The effect of estrogen depletion on skeletal muscle metabolism

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Abstract

Skeletal muscle plays a significant role in altering metabolic activity of the body by influencing blood lipid profiles and insulin sensitivity through enzymatic processes. Hormones such as estrogen have been shown to effect skeletal muscle function. Research indicates the metabolic influence of skeletal muscle may be altered by the depletion of estrogen. Through its role in energy metabolism and expenditure, skeletal muscle likely influences the development of cardiovascular risk factors; including hypertension, diabetes, dyslipidemia, and obesity. To study the effects of estrogen depletion on skeletal muscle metabolic activity, seventeen female C57BL/6 mice have been randomly divided into two groups for the duration of 60 days; ovariectomized (OVX) and control. Circulating concentrations of total cholesterol, high–density lipoprotein, and glucose were measured prior to surgery and at 20, 40, and 60 days post-surgery. Pre and post trial measurements of body composition were measured on the EchoMRI. Body weight was measured weekly and food intake was calculated during a one week period. Twenty-four hour cage activity was monitored in activity chambers at 50 days. Enzymatic activity of skeletal muscle will be measured to assess oxidative capacity and rates of fatty-acid breakdown. Early results indicate that the OVX mice are gaining weight and have increased total cholesterol over baseline values. I hypothesize that estrogen-depletion will increase circulating cholesterol and glucose levels, decrease circulating high-density lipid, increase body mass and percent body fat, decrease cage activities, and decrease skeletal muscle oxidative capacity and lipid metabolism through reduced enzymatic activity.

Introduction

The benefits of hormone replacement have been controversial despite studies that have shown an increase in CVD risk following the onset of menopause and fall in estrogen levels (American Heart Association). In 2002 the Women's Health Initiative raises concern about possible increased cardiovascular disease (CVD) risk in menopausal women following hormone replacement therapy [1]. Additionally, the Heart and Estrogen/Progestin Replacement Study (HERS) indicates there is no cardio-protective effect of hormone therapy [2]. Moreover, basic science research indicates a protective effect of estrogen on the cardiovascular system [3, 4].

Universal risk factors of CVD include hypertension, diabetes, dyslipidemia, and obesity, all of which are directly influenced by metabolic activity. Skeletal muscle plays a significant role in altering metabolic activity by influencing blood lipid profiles and insulin sensitivity through enzymatic processes [5, 6]. Changes in blood lipid profiles have shown that Ovariectomy (OVX) in rats and mice, results in higher total cholesterol and low density lipoprotein (LDL) levels which further suggest that estrogen may influence regulation and uptake of cholesterol within the body [4, 7].

Estrogen loss also results in increased body mass in OVX rodents [4, 7-10]. In addition, estrogen influences body fat distribution resulting in subcutaneous fat accumulation in females. Consequently a loss of estrogen results to a visceral fat distribution which is highly correlated to increased CVD risk.

The increase in body fat observed in OVX animals may be attributed to decreased energy expenditure. A reduction in activity levels has been suggested to be the result of decreased estrogen receptor binding activity. A study by Musatov et al revealed that mice with silenced α estrogen receptors demonstrated reduced rearing and ambulation activity in open field tests and on running wheels [11]. Estrogen loss is associated with decreased voluntary wheel running while estrogen replacement to OVX mice resulted in more running, as our lab has previously reported [9].

By gaining a better understanding of the effects of estrogen depletion on skeletal muscle's overall function, future hormone treatment that specifically targets skeletal muscle may become important in the effort to improve quality of life for menopausal and post-menopausal women.

Methods

Animals. 3 mo old female C57BL/6 mice were used in this study. Two weeks following their arrival, mice were randomized into three groups: ovariectomized (OVX), sham-operated, or control (n=8, n=4, and n=5, respectively). Briefly, mice were anesthetized with isoflurane and two incisions were made to remove both ovaries in ovariectomized mice; in sham mice the ovaries were located and replaced.

Vaginal Cytology. Vaginal smears were obtained to visibly measure estrus cycle changes that occur in female mice. Mouse cycles consist of four stages: diestrus, proestrus, estrus, and metestrus. All Non-OVX mice were determined to be in estrus before blood could be collected. OVX mice remained in diestrus for the duration of the study.

Body Composition. Mice were weighed on a weekly basis. Measurements of body composition were done on an EchoMRI-900TM to obtain measurements of lean, fat, total body water, and free body water mass.

Food Intake. Food was weighed each morning and the total amount consumed per day per mouse was calculated from the food consumed divided by the total time divided by the number of mice per cage. Daily body weight was measured to calculate the grams of food consumed per gram of body weight per mouse

Blood Chemistry. Mice were fasted for 6 hours before blood was collected via facial vein bleeds and placed into coated heparin tubes on ice. Blood profiles were measured in mg/dL using the CardioChek PA Professional blood testing device and PTS panel test strips specific for total cholesterol, HDL, and glucose.

Grip Strength. Mice grasp the ring connected to the grip apparatus and are then pulled horizontally until release occurs and the reading from the strain gauge is recorded. Each mouse performed 5 times with 5-10 second rest between trials. The average measurement was normalized to body mass.

Whole Body Tension. Forward pulling force evoked by a light tail pinch, described as whole body tension, was measured using a force transducer. Peak and top five averaged forces developed during a ~5 minute protocol are reported.

Activity Chambers. The 24 hour activity chambers have three infrared ranges in the x-, y-, and z- directions which measure cage activities including distance traveled in the chamber (ambulation), rearing, jumping counts, time spent ambulating, time spent jumping, and total active time.

Statistical Analysis. The data was analyzed by one-way ANOVA with Holm-Sidak post-hoc test, using SigmaStat version 2.03 (Systat Software). Repeated measures (i.e. body weight and blood chemistry) were analyzed by repeated measures ANOVA. Significance was accepted at the $\alpha \leq 0.05$ level. Data is reported as mean ± SE.

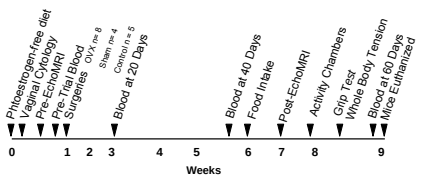

Figure 1. Study design and timeline for methods. No differences were found between Sham and control mice; thus they were combined into the group termed Non-OVX.

Body mass in OVX increases over 60 days more than the non-OVX

2-Way Repeated ANOVA: Group x Time P<0.001

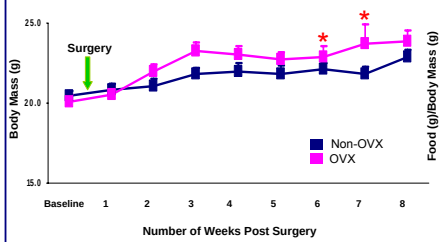

Figure 2. Comparisons of body mass by group over the 8 week period of the study. The green arrow represents the time of surgery. Asterisk (*) represents significant differences (p<0.05) between groups at the given time point.

Food intake was not different between OVX and Non-OVX

2-Way Repeated ANOVA: Group P=0.088; Time P=0.308; Group x Time P=0.648

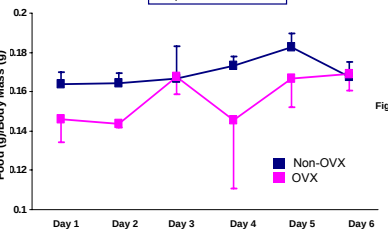

Figure 3. Comparisons of individual food intake for a 6-day period following 40 day blood collection. Asterisk (*) represents significant differences (p<0.05) between groups as measured by a t-test. Food consumed per 24 hours was also measured (not pictured) and was not different over time or between groups.

Lean but not fat mass changed over time

2-Way Repeated ANOVA

	Fat	Lean
Group	P=0.466	P=0.249
Time	P=0.514	P<0.001
Group x Time	P=0.114	P=0.313

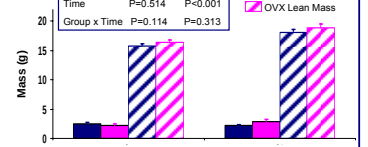

Figure 4. Comparison fat and lean masses pre surgery and 50 days post surgery.

% Body fat was not different

2-Way Repeated ANOVA: Group P=0.909; Time P=0.661; Group x Time P=0.158

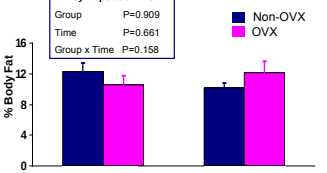

Figure 5. Comparison of % body fat pre surgery and 50 days post surgery.

Total Cholesterol and HDL levels increased slightly over time

2-Way Repeated ANOVA: Total Cholesterol

Group	P=0.113
Time	P=0.010
Group x Time	P=0.051

2-Way Repeated ANOVA: HDL

Group	P=0.688
Time	P=0.004
Group x Time	P=0.061

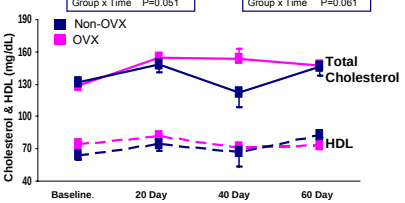

Figure 6. Comparisons of total cholesterol and HDL levels at pre-surgery, 20 days post, 40 days post, and 60 days post surgery.

Cholesterol ratio tended to increase over time for OVX

2-Way Repeated ANOVA: Group x Time P=0.046

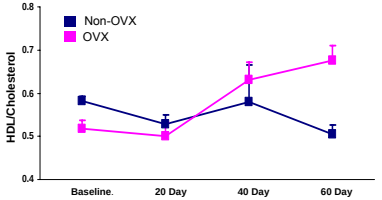

Figure 7. Comparisons the ratio of HDL to total cholesterol at pre-surgery, 20 days post, 40 days post, and 60 days post surgery.

Glucose levels are greater in OVX than Non-OVX

2-Way Repeated ANOVA

Group	P=0.041
Time	P=0.035
Group x Time	P=0.140

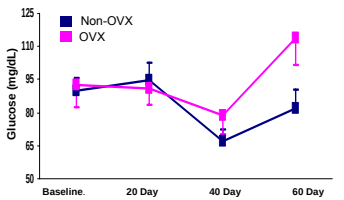

Figure 8. Comparisons fasting glucose levels pre-surgery, 20 days post, 40 days post, and 60 days post surgery.

OVX cage activity tended to be less than Non-OVX

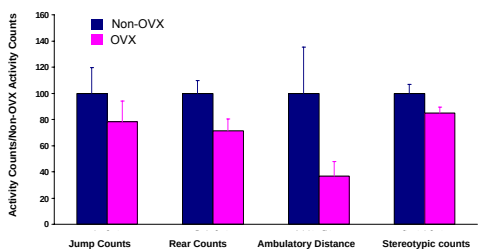

Figure 6. Comparisons cage activity in terms of jump count, rearing count, ambulatory distance, and stereotypic counts (P≥0.067).

Grip strength was not different in OVX

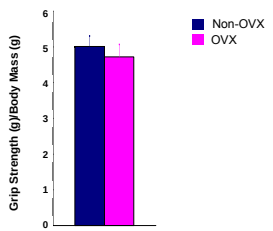

Figure 7. Comparisons of grip strength normalized to body mass (P=0.513).

OVX tended to have less whole body tension

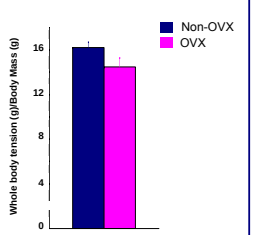

Figure 8. Comparison of whole body tension. Values were reported as the average of the top peak tensions normalized to body mass (P=0.068).

Follow-up measurements that will be made

In vivo muscle function. *In vivo* experiments allows us to evaluate the contractile properties of skeletal muscles on intact, anesthetized mice. Peak isometric torque of the anterior hindlimb muscles will be measured to assess maximal muscle strength.

Plasma estradiol and uterine mass. Plasma estradiol will confirm physiological levels of estrogen in control mice as well as verify estrogen depletion in OVX mice. Uterine mass will also be used as a secondary indicator of estradiol status.

Muscle and fat masses.
Dry and wet weights of the following hindlimb muscles will be determined:
- Tibialis anterior muscle
- Gastrocnemius muscle
- Extensor digitorum longus muscle
- Soleus muscle

Wet weights of the following organs will be determined:
- Uterus
- Paraovarian fat pad
- Heart

Muscle enzyme assays. Beta-hydroxy acyl Co-A dehydrogenase activity (β-HAD) is a marker for the beta oxidation pathway. Through this pathway fatty acids are broken down to generate acetyl Co-A, the entry molecule for the tricarboxylic cycle (TCA).

Citrate synthase enzyme assay will be used to assess enzymatic activity within the TCA cycle. This cycle uses oxidative phosphorylation to generate adenosine triphosphate molecules (ATP) which is the useable form of chemical energy in muscle.

Intramyocellular fat content in skeletal muscle. Histology is a morphological parameter which assesses the presence or absence of intracellular components such as intramyocellular fat deposits. The oil red staining procedure will stain neutral lipids, mostly triglycerides, a red-orange hue. Immunohistological staining will be done concurrently with the oil red O stain to indicate fiber distribution as well as possible fiber preference of lipid deposits.

Conclusion

	Non-OVX, normal estrogen	OVX, estrogen deficient
Body Weight	Increase over time less than OVX	**Increase over time more** than Non
Food Intake	Not different from OVX	Not different from Non
Fat/Lean Mass	Increase in lean mass over time	Increase in lean mass over time
% Fat	Not different over time or between groups	Not different over time or between groups
Total Cholesterol	Increase slightly over time	Increase slightly over time
HDL	Increase slightly over time	Increase slightly over time
HDL/Total	No change over time	Tended to **increase over time**
Glucose	Less than OVX	**Greater** in OVX
Cage Activity	Within normal ranges for wildtype	Tend to have **less activity**
Whole Body Tension	Within normal ranges for wildtype	Tend to have **less WBT**
Grip Strength	No Difference	No Difference

I hypothesized that estrogen-depletion would increase circulating cholesterol and glucose levels, decrease HDL levels, increase body mass and percent body fat, decrease cage activities, and reduce grip strength and whole body tension. My hypothesis was only partially supported in that OVX exhibited an increase in body mass over time, increased HDL to total cholesterol ratios, and elevated glucose levels. Data on estrogen-depleted mouse activity showed a general trend in which OVX demonstrated reduced cage activity and less whole body tension. This is only part of the study and on-going measurements will be done, specifically *in vivo* strength measurements and whole muscle analysis. Follow-up measurements will give us a better understanding of what is occurring at the cellular level in skeletal muscles of estrogen-depleted mice. Perhaps the measurements already obtained only reveal subtle, acute changes in OVX and analysis of cellular adaptations may uncover more drastic changes in muscle function that have not yet been translated to whole-body effects.

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