure to Lidocaine or Novocaine anesthesia or by having a physician available during the biopsy procedure for those subjects who have not had previous exposure to "caine-type" anesthetics. To your knowledge, you are not allergic to "caine-type" anesthetics. For example, you have not had an allergic reaction to an injection at the dentist's office. Robert Hickner, Ph.D, will perform the biopsies.

We will ask that you do not change your activity level, dietary habits or any medication you are taking during the time you are taking part in this study. If this becomes a concern during your participation we ask you to contact us and let us know as soon as possible.

#### **What possible harms or discomforts might I experience if I take part in the research?**

There are always risks (the chance of harm) when taking part in research. We know about the following risks or discomforts you may experience if you choose to volunteer for this study. These are called side effects. The following side effects are known to occur in some people:

- Medical history/circumferences – Risk of feeling embarrassed or a loss of privacy. To minimize these risks, testing will be conducted in a private area and all information collected will be kept confidential.
- Body composition assessment (DXA) - Exposure to low levels of radiation. Risks will be minimized by using an FDA-approved bone density machine (Prodigy, GE Lunar Corp., Madison, WI). This procedure involves a minimal amount of radiation. 1-3 microSieverts) that is within an acceptable range as provided by "North Carolina Regulations for Protection Against Radiation". The amount of radiation (1-3 microSieverts) exposure of one procedure is quite minimal. For example, radiation exposure is approximately 80 microSieverts on a transatlantic airline flight of 8 hours, 50 microSieverts living in Denver, Colorado, at an elevation of 5,000 feet for approximately 4 weeks, or 30 to 40 microSieverts during a typical chest x-ray.
- Exercise testing – Labored breathing, dizziness, ventricular arrhythmia (odd heart beats), and in very rare instances death can occur during exercise. These risks are very small, with an incidence of fewer than 1 in 10,000 deaths in patients who are known to, or suspected of, having heart disease. Anyone suspected of having heart disease will

UMCIRB Number: 11-077

Page 4 of 8

Consent Version # or Date: Revision 2 06/07/11

UMCIRB Version 2011.1.10

Participant's Initials

*Title of Study:* Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women

be disqualified from participating in this study. To minimize these risks during exercise, we monitor your heart rate and blood pressure throughout the test and you will be able to stop the test at any time if you feel ill or uncomfortable for any reason. Wearing the mouthpiece needed to collect the air you breathe out may become uncomfortable. You may experience a dry mouth and throat while wearing the mouthpiece, water will be available to drink before and after the test.

- Overnight fasting may cause hunger and possibly dizziness.
- Resting energy expenditure test – Laying completely still for 30 minutes may prove to be difficult and wearing the mouthpiece needed to collect the air you breathe out may become uncomfortable. You may experience a dry mouth and throat while wearing the mouthpiece. Water will be available for you to drink before and after the test.
- Risks associated with an IV catheter and blood draw are small, and include hematoma (minor swelling and bruising) and infection. The insertion of the IV catheter will be performed by qualified personnel to reduce these risks.
- Fat Biopsies - Robert Hickner, Ph.D, will perform the fat biopsies with Dr. James deVente, MD providing medical coverage. There is a small risk of hematoma or infection around the biopsy site, as well as mild tenderness and bruising. The risk will be minimized by using sterile procedures and applying pressure to the biopsy site until bleeding has stopped. You will feel mild pain, similar to the pain you feel when receiving a shot in the arm, during injection of numbing medication (Lidocaine) under the skin over their abdominal and gluteal adipose tissue before the adipose tissue biopsy. Because there is an extremely remote risk of allergic reaction to the Lidocaine anesthesia, risk will be minimized by using subject's who have had prior exposure to Lidocaine or Novocaine anesthesia. To your knowledge, you are not allergic to "caine-type" anesthetics. For example, you have not had an allergic reaction to an injection at the dentist's office. You cannot participate in this research if you knowingly have heart disease or any condition that could result in excessive bleeding.

There is also the risk of potential embarrassment with adipose tissue biopsy of the upper buttock area. This risk will be minimized by the involvement of minimal staff associated with the procedure and conducting the procedure in a private room or behind a privacy curtain.

- Insertion of microdialysis probes (thin plastic tubing) - You will feel mild pain, similar to the pain you feel when you get a blood draw when the probes are being placed, but we will use a cold spray to diminish your discomfort. You should not feel pain from the microdialysis probe after insertion of the probe into your fat. You will feel no discomfort from the substances (for example, alcohol) pumped through the microdialysis probe. Risks associated with this procedure, as well as the blood draw, are small and include hematoma (minor swelling and bruising) and infection. To minimize the risk of bruising or infection, insertion of the microdialysis probes will be performed using sterile techniques. The microdialysis probes are made of materials that will not cause allergic reactions. The probes will be removed immediately post-exercise and pressure will be applied to the area to eliminate any potential bleeding. There is also the risk of potential embarrassment with microdialysis of the upper buttock area. This risk will be minimized by the presence of minimal staff necessary to insert the probes and conducting the procedure in a private room or behind a privacy curtain.

The risks from compounds added to the liquid that will be pumped through the probes are minor because the compounds in the small amounts used do not affect your whole-body circulation or metabolism. There is a risk of infection or allergic reaction from the chemicals, but this risk is minimized by using sterile techniques and filtering the solution the day of the experiment.

*UMCIRB Number:* 11-077

*Page 5 of 8*

*Consent Version # or Date:* Revision 2 06/07/11

*UMCIRB Version 2011.1.10*

*Participant's Initials*

*Title of Study:* Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women

There is always a chance that any medical intervention may cause you some discomfort or harm and the procedures in this study are no different. We will do everything possible to keep you from being harmed. There may be other risks or side effects that occur which we do not know about at this time.

It is important for you to tell us as quickly as possible if you experience a side effect.

### **Are there any reasons you might take me out of the research?**

During the study, information about this research may become available that would be important to you. This includes information that, once learned, might cause you to change your mind about wanting to be in the study. We will tell you as soon as we can. This might include information about the side effects that are caused by taking part in this study. If that happens, we can tell you about these new side effects and let you decide whether you want to continue to take part in the research.

There may be reasons we will need to take you out of the study, even if you want to stay in. We may find out that it is not safe for you to stay in the study. It may be that the side effects are so severe that we need to stop the study or take you out of the study to reduce your risk of harm. If we find that the research might harm you or that it is not providing enough of a benefit to justify the risks you are taking, we will contact you immediately with an explanation of why you can no longer participate and give you copies of results from any screening or research tests that would be of interest to you or your primary care physician. We may also find that you cannot come for your study visits as scheduled. If that is found to be true, we will need to take you out of the study.

### **What are the possible benefits I may experience from taking part in this research?**

We do not know if you will get any benefits by taking part in this study. That is why we are doing this research. This research should help us learn more about the female sex hormone, estrogen, and fat storage and breakdown in premenopausal women.

There may be no personal benefit from your participation but the information gained by doing this research may help others in the future.

### **Will I be paid for taking part in this research?**

We will pay you for the time you volunteer while being in this study. Compensation will be provided according to the visits you complete as part of the study. \$25 will be provided for the screening visit, \$100 for the microdialysis visit and \$50 for the fat biopsy visit for a possible total of \$175. Appropriate compensation will be provided after completion of the study or after your participation in the study has stopped for any reason.

### **What will it cost me to take part in this research?**

It will not cost you any money to be part of the research, it will only cost your personal time. You will be responsible for providing your own transportation to and from the FITT Building and ECHI for each study related visit.

### **Who will know that I took part in this research and learn personal information about me?**

To do this research, ECU and the people and organizations listed below may know that you took part in this research and may see information about you that is normally kept private. With your permission, these people may use your private information to do this research:

- The research team, including the Principal Investigator, study coordinator, research nurses, and all other research staff or students involved in conducting the study.

*UMCIRB Number:* 11-077

*Page 6 of 8*

*Consent Version # or Date:* Revision 2 06/07/11

*UMCIRB Version 2011.1.10*

*Participant's Initials*

*Title of Study:* Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women

- All of the research sites' staff. This includes the research and medical staff at both East Carolina Heart Institute and Human Performance Lab's FITT Building.
- Any agency of the federal, state, or local government that regulates this research. This includes the Department of Health and Human Services (DHHS), the Food and Drug Administration (FDA), the North Carolina Department of Health, and the Office for Human Research Protections
- The ECU University & Medical Center Institutional Review Board (UMCIRB) and the staff who have responsibility for overseeing your welfare during this research, and other ECU office staff who oversee this research.

### **How will you keep the information you collect about me secure and how long will you keep it?**

The confidentiality of information collected from volunteers of this study will be ensured by numeric coding of all data; only the P.I. and co-investigators will have access to the code. Data will be secured on password encrypted computers, in a locked filing cabinet in the office of the P.I. or in a storage facility in the Human Performance Laboratory. The data will be kept for at least 10 years. Samples will be stored in freezers at the Human Performance Laboratory for at least 10 years. As a volunteer for this study you can request destruction (discarded into biohazard containers and disposed of by ECU biohazard personnel) of your samples at any time. It is possible that the information collected in this study will be used in professional publications, conference presentations or for future research projects, but all data will be stripped of any personal identifiers so no one will associate you with the research project.

### **What if I decide I do not want to continue in this research?**

Participating in this study is voluntary. If you decide not to be in this research after it has already started, you may stop at any time. You will not be penalized or criticized for stopping. You will not lose any benefits that you should normally receive.

### **What if I get sick or hurt while I am in this research?**

#### **If you need emergency care:**

Call 911 for help. It is important that you tell the doctors, the hospital or emergency room staff that you are taking part in a research study and the name of the Principal Investigator. If possible, take a copy of this consent form with you when you go.

Call the principal investigator as soon as you can. She needs to know that you are hurt or ill. Call Kathleen Gavin at (716) 713-4226 or Bob Hickner, PhD at (252) 737-4677.

#### **If you do NOT need emergency care, but have been hurt or get sick:**

Call the principal investigator, Kathleen Gavin at (716) 713-4226 as soon as you can. As necessary, go to your regular doctor. It is important that you tell your regular doctor that you are participating in a research study. If possible, take a copy of this consent form with you when you go.

The ECU Medical Clinics may be able to give you the kind of help you need. However, you may need to get help from a different type of medical facility and your Principal Investigator will know best what you should do.

#### **If you are harmed while taking part in this study:**

If you believe you have been hurt or if you get sick because of something that is done during the study, you should call Kathleen Gavin at (716) 713-4226 immediately. There are procedures in place to help attend to your injuries or

*UMCIRB Number:* 11-077 \_\_\_\_\_

*Page 7 of 8*

*Consent Version # or Date:* \_\_\_ Revision 2 06/07/11 \_\_\_

*UMCIRB Version 2011.1.10*

\_\_\_\_\_  
*Participant's Initials*

*Title of Study:* Role of Subcutaneous Adipose Tissue Estradiol in Regional Lipolysis in Premenopausal Women

provide care for you. Costs associated with this care will be billed in the ordinary manner, to you or your insurance company. However, some insurance companies will not pay bills that are related to research costs. You should check with your insurance about this. Medical costs that result from research-related harm may also not qualify for payments through Medicare, or Medicaid. You should talk to the Principal Investigator about this, if you have concerns.

### **Who should I contact if I have questions?**

The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator, Kathleen Gavin at (716) 713-4226 days or nights and weekends or Bob Hickner, PhD at (252) 737-4677 (days) or (252) 414-9311 (nights and weekends).

If you have questions about your rights as someone taking part in research, you may call the ECU Institutional Review Board Office at phone number 252-744-2914 (days). If you would like to report a complaint or concern about this research study, you may call the Director of UMCIRB Office, at 252-744-1971.

### **Is there anything else I should know?**

You will be given a signed copy of your authorization to release medical information for your records. You can limit the amount and type of information that is shared and you must make this request in writing; however, the researcher is able to use any and all information collected prior to the request not to disclose information. Although you can limit the release of your medical information, withholding some information may cause you to become ineligible for this research project. Research information continues to be looked at after study completion, so it is difficult to say when use of your information will stop. There is not an expiration date for the use of your information for this study.

### **I have decided I want to take part in this research. What should I do now?**

The person obtaining informed consent will ask you to read the following and if you agree, you should sign this form:

- I have read (or had read to me) all of the above information.
- I have had an opportunity to ask questions about things in this research I did not understand and have received satisfactory answers.
- I understand that I can stop taking part in this study at any time.
- By signing this informed consent form, I am not giving up any of my rights.
- I have been given a copy of this consent document, and it is mine to keep.

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Participant's Name (PRINT)	Signature	Date
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**Person Obtaining Informed Consent:** I have conducted the initial informed consent process. I have orally reviewed the contents of the consent document with the person who has signed above, and answered all of the person's questions about the research.

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Person Obtaining Consent (PRINT)	Signature	Date
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Kathleen M. Gavin		
Principal Investigator (PRINT)	Signature	Date

(If other than person obtaining informed consent)

UMCIRB Number: 11-077

Page 8 of 8

Consent Version # or Date: Revision 2 06/07/11

UMCIRB Version 2011.1.10

Participant's Initials

# APPENDIX C: MEDICAL HISTORY FORM

East Carolina University

Human Performance Laboratory

## MEDICAL HISTORY QUESTIONNAIRE PLEASE PRINT AND FILL OUT COMPLETELY

1. Banner ID # (if you have one): \_\_\_\_\_ Date: \_\_\_\_\_  
Name: \_\_\_\_\_  
Address: \_\_\_\_\_  
City: \_\_\_\_\_ State \_\_\_\_\_ Zip \_\_\_\_\_  
Phone: (home) \_\_\_\_\_ (cell) \_\_\_\_\_ (work) \_\_\_\_\_  
E-mail: \_\_\_\_\_

2. Date of birth (dd/mm/yyyy): \_\_\_\_\_ Age: \_\_\_\_\_  
Race: \_\_\_\_\_  
Current height (feet and inches) \_\_\_\_\_ Current weight (pounds) \_\_\_\_\_  
Approximate weight 6 months ago: \_\_\_\_\_

3. **General Medical History** Circle one

Do you have any allergies to medications? yes no  
If yes, list: \_\_\_\_\_

Do you have any other allergies (foods, tape, etc)? yes no  
If yes, list: \_\_\_\_\_

Any medical complaints presently? (if yes, explain) .... yes no  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Any major illnesses in the past? (if yes, explain) ..... (date) \_\_\_\_\_ yes no  
\_\_\_\_\_  
\_\_\_\_\_

Any hospitalization or surgery? (if yes, explain) ..... (date) \_\_\_\_\_ yes no  
\_\_\_\_\_  
\_\_\_\_\_

Have you ever had an EKG (electrocardiogram) ? ..... (date) \_\_\_\_\_ yes no

Have you ever had asthma, difficulty breathing, shortness of breath  
or any respiratory illness ? yes no  
(date) \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Are you diabetic? If yes, what type (I or II) and at what age did you develop  
diabetes: \_\_\_\_\_ yes no

Are you currently taking any medications/supplements (prescription, over the counter, vitamins/herbs)? yes no

If yes:

<u>Medication</u>	<u>Dosage</u>	<u>Reason</u>	<u>Taken how often</u>

Have you stopped taking any medications in the last year? yes no

If yes:

<u>Drug name</u>	<u>When you stopped</u>	<u>Why you stopped</u>

4. **Cardio-Respiratory History** (Do you currently or have you ever?)

- Any heart disease?..... yes no
- Heart murmur?..... yes no
- Occasional chest pains?..... yes no
- Chest pains on exertion?..... yes no
- Fainting?..... yes no
- Daily coughing?..... yes no
- High blood pressure?..... yes no
- Shortness of breath --
  - at rest..... yes no
  - lying down..... yes no
  - sleeping at night..... yes no
  - after 2 flights of stairs..... yes no

5. **Muscular History**

- Any muscle injuries or illnesses now?..... yes no
- Any muscle injuries in the past?..... yes no
- Muscle pain at rest?..... yes no

Muscle pain on exertion?..... yes no

**6. Bone-Joint History**

Any bone or joint (including spinal) injuries or illnesses now?..... yes no

Any bone or joint (including spinal) injuries or illnesses in the past?..... yes no

Ever had painful joints?..... yes no

Ever had swollen joints?..... yes no

**7. Health Habits History**

Have you participated in any weight-bearing exercise (for example, walking, jogging) that produces sweating in the last month?..... yes no

If yes, how many days each week (0 to 7 days) \_\_\_\_\_

If yes, how many minutes each time? \_\_\_\_\_

Have you participated in any weight-lifting exercise in the last month?..... yes no

If yes, how many days each week (0 to 7 days) \_\_\_\_\_

Do you participate in any other kind of physical exercise?..... yes no

If yes, describe type \_\_\_\_\_

and how frequently each week (0 to 7 days) \_\_\_\_\_

Do you currently smoke?..... yes no

Did you smoke in the past?..... yes no

If yes, when did you quit? \_\_\_\_\_ (mm/yy)

Do you drink caffeinated beverages (coffee, tea, soda)?..... yes no

If yes, how much (circle one)? 1-2 cups/day 3-5 cups/day >5 cups/day

Do you drink any alcohol?..... yes no

If yes, how much (circle one)?

>5 drinks/day 3-4 drinks/day 1-2 drinks/day 1-2 drinks/week 1-2 drinks/month 1-2 drinks/yr

Do you consume soy products or flax seed regularly?..... yes no

If yes, please explain how often and the usual quantity consumed \_\_\_\_\_

**8. Gynecological History**

How old were you when you had your first menstrual period? \_\_\_\_\_ years old

Have you ever been pregnant?..... yes no

If yes, how many times? \_\_\_\_\_  
 If yes, when was the last time you were pregnant? \_\_\_\_\_ (mm/yy)  
 Did you ever have any miscarriages?..... yes no

When was your last pap smear? \_\_\_\_\_ (mm/yy)  
 Have you ever had an abnormal pap smear?..... yes no  
 If yes, what was done? (circle one)  
 Repeat was OK / Biopsy / Other \_\_\_\_\_

Have you ever had a mammogram?..... yes no  
 If yes, when was your last mammogram? \_\_\_\_\_ (mm/yy)  
 Have you ever had an abnormal mammogram?..... yes no  
 If yes, what was done? (circle one)  
 Repeat was OK / Ultrasound / Biopsy / Other \_\_\_\_\_

Are you sexually active with men?..... yes no  
 If yes, what type of contraception do you currently use? (circle all appropriate)  
 oral ("the pill")      transdermal ("the patch")      tubal ligation ("tubes tied")  
 vaginal ring      depoprovera      spermicide      norplant  
 vasectomy      condoms      sponge diaphragm      IUD      none

Have your menstrual periods been regular in the last year?..... yes no  
 If NO, please explain: \_\_\_\_\_

How many days from the first day of bleeding in a menstrual period to the first day of bleeding in the next period? \_\_\_\_\_ (# of days, example: 28)  
 In the past year, what was the # of days in your:  
 shortest cycle: \_\_\_\_\_ longest cycle: \_\_\_\_\_

How many days do you usually bleed for during your period? \_\_\_\_\_ (# of days, example: 4)

Do you ever have bleeding or spotting in between your monthly periods?..... yes no

Do you usually get any of the following symptoms before your period?  
 Bloating..... yes no  
 Breast Tenderness..... yes no  
 Irritability/moodiness..... yes no  
 Food cravings..... yes no

Do you usually have cramps with your periods?..... yes no  
 If yes, do you take any medicines or herbs for the pain? \_\_\_\_\_

Have you had hot flashes in the last year?..... yes no

When was your last menstrual period? \_\_\_\_\_ (mm/dd/yy)

Have you ever used hormonal contraceptives?..... yes no  
 If YES, when did you stop? \_\_\_\_\_ (mm/yy)  
 How long did you take it? \_\_\_\_\_ (yrs)  
 Why did you stop? \_\_\_\_\_  
 Which form did you take? \_\_\_\_\_  
 (e.g. Orthocyclen, Ortho Evra, NuvaRing, Norplant, Loestrin, etc.)

**9. Sleeping Habits**

Do you ever experience insomnia (trouble sleeping)?..... yes no  
 If yes, approximately how often: \_\_\_\_\_  
 How many hours of sleep do you usually average per night: \_\_\_\_\_

**10. Family History**

	Age	Age of death	Cause of death
Father	_____	_____	_____
Paternal Grandmother	_____	_____	_____
Paternal Grandfather	_____	_____	_____
Mother	_____	_____	_____
Maternal Grandmother	_____	_____	_____
Maternal Grandfather	_____	_____	_____

Do you have a family history of: (Blood relatives only: give age of occurrence if applicable)

	Relationship	Age of Occurrence
• High blood pressure .....	yes no	_____
• Heart attack.....	yes no	_____
• By-pass surgery.....	yes no	_____
• Stroke.....	yes no	_____
• Diabetes.....	yes no	_____
• Gout.....	yes no	_____
• Obesity.....	yes no	_____
• sudden death caused by heart attack or other heart related condition prior to the age of 55.....	yes no	_____
• Cancer.(please list type)..	yes no	_____

11. Does anyone in the household smoke?..... yes no  
 If yes, do they smoke in the house or in the car? \_\_\_\_\_

12. Is there reason not mentioned here why you should not participate in physical activity?  
 yes no

**13. Family Physician**

Name: \_\_\_\_\_  
 Address: \_\_\_\_\_  
 Phone: \_\_\_\_\_

