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TITLE: Effects of Moderate Aerobic Exercise Combined with Caloric Restriction on Circulating Estrogens and IGF-I in Premenopausal Women

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14. ABSTRACT

This proposal entitled "Effects of moderate aerobic exercise combined with caloric restriction on circulating estrogens and IGF-1 in premenopausal women" examined the effects of exercise training combined with caloric restriction, resulting in weight loss, on two hormonal biomarkers for breast cancer i.e., circulating estrogens and insulin-like growth factor I (IGF-I). In 33 women who completed the study, exercise 4 X per wk at 79 ± 6 % of maximal heart rate combined with an 32% decrease in caloric intake over four menstrual cycles produced significant increases in aerobic capacity (27 ± 5%), decreases in body weight loss ranging from 1-9 kg, and loss of body fat ranging from 5 to 12% of initial percent fat. Light conditioning resulted in significant gains in aerobic capacity (30±5%), but only produced a trend toward a decrease in body fat percent (-1.2%), and only a small change in body weight (-0.8 kg). Despite the highly significant changes in body composition and body weight in the exercising group, preliminary results indicate no significant changes in serum estradiol or serum estrone. IGF-I did not change significantly either, indicating that chronic exercise and dieting do not result in favorable changes in two hormonal biomarkers for breast cancer in this age group.

15. SUBJECT TERMS
Breast cancer, menstrual cycle, IGF-1, estradiol, exercise

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INTRODUCTION

This proposal entitled "Effects of moderate aerobic exercise combined with caloric restriction on circulating estrogens and IGF-I in premenopausal women" was designed to provide important scientific contributions with respect to the primary prevention of breast cancer in women. Specifically, this study examined potential mechanisms relating to the role of physical activity in the reduction of the risk of breast cancer by experimentally testing whether moderate aerobic exercise can reduced the levels of two hormonal biomarkers, circulating estrogens and insulin-like growth factor I (IGF-I). Since elevated levels of both of these hormones have been associated with an increased risk of breast cancer, and because exercise and weight loss has been shown to induce disturbances in menstrual cyclicity that may reduce circulating estrogens, we wished to extend previous findings from epidemiological and cross-sectional studies by performing a tightly controlled, prospective clinical study that addressed previously unanswered questions related to the role of exercise in the modulation of estrogen and IGF-I. Although previous studies have shown that negative energy balance, and not other stressful aspects of physical exercise, can modulate reproductive function and reduce circulating estrogen levels, no studies to date have determined the magnitude of energy deficit required for these changes during long-term training, and no studies have attempted to differentiate between the exercise-induced changes in ovarian versus adipose sources of circulating estrogens. Since both estradiol (ovarian) and estrone (adipose tissue) are biologically active, and because the importance of estrone as a risk factor increases with age and adiposity, it is important to consider the degree to which exercise which creates a negative energy balance affects both of these sources of circulating estrogens.

Circulating levels of IGF-I correlate with breast cancer risk, yet studies examining the responses of this hormone and its binding proteins to chronic exercise are lacking. Since IGF-I levels are very sensitive to nutritional status, previously reported stimulatory effects of exercise on IGF-I can be overridden if exercise is performed in the face of negative energy balance. In this regard, exercise that promotes weight loss can be viewed as a way to reduce levels of IGF-I, and therefore potentially reduce the risk of breast cancers. To date, no studies have addressed whether a program of moderate aerobic exercise and dietary restriction producing a negative energy balance that is carried out over a long duration will significantly alter IGF-I levels. Further, the degree to which these levels might be altered in individuals of differing initial energy stores has not been addressed.

Metabolic energy availability is an important contributing factor in the development of reproductive cancers. However, current methods for assessing energy availability, which include anthropometric measures, calculations of energy balance, evaluation of various serum and urinary biomarkers are prone to measurement error, not sensitive to alterations in energy availability, and are sometimes affected by disease states. The current project includes an introduction of a novel approach to estimating energy status by measuring metabolic hormones in plasma, T3, IGF-I and leptin. Recently, dried blood spot (DBS) sample collection techniques have allowed for endocrine based population studies examining a wide variety of ecological factors that contribute to variation in human reproduction. In order to use the proposed method of energy status assessment in large population-based applications, such as those addressing the role of physical activity and or diet in the risk of breast cancer, the battery of metabolic hormones that comprise the proposed method must be amenable to collection and assays. Although the DBS technique has been partially validated for some hormonal assays, it has not yet been properly validated for T3, IGF-I, and leptin, and it is unclear whether the technique is responsive to physiological changes of these compounds. Therefore, the current work calls for the validation of the DBS sampling technique for these assays under physiological conditions.

The proposed studies will yield new and important information regarding the degree to which an exercise and diet program that results in an energy deficit will reduce the risk of breast cancer.

BODY

Study Design: The study utilized a prospective, randomized design that tested the effects of a moderate exercise program (4X/wk; 4 months) combined with moderate dietary restriction that results in an average daily energy deficit of -20%-40% kcals (**Figure 1**). Previously sedentary, eumenorrheic women aged 25-40 years were assigned to exercise or light conditioning groups. Initially, we had targeted both normal weight (BMI 21-25 kg/m^2) and overweight (BMI 26-30 kg/m^2) women to be assigned to either the Exercise (exercise 4 X per week; 20-30% dietary restriction) or Light Conditioning groups (exercise 2X/wk; no dietary restriction) groups; 4 groups, n=15 each group. Subjects were studied for a total of six menstrual cycles, i.e., 2 control followed by 4 cycles with training and dietary restriction.

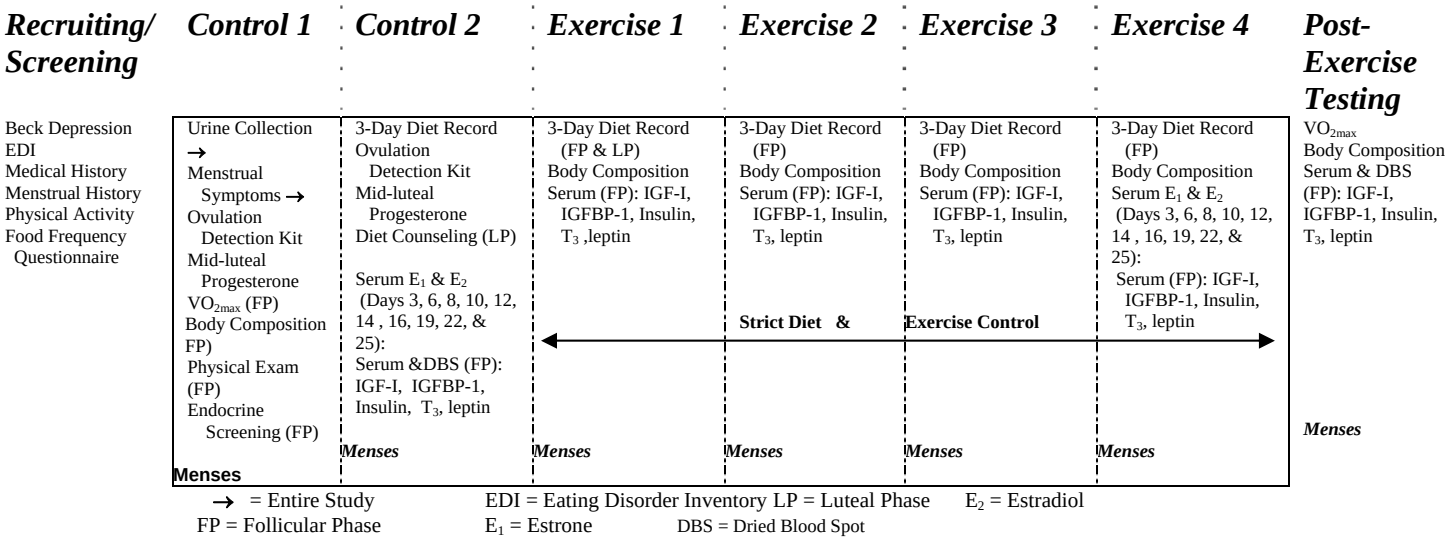

Figure 1. Study Design

Progress According to the Approved Statement of Work (2004-2005):

(See previous Annual Summary for 2003-2004)

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Proposed Months 38-42, October, 2004-February, 2005:

- Requested extension for final report for this study (IDEA Award) from DAMD;
- Perform urinary LH, E1G, and PDG assays, and serum metabolic assays when final cohort is finished;
- Perform insulin, IGFBP-2, estradiol, and estrone on remaining completed subjects;
- Send completed DBS samples from completed subjects to Salimetrics
- Perform data analysis;
- Obtain results of DBS samples from Salimetrics,
- Submit abstract for ERA of HOPE Meeting in December, 2004

Actual Months 38-42, October, 2004-February, 2005:

- Extension requested and granted for final report for this study (IDEA Award); **We are unclear why another request for a Final Report was made in October of 2005.**

- Urinary assays for LH, E1G, and PdG assays from 47 subjects were performed during February – March of 2005.

- Assays for estrone, estradiol, and insulin-life growth factor -1 performed on samples
- Results obtained from DBS samples sent to Salimetrics
- Data Analysis Performed
- Abstract submitted for ERA of HOPE meeting

Proposed Months 43-48, March 2005- May 2005:

- Write and submit manuscripts:

Actual Months 43-48, March 2005- May 2005; including June –October 2005

See results and discussion section for information regarding:

Manuscript #1: In Review in Medicine, Science, Sports and Exercise, Fall, 2006: Effect of Exercise Training on Estrogen Metabolism in Premenopausal Women. Kim C. Westerlind, Jennifer Bell, Nancy I. Williams

Manuscript #2: In Progress: Susceptibility to energy-related menstrual disturbances is reduced with increased age. Nancy I. Williams, Heather Leidy, Brandy Weller. Penn State University, University Park, PA. 16802.

Report of Final Results:

Subject Recruitment:

Over the three years in which the study took place, 572 contacts through phone or email were made with women interested in the study. Of those 572 women, 85 signed informed consents for the study, 52 were excluded from the study at various time points for the following reasons: 10 for medical reasons, 17 because of time constraints or other personal reasons, 3 became pregnant during the study, 1 had abnormal prolactin levels, 6 exceeded limits for body composition or body weight, 2 dropped out because they were not able to receive financial compensation due to visa restrictions, 11 had existing menstrual abnormalities, and 2 were asked to leave because of non-compliance. Forty seven women began the experimental protocol **(Table 1)**. There were 33 women who completed the study **(Table 2)**.

Table 1. Initial Subject Characteristics

		Age (yrs)	Weight (kg)	Height (cm)	BMI (kg/m2)	Body Fat (%)	VO2 Max (ml/kg/min)
Light Conditioning (n = 16)	Mean	33.69	65.21	163.36	24.61	34.35	29.31
	Std Deviation	3.66	5.46	6.51	3.19	5.17	4.03
Exercise (n=31)	Mean	31.90	64.84	163.44	24.29	32.73	31.66
	Std Deviation	4.13	9.70	6.10	3.32	5.41	6.33

Compliance

- Began Study (n=47)
- Drop Outs (n=14): Light Conditioning = 7, Exercise = 7, (no differences between drop-outs and finishers in age, weight, BMI, body fat %, or VO_{2max})

Table 2. Descriptive Characteristics of the Completed Subjects

VARIABLE	OVERALL (n=33)	Light Conditioning (n=9)	Exercise (n=24)	SIG
Age (yrs)	32.4 + 0.8	35 + 1.0	31.5 + 0.9	P=0.049
Height (cm)	164.0 + 1.1	164.8 + 2.5	163.7 + 1.3	P=0.663
Weight (kg)	64.1 + 1.4	64.5 + 1.9	63.3 + 1.8	P=0.728
BMI	23.7+ 0.5	23.9 + 1.3	23.6 + 0.6	P=0.811
Body Fat (%)	32.3 + 0.9	34.3 + 1.8	31.6 + 1.0	P=0.192
VO2 max (ml/kg/min)	31.7 + 1.0	28.8 + 1.1	32.4 + 1.1	P=0.027

Data are presented as Mean + SE. Significance determined by t-test between Control and Exercise groups.

Dietary Intake during the Intervention:

The target macronutrient composition for all subjects during the intervention was 55% carbohydrate, 30% fat, and 15% protein. Using the average total daily caloric intake from the each subject's three day diet log, in combination with the Harris Benedict equation as adjusted for daily physical activity (Harris et al.,1919), an estimate of the eucaloric (weight maintenance) energy needs of each subject were calculated. In order to promote weight loss, this level of dietary intake was reduced by 20-30 % for exercising subjects, and this new level was their target daily caloric intake throughout the intervention. For light conditioning subjects, the target level of daily dietary intake during the intervention remained that which represented their eucaloric energy needs. Prior to the intervention the GCRC dietician taught all subjects how to use the food exchange system (American Diabetes Association, 2003 Edition, Chicago, IL) to obtain their targeted caloric intake, and the targeted macronutrient composition. Subjects kept track of their daily food exchanges by recording them on monitoring forms for seven days at a time, every two weeks. Estimates of daily caloric intake from food exchange monitoring sheets were determined using Dietician's Assistant v. 2.99 (Compu-Cal Inc., Olympia, WA). Once per menstrual cycle during the intervention subjects also completed a 3 day diet log. Total calories and macronutrient content was determined using Nutritionist Pro (First Data Bank, Indianapolis, IN). If difficulties arose with subjects meeting their targeted caloric intake, the dietician counseled on how to overcome these challenges. If subjects in the Light Conditioning group were experiencing weight changes, or if subjects in the Exercise group were not losing weight, appropriate changes to the target caloric intake were made. Dietary counseling sessions for both Light Conditioning and Exercise groups included discussion of food education modules including Shopping Tips, Low Fat/Low Calorie food, Food Preparation, Dining Out, Iron, Calcium, Fiber, and Vitamins in Food.

Exercise Training During the Intervention

Subjects in both groups attended supervised exercise sessions in the training room in Noll Laboratory that were monitored by a Head Trainer and several personal trainers who had experience in fitness assessment and personal training. Workouts for the exercise group were four times per week and consisted of a 5 minute warm-up followed by approximately 40-90 min of aerobic activity at a heart rate 60-90% (average 79 ± 0.7/ 6.0 % (sem/SD)) of the maximal heart rate (obtained from the VO_2 max test achieved during exercise sessions),

followed by a 10 min cool-down. Modes of aerobic activity included treadmill walking, stationary cycling, and stair stepping. Average attendance for subjects in the Exercise group was 96 ± 0.9/4.6 (sem/sd) % of the total workouts. The duration of exercise for the Exercise group was equal in minutes to that required to expend a target amount of calories, determined to be approximately 20% of the subjects' eucaloric intake. For example, if the pre-determined eucaloric intake was 2000 calories, then this subject's exercise calorie target would be 400 calories. The total amount of calories expended during each exercise session was measured using the OwnCal feature on the Polar S610 heart rate monitor (Polar Electro Oy, Kempele, Finland). Throughout the study the heart rate monitors were continually reinitialized with the most recent values of weight, maximum heart rate, maximal aerobic capacity, and age.

Light conditioning subjects exercised 2 times per week; sessions were comprised of 36 ± 2.9/8.3 (sem/sd) minutes of aerobic activity at 77 ± 0.8/2.3% (sem/sd) of maximum heart rate, and 10 minutes of light stretching and calisthenics. Compliance to these sessions was 94 %. These sessions were designed to provide a sufficient training stimulus for subjects to see some improvement in aerobic capacity and strength and flexibility, but not to expend a significant amount of calories such that weight loss would be observed. Earlier attempts at recruiting subjects who would agree to be randomized to a non-exercising control group were unsuccessful.

Results for Body Weight and Body Composition

Descriptive characteristics for completed subjects are presented in **Table 2.** No differences were observed in any of the parameters after women were randomized to Light Conditioning versus the Exercise groups with the exception of age (P= 0.049; independent t-test). Light Conditioning women were significantly older when compared to the exercise women.

The effects of the intervention on body composition, body weight, and fitness are shown in **Table 3**. More detailed effects of the intervention on body weight are shown in Figure2. In general significant improvements were observed in all variables over the course of the intervention, with the exception of no change in fat free mass. Significant group interactions were observed for all variables where significant changes occurred over time, except VO_{2max}, indicating that although changes were favorable in both groups, the Exercise group exhibited significantly greater gains as a result of the intervention. Average percent changes from baseline for $VO_{2\ max}$ for Light Conditioning and Exercise groups respectively were +30 ±5%, and +27 ± 5%. Average percent changes from baseline for percent body fat for Light Conditioning and Exercise groups respectively were -3.8 ±1.7 %, and -15 ± 2.3%. Light Conditioning subjects lost 1.2 ± 0.6 % of their initial body weight, while Exercise subjects lost 6.0 ± 0.9%. Daily caloric intake from the average of three day diet logs recorded during the baseline period, and during the fourth menstrual cycle exhibited a significant decrease over time (P<0.001), and this difference varied between the Light Conditioning and Exercise groups (interaction effect P = 0.004) such that the Exercise group experienced a 32% decrease and the Light Conditioning group experienced an 8% decrease. Average macronutrient content across the intervention was not different between groups with the exception of the percent of daily calories derived from fat. Light Conditioning subjects consumed 34.8 ± 4.5 % fat versus the Exercisers who consumed 27.5 ± 5.2 % (P = 0.035; one – way ANOVA on average 3 day diet macronutrients for Exercise 1, Exercise 2, and Exercise 3, and Exercise 4). The overall macronutrient distribution for both groups during the intervention was 55 ± 1.2 % carbohydrate, 15.7 ± 0.7% protein, 26.1 ± 1.3 % Fat, and 3.5 ± 0.8 % alcohol.

Table 3. Weight, Body Composition and Aerobic Capacity at Baseline and Post-Intervention

VARIABLE	Light Conditioning (n=9)		Exercise (n=24)	
	Baseline	Post-Intervention	Baseline	Post-Intervention
Weight (kg)[a,b]	64.5 ± 1.9	63.8 ± 2.8	63.2 ± 1.7	59.5 ± 1.6
BMI[a,b]	23.9 ± 1.1	23.6 ± 1.0	23.6 ± 0.6	22.1 ± 0.6
Body Fat (%)[a,b]	34.3 ± 1.8	33.1 ± 2.1	31.6 ± 1.0	27.0 ± 1.2
Fat Mass (kg)[a,b]	22.2 ± 1.9	21.3 ± 1.8	20.2 ± 1.1	16.3 ± 1.0
Fat Free Mass (kg)	42.1 ± 1.6	42.3 ± 1.7	43.1 ± 0.9	43.2 ± 1.0
VO2 max (ml/kg/min)[a]	28.8 ± 1.9	37.3 ± 3.0	32.4 ± 1.1	40.8 ± 1.8

Data are presented as Mean ± SE. Significance determined by ANOVA with repeated measures with main effect of Group
[a] Significant Effect of Time
[b] Significant Interaction Effect between Conditioning and Exercise

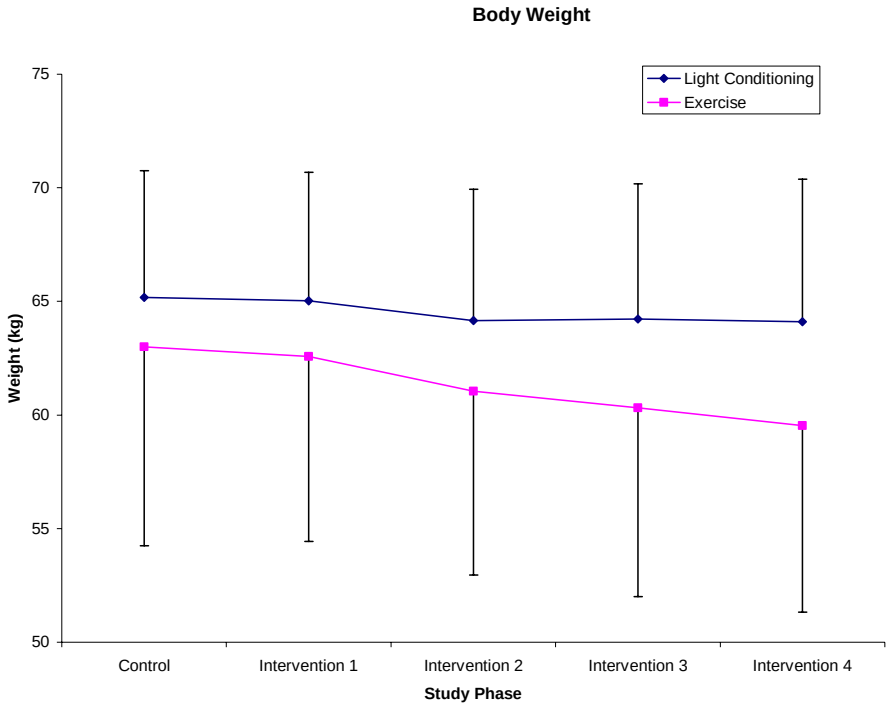

Figure 2. Average body weights for experimental groups throughout the intervention

Results for Serum Estradiol and Urinary Estrogen (E1G)

A composite graph of changes across the menstrual cycle in serum estradiol, depicted according to cycle day at baseline and during the fourth intervention month is depicted in **Figures 3 and 4**. Average serum estradiol for the Control Cycle 2 and the Exercise 4 cycle are shown in **Figure 5**. When serum measurements of estradiol are examined across Control Cycle 2 (n=10 measurements per subject), and Exercise 4 cycles (n=10 measurements per subject) are averaged, and then compared with paired samples T-tests, no differences are observed in either the light conditioning (p = 0.43) or exercising groups (p=0.32), despite the loss of body fat. Changes in average urinary E1G in both experimental groups are depicted in **Figure 6**. There are no significant effects of time or group on urinary E1G (ANOVA with repeated measures, time effect p= 0.15; group effect p = 0.23), despite the significant loss of body weight and body fat. With respect to estrone, **Figure 7** illustrates the close correlation between urinary E1G, serum estradiol, and serum estrone in a representative subject. Similar results were obtained for all other subjects, illustrating that serum estrone concentrations in premenopausal women are of ovarian origin versus peripheral production in adipose tissue. **Figure 8** illustrates the changes in serum estrone in exercising subjects. There were no significant effects of the intervention on these levels (paired t-test: p=0.689).

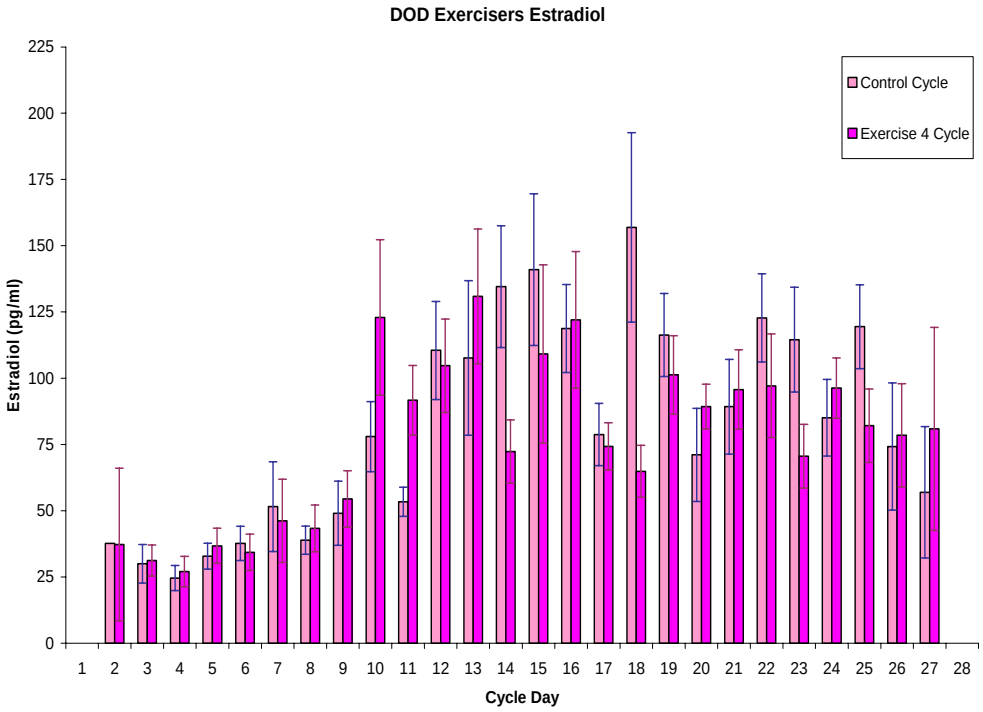

Figure 3. Composite graph of serum estradiol measurements from Control Cycle 2 (Pre) and Exercise 4 (Post) cycles in Exercising Subjects.

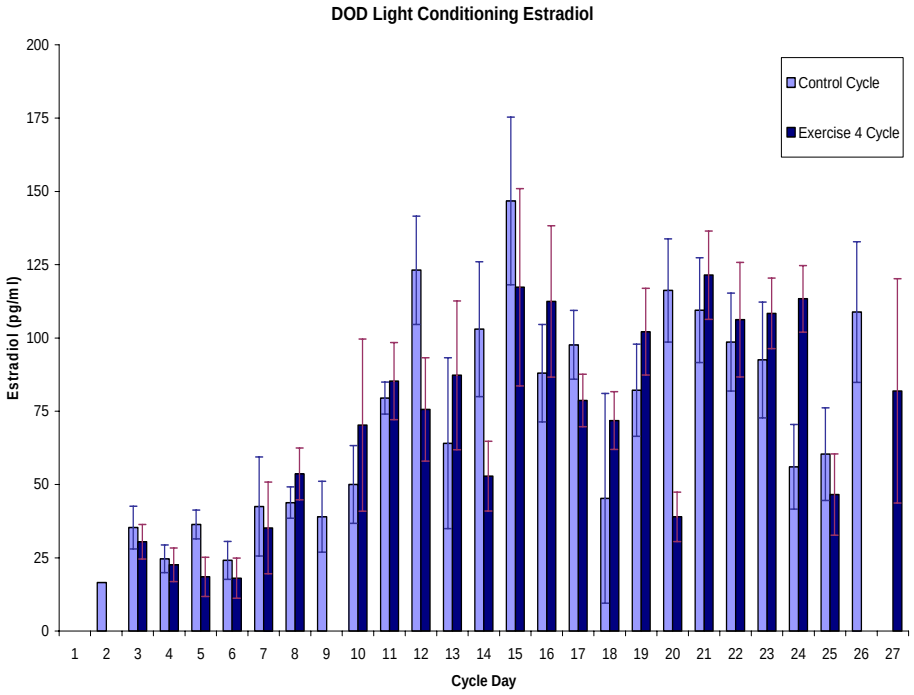

Figure 4. Composite graph of serum estradiol measurements from Control Cycle 2 (Pre) and Exercise 4 (Post) cycles in Light Conditioning Subjects

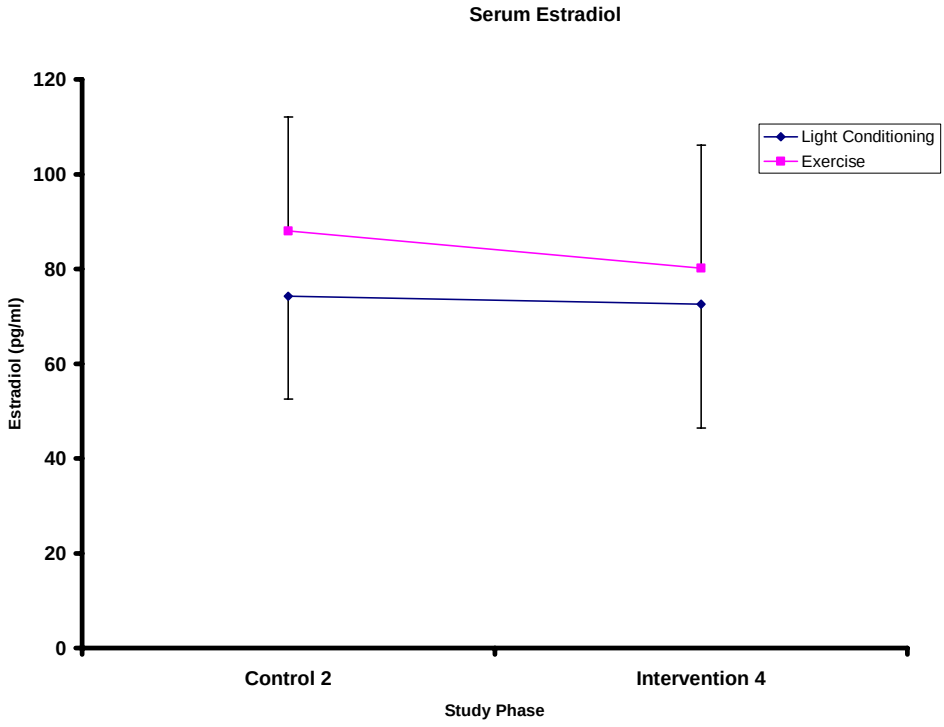

Figure 5. Average serum estradiol from the Control 2 and Intervention 4 phases of the study for each experimental group.

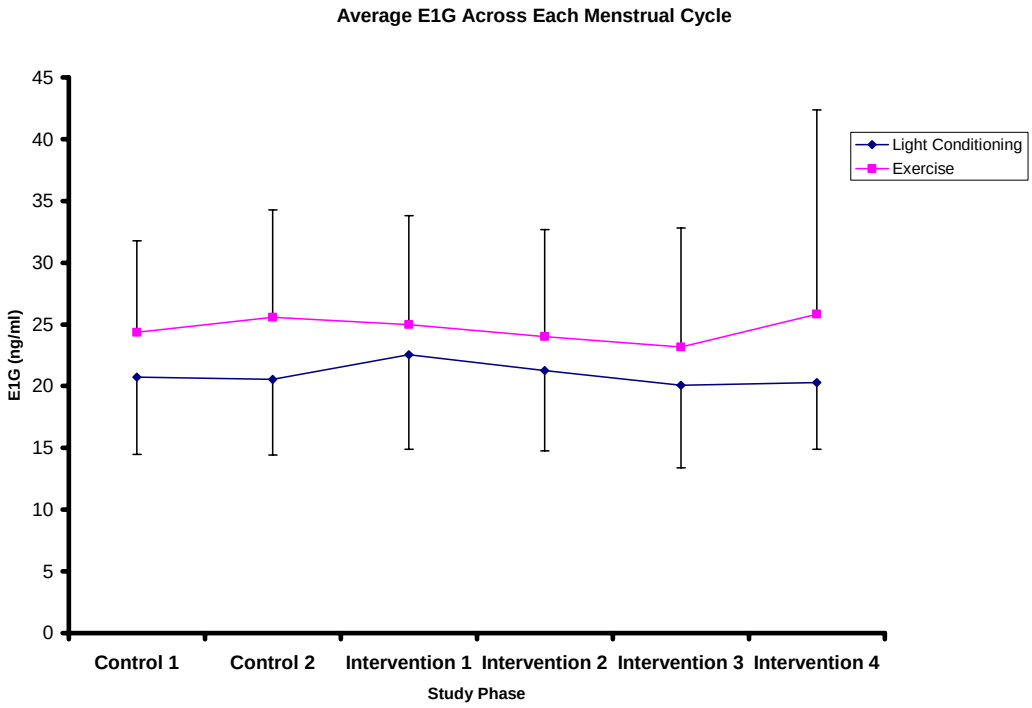

Figure 6. Changes in average E1G for each menstrual cycle across the intervention in both experimental groups.

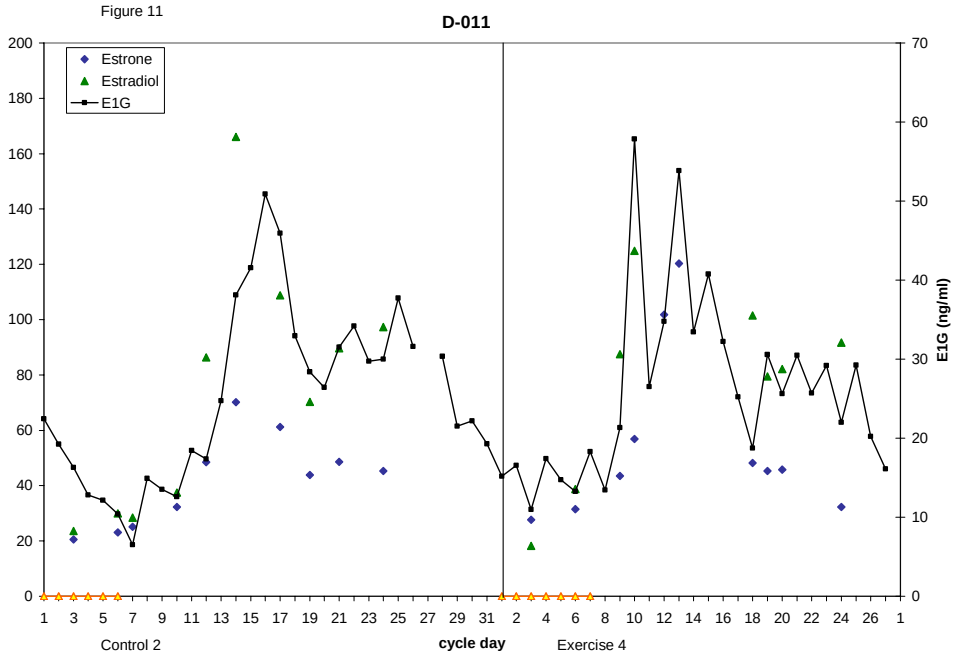

Figure 7. Representative example of a single subject's urinary (E1G) and serum estrogens (estrone and estradiol) before (Control 2) and after (Exercise 4) exercise training combined with caloric restriction.

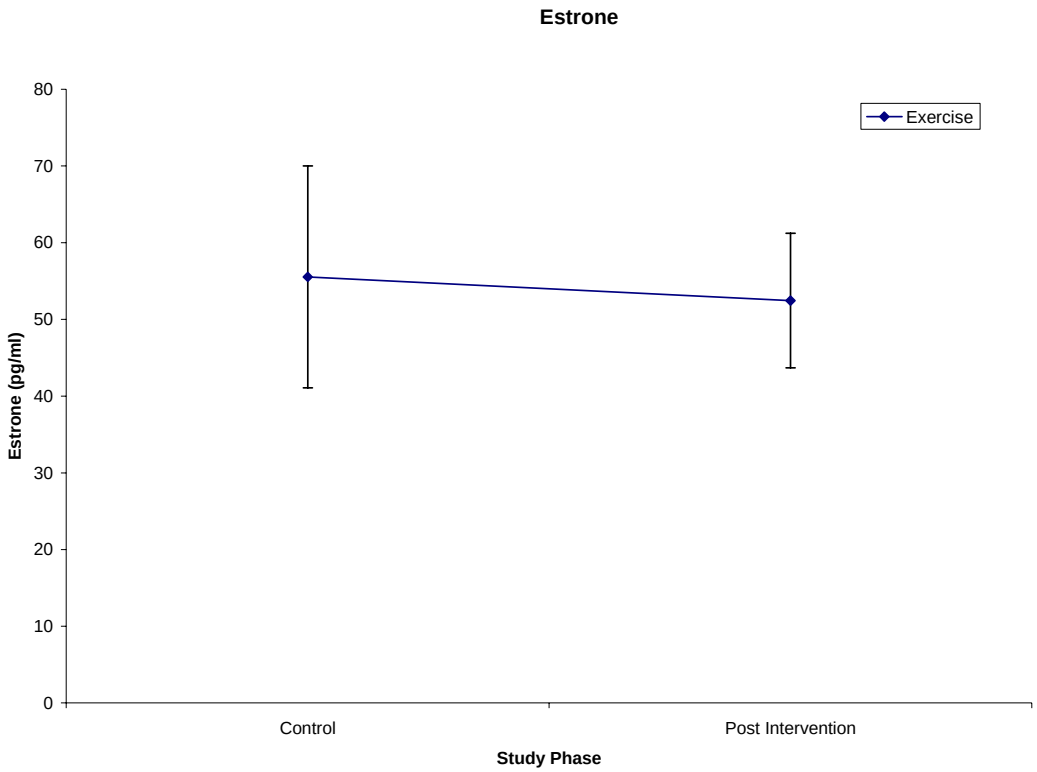

Figure 8. Serum Estrone in subjects before and after the intervention.

Results for IGF-1:

Figure 9 illustrates the changes in IGF-1 across the intervention. IGF-1 does not decline despite significant fat and weight loss in Exercise or Light Conditioning (ANOVA with repeated measures, time effect: $p = 0.139$, group effect $p = 0.396$)**,** and there is no correlation between change in body weight and change in IGF-1 from pre to post (Linear regression $p > 0.05$; $R^2 = 0.002$)**(Figure 10)**.

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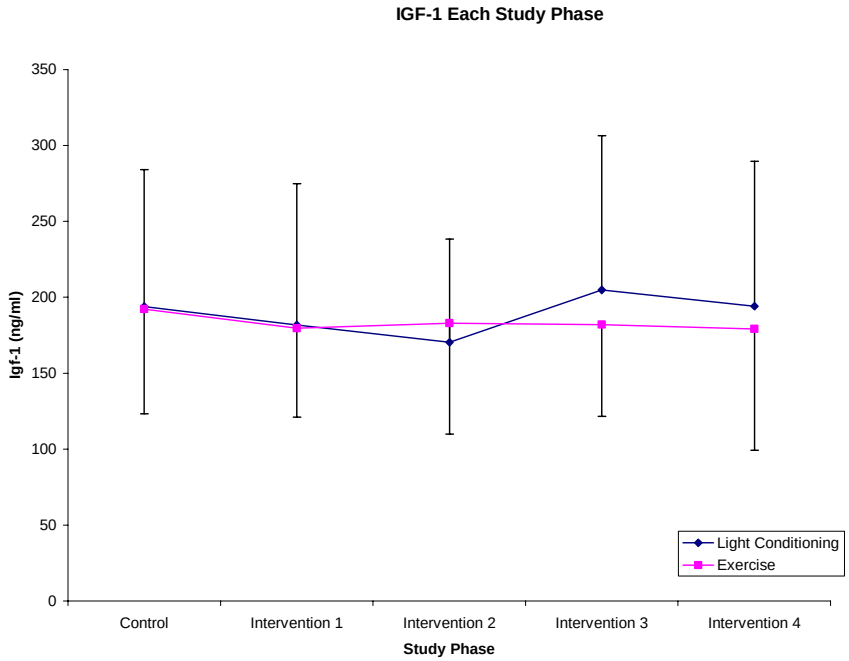

Figure 9. Changes in Circulating IGF-I. During Exercise Combined with Diet Intervention:

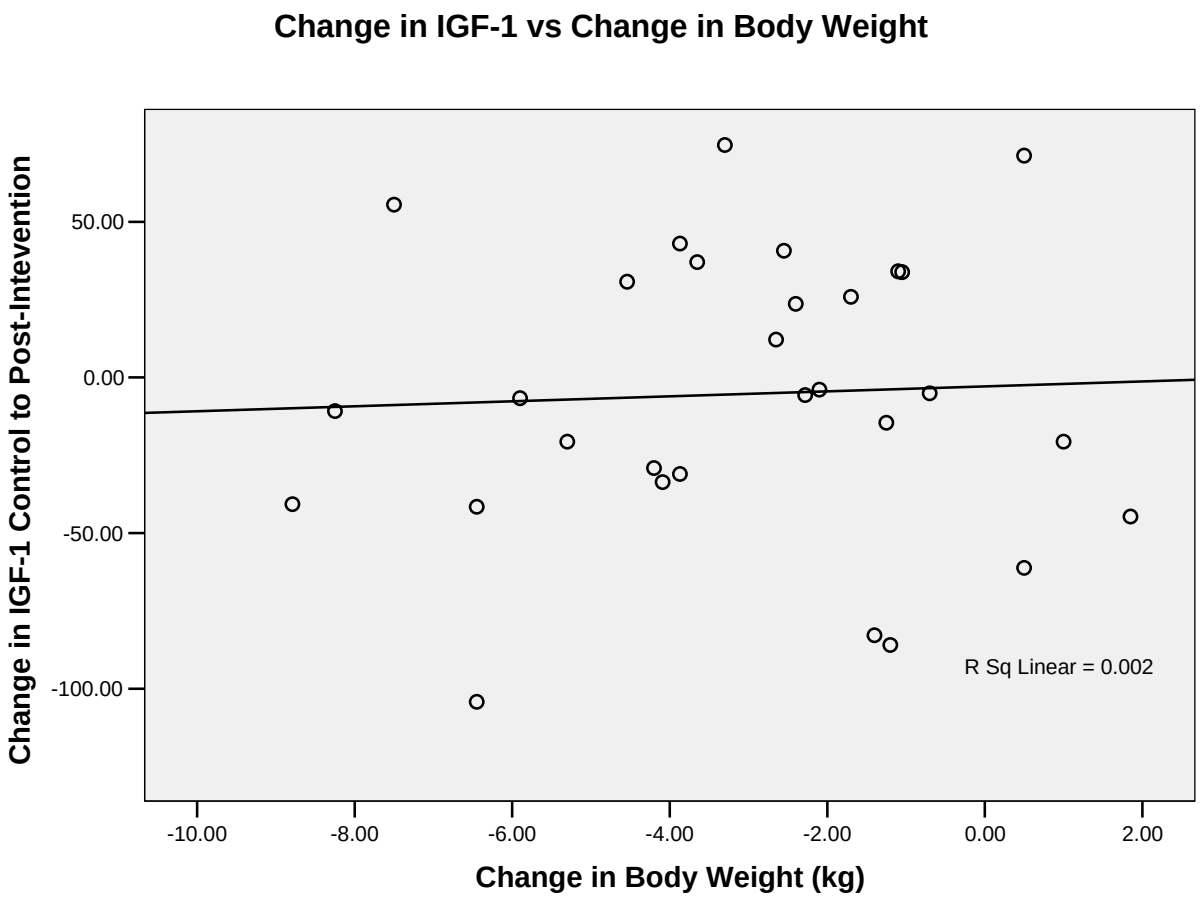

Figure 9. Regression results for relation between the change in body weight and the change in circulating IGF-1 for all subjects.

Results for Insulin, IGFBP-3, and SHBG:

Additional funding has been secured by the PI to assay these hormones and binding proteins during the Spring of '07 in her laboratory at PSU. Currently there are no data for these analytes. The final report mistakenly listed insulin assays as having been completed.

Results for Dried Blood Spot Samples:

Samples for serum versus dried blood spot analyses were analyzed in the PI's lab (serum hormone concentrations) or by Salimetrics, Inc., Penn State University (Blood Spot assays). Results for the comparison between serum and dried blood spot samples are illustrated in **Tables 4, 5, and 6,** and **Figures 9, 10, and 11**. Leptin venipuncture concentrations were significantly correlated with leptin dried blood spots concentrations ($R = 0.677$; $p = 0.011$). Results for T3 indicated no correlation between serum and blood spot values ($R= 0.320$; $p=0.211$). IGF-1 venipuncture concentrations were significantly correlated with blood spot values ($R= 0.778$; $p < 0.0001$).

Table 4. Correlation between serum and Dried Blood Spot sample for leptin

		Av Control Month Leptin (ng/ml)	PreLeptinBlood Spot(ng/ml)
Av Control Month Leptin (ng/ml)	Pearson Correlation	1	.677(*)
	Sig. (2-tailed)	.	.011
	N	36	13
PreLeptinBloodSpot (ng/ml)	Pearson Correlation	.677(*)	1
	Sig. (2-tailed)	.011	.
	N	13	13

* Correlation is significant at the 0.05 level (2-tailed).

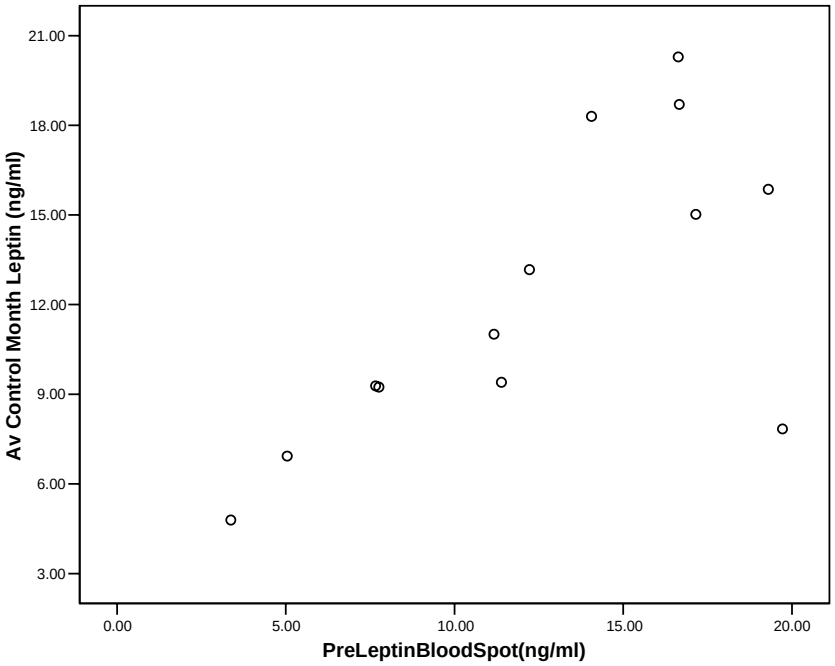

Figure10. Scatterplot of leptin dried blood spot (X axis) vs leptin (Y axis) venipuncture results

Table 5. Correlation between serum and Dried Blood Spot sample for T3

Correlations

		SerumT3ALL	Bloodspot T3All
SerumT3ALL	Pearson Correlation	1	.320
	Sig. (2-tailed)		.211
	N	50	17
BloodspotT3All	Pearson Correlation	.320	1
	Sig. (2-tailed)	.211	
	N	17	44

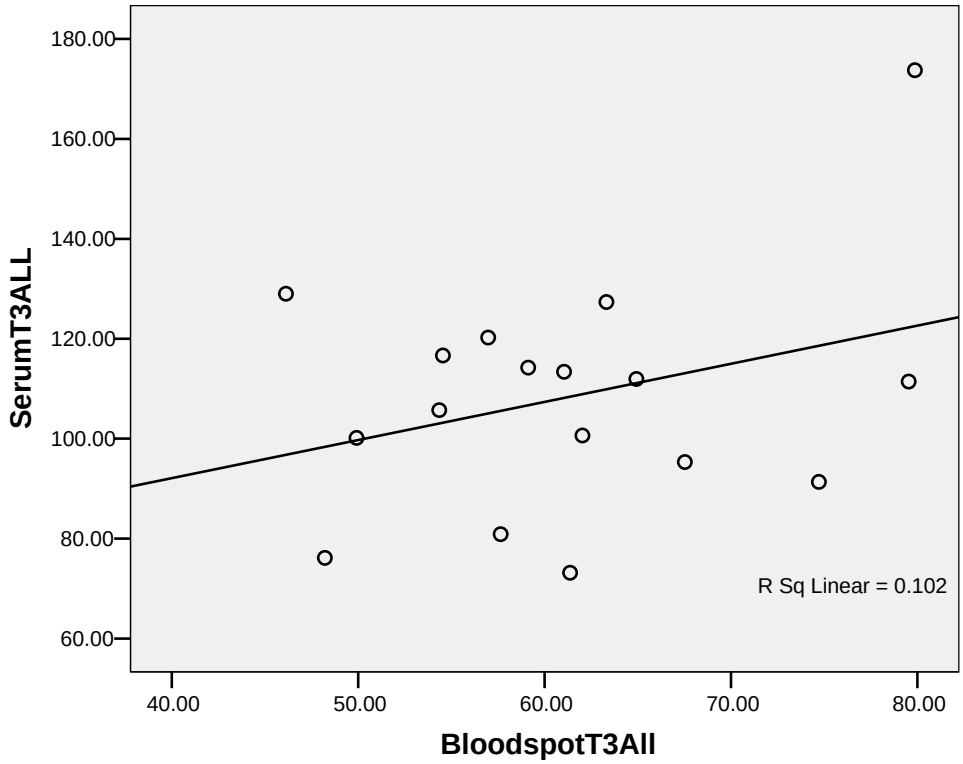

Figure11. Scatterplot of dried blood spot T3 (X axis) vs serum T3 (Y axis) venipuncture results

Table 6. Correlation between serum and Dried Blood Spot sample for T3

Correlations

		serumigfall	bloodspot igf1all
serumigfall	Pearson Correlation	1	.778**
	Sig. (2-tailed)		.000
	N	54	18
bloodspotigf1all	Pearson Correlation	.778**	1
	Sig. (2-tailed)	.000	
	N	18	45

**. Correlation is significant at the 0.01 level (2-tailed).

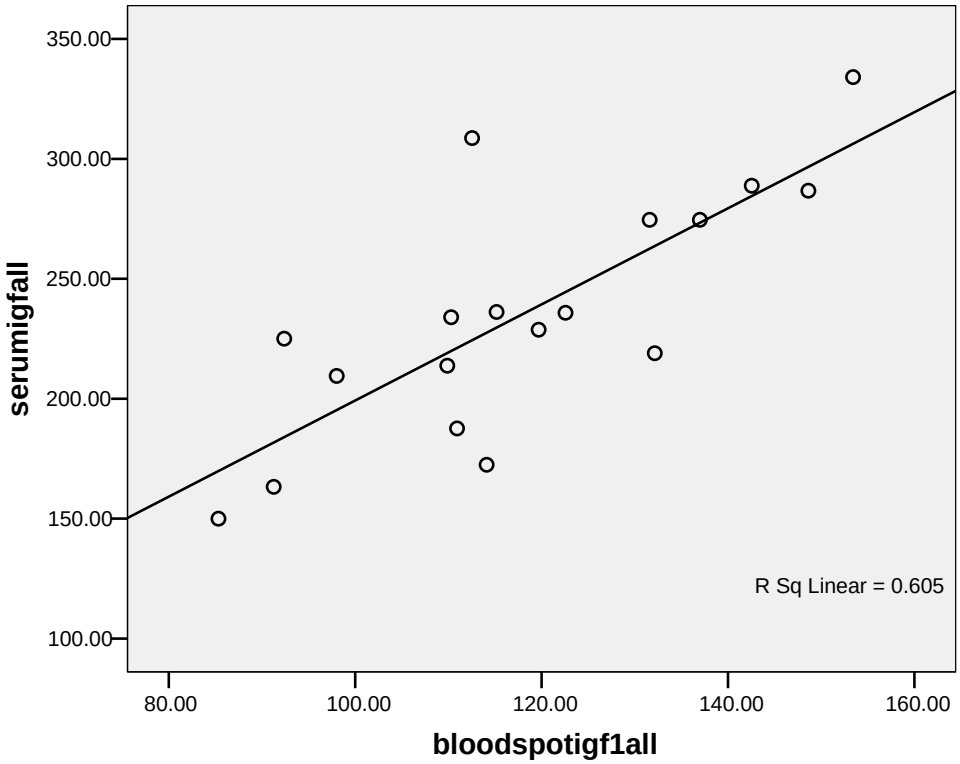

Figure12. Scatterplot of IGF-1 dried blood spot (X-axis) vs IGF-1 venipuncture (Y axis) results

Results of Additional Projects:

Findings from two additional projects using samples and or data from the primary study are reported below.

Additional Project #1: "Effect of Exercise Training on Estrogen Metabolism in Premenopausal Women"

Manuscript #1: In Review in Medicine, Science, Sports and Exercise, Fall, 2006: Effect of Exercise Training on Estrogen Metabolism in Premenopausal Women. Kim C. Westerlind, Jennifer Bell, Nancy I. Williams

This manuscript is in review, written in collaboration with Dr. Kim Westerlind, AMC Cancer Center, Boulder, Colorado. An increasing body of data provides evidence that being physically active reduces a woman's risk for developing breast cancer. To date, the mechanism for this reduction in risk remains unknown. The original study that is the subject of this Final Report was designed to examine how exercise combined with caloric restriction alters circulating estradiol and estrone, and circulating concentrations of IGF-I. Another hypothesis is that exercise may exert more subtle changes in steroid hormone profiles, specifically that exercise may result in an increase in the hydroxylation of the parent estrogens through the 2-hydroxylation pathway with a concomitant decrease through the 16 OHE pathway. Higher levels of 2OHE1 have been associated with decreased breast cancer risk. In animal tumor models, increased 20HE1 results in reduced tumorigenesis. In contrast, 16OHE1 has potent estrogenic effects and has been reported to be elevated in normal and malignant tissue from women with breast cancer. We proposed that exercise increases the ratio of 2/16 OHE1. We reasoned that this in turn might be a factor in the physical activity-mediated reduction in breast cancer risk that has been observed in epidemiological and some animal studies. We sought to examine the effect of exercise

training on estrogen metabolism in the same subjects as were used in the current study. We hypothesized that exercise training would result in an increase in the 2/16 ratio.

Urine samples from the mid-follicular (length of follicular phase divided by 2 = day of cycle on which urine sample was analyzed) and mid-luteal phases (length of luteal phase divided by 2 = day of cycle on which urine sample was analyzed) from each cycle from each subject in the study were sent for analysis by Dr. Westerlind. Urine was collected daily by subjects and represented an aliquot from their first morning void. Samples were shipped on dry ice to the AMC Cancer Research Center where they were stored at -80 until all samples from a given subject were available to be assayed. The funds for these assays were awarded from a separate grant that Dr. Westerlind wrote in collaboration with Dr. Williams. Estrogen metabolite levels were measured using a commercially available competitive, solid-phase enzyme-linked immunoassay (ESTRAMET, ImmunaCare, Corp) and the ratio of 2/16 computed. The frozen urine samples were brought to room temperature before testing. Samples were diluted 1:4 prior to testing with manufacturer-supplied diluent. In urine, 2-OHE1 and 16OH are found in the glucuronide conjugate from and require removal of the sugar moiety before recognition by the monoclonal antibodies. Samples are deconjugated with b-glucoronidase and arylsulphatase, then neutralized. Samples are incubated for 3 hrs at room temperature then kinetically read every 2 min for 20 minutes using a Thermomax Microplate Reader (Molecular Devices, Sunnyvale, CA). Estrogen metabolite values were determined from a calibration curve derived from six standards with the kit (0.625 – 15.0 ng/ml). All samples, controls, and standards are assayed in triplicate and all of the samples from a given subject were batch assayed to minimize interassay variability. Any sample outside of the range of the standard curve or with a coefficient of variation greater than 10% was reassayed. In house and manufacturer-supplied controls were included in each of the assays performed. Urinary 2OHE1 and 16OHE1 were normalized to urinary creatinine concentration and expressed in ng/ml/mg creatinine. When the ratio was computed the values used were uncorrected. Urinary creatinine was measured in duplicate using a Diagnostic Chemicals Limited Assay (company, state). The ratio of 2/16 OHE1 was computed by dividing the absolute concentration of 2OHE1 by 16OHE1. Results were analyzed using SPSS Statistical software, version 13.0. Statistical tests included t-tests and repeated measures ANOVAs or ANCOVAs.

Preliminary results from the analyses of these samples indicate that the ratio of 2/16 was significantly higher in the exercise women (1.96) compared to the controls (1.41). This was factored into the statistical analyses. Estrogen metabolite values for the subjects overall did not change significantly as a result of the intervention. 2OHE1 and 16OHE were higher during the luteal phase than the follicular phase as would be expected as the concentration of the metabolites parallels the changes in estradiol during the menstrual cycle. The 2/16 ratio was not significantly different between the follicular and luteal phase for any of the months of assessment (P values ranged from 0.30 to 0.99). In order to evaluate the change in 2/16 over time, the percentage change between each of the months of the studies was calculated. Data for the 2/16 were evaluated by calculating the percent change over the course of the intervention programs. 2/16 increased linearly in the follicular phase ($p=0.004$) but did not differ between groups ($p=.91$). In the luteal phase, initial 2/16 ratios were lower in the Light Conditioning group (1.41) vs the Exercise group (1.94) ($p=0.04$). After adjusting for initial differences, significant increases over time were observed ($p<0.001$) but no difference was observed between groups ($P=0.53$). Evaluating the data further, we analyzed the relationship between a woman's initial 2/16 values in both the follicular and luteal phases and her exercise-associated changes in 2/16. Correlations between baseline follicular and luteal 2/16 ratios was $r = 0.79$. Baseline values for 2/16 were significantly associated with the percent change in the ratio over the exercise periods. Most dramatically, the 2/16 ratios in the follicular and luteal phase of the baseline month were correlated $r = -0.59$ ($p=0.002$) and $r = -0.72$ ($p<0.001$) with the total percent change (defined as the percent change between baseline and the fourth exercise month) in 2/16 luteal phase. When we divided the women into quartiles (n=6/quartile) based on either their baseline follicular or luteal 2/16 ratios with experimental groups combined (**Tables 7 and 8)**, we observed a significant effect of exercise on the ratio of 2/16. Women within the lowest quartile, i.e., those with the lowest ratio of 2/16, had the largest increase in the ratio in response to exercise. Absolute estrogen metabolite values as well as percentage change over time were not associated with any change in body composition, BMI, or weight.

Table 7. Study 2: Total Percent Change in 2/16OHE1 based on baseline follicular 2/16OHE1 ratio

	Quartiles 2/16 OHE1 (n=6/quartile)				
	1 (X = 0.78)	**2 (X = 1.57)**	**3 (X = 2.20)**	**4 (X = 3.18)**	**SIG**
% Change 2/16 Follicular phase	**116 + 29%[a]**	**-6 + 11 %**	**30 + 28%**	**29 + 28%**	**P=0.007**
% Change 2/16 Luteal phase	**75 + 14%[b]**	**32 + 10%**	**-8 + 24%**	**2 + 5%**	**P=0.004**

Overall significance determined by ANOVA with post-hoc LSD
[a] significantly different than quartiles 2, 3, 4
[b] significantly different than quartiles 3 and 4; NS between quartile 1 and 2 (p=0.54)

Table 8. Total Percent Change in 2/16OHE1 based on baseline luteal 2/16 OHE1 ratio

	Quartiles 2/16 OHE1 (n=6/quartile)				
	1 (X = 0.77)	**2 (X = 1.53)**	**3 (X = 2.38)**	**4 (X = 3.16)**	**SIG**
% Change 2/16 Follicular phase	**88 + 36%**	**22 + 27%**	**16 + 26%**	**43 + 18%**	**P=0.226**
% Change 2/16 Luteal phase	**70 + 17% [a]**	**37 + 19% [b]**	**3 + 10%**	**-9 + 22%**	**P=0.007**

Overall significance determined by ANOVA with post-hoc LSD
[a] significantly different than quartiles 2, 3, and 4
[b] significantly different than quartile 4

In conclusion, the data suggest that women who have low 2/16 ratios may respond positively to exercise training with an increase in 2/16. Literature suggests that women with low 2/16 ratios may be at higher risk for developing breast cancer. Thus, exercise training may be of significant benefit to reducing breast cancer risk, particularly for those women at greatest risk. This manuscript is in Review in Medicine, Science, Sports, and Exercise. An abstract describing this work was presented at the American College of Sports Medicine, June, 2006 in Boulder, Colorado.

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Estrogen Metabolism is Altered with Exercise Training. Kim C. Westerlind, FACSM, Jennifer Bell, and Nancy I. Williams, FACSM. AMC Cancer Research Center, Denver, CO and Noll Laboratory, Penn State University, University Park, PA

Additional Project #2: "Susceptibility to energy-related menstrual disturbances is reduced with increased age"

Although energy deficiency is known to disrupt reproductive function in exercising women, no studies have examined whether the susceptibility to menstrual disturbances is altered with increased age. Therefore, the purpose of this study was to determine whether increased age is associated with reduced susceptibility to menstrual disturbances caused by chronic energy deficiency. A subset of twenty-four premenopausal women (12 young and 12 middle aged) from two larger studies (the DOD study that is the subject of this report, and an NIH funded study examining weight loss effects in younger (18-25 yrs) women) were matched according to the amount weight lost during a three month diet and exercise intervention. Menstrual status was assessed with daily urine samples and measurement of estrogen (E1G) and progesterone (PdG) conjugates using enzymatic immunoassays. The occurrence of menstrual disturbances, i.e., short luteal phases, inadequate luteal phases, oligomenorrheic cycles, and anovulatory cycles was quantified according to the number of defects/number of cycles observed. Independent samples t-tests were conducted to determine if there was a significant difference between younger and older women in the occurrence of menstrual disturbances.

Table 9. Effects of Energy Deficiency on Menstrual Disturbances in Young and Middle Age Women

	Age	Gynecologic Age	Occurrence of Defects	Weight Loss	% Energy Deficit
Young (n=12)	20.2 ± 1.9	8.0 ± 2.0	1.92 ± 2.02	-4.6 ± 1.4	-20.0 ± 17.2
Middle Aged (n=12)	33.5 ± 4.2	21.4 ± 4.8	0.58 ± 0.90	-4.6 ± 1.4	-37.7 ± 11.3
	p<0.00*	p<0.00*	p<0.05*	p<0.95	p<0.05*

In conclusion, older age was associated with significantly fewer disturbances in response to weight loss resulting from a diet and exercise intervention **(Table 9)**. These results suggest that factor(s) associated with increased age confer protection against menstrual disturbances caused by energy deficiency. An abstract describing this work was presented at the American College of Sports Medicine, June, 2006 in Boulder, Colorado, and a manuscript is in review:

Susceptibility to energy-related menstrual disturbances is reduced with increased age. Brandy Weller, Ann Albert, Matthew Johnson, Nancy Williams, Penn State University, University Park, PA 16802

Overall Discussion:

The results of this study are unexpected, as it was hypothesized that significant weight loss produced through a combination of diet and exercise would result in significant declines in circulating biomarkers of breast cancer. The results thus far indicate that several measures of estrogens, i.e., serum estradiol spread out across the menstrual cycle, average serum estradiol, urinary metabolites reflecting estradiol (E1G), and serum estrone (E3) do not change with weight loss, despite significant losses of both body weight and body fat. Previous prospective exercise training studies promoting weight loss in younger women age 18-25 yrs (Bullen et al. 1985, Williams et al. 2004, Williams et al. 2005) have demonstrated significant disturbances in menstrual cyclicity with concomitant changes in measures of estrogens with a range of weight loss that approximates that observed in the current study conducted in older (25-40) women. These results suggest that there is an age-related decline in the responsiveness of the hypothalamic-pituitary-gonadal axis to an energy imbalance such

that age protects against energy perturbations that might compromise reproductive function. This finding is novel. One other study has directly compared the responses to a negative energy balance between young (18-25 yrs) and older (26-30 yr) of the reproductive axis and found that 5 days of low energy availability produced significant decreases in LH pulse frequency only in the younger subjects (Loucks, 2006). The findings from the the current study extend those of Loucks to the entire menstrual cycle over a prolonged period of energy deficiency. The findings are currently the subject of a manuscript from the PI's laboratory (results reported as Additional Project #2) where young and older subjects were matched for weight loss and other baseline characteristics, and the effects of weight loss over 3 months were directly compared. Data from the DOD funded study are compared to those from an NIH funded study conducted in younger women. This manuscript is in review. The PI also plans to use these data as preliminary data for a NIH grant proposal.

Another unexpected finding is that for IGF-1, which showed no significant changes in response to significant weight loss. We anticipated a reduction in IGF-1, as this hormone is often found to decrease with energy deprivation (Thissen, 1994). One explanation might be that exercise training, which has been reported to increase with exercise training (Koziris et al., 1999) may have represented a competing influence on IGF-1 production and thus in combination, weight loss and exercise training resulted in no change in IGF-1 levels. We plan to perform further analyses of IGFBP-3 to determine if the bioavailability of IGF-1 was altered with our intervention. This result suggests, that although physical activity has been associated with a reduced risk of breast cancer, reductions in IGF-1 are not likely to be a mechanism for this reduced risk.

A novel and unexpected finding from Additional Project #1 is that the ratio of estrogen metabolites 2OHE1 /16OHE was increased with exercise training in subjects who were ranked in the lowest quartile, those with the lowest ratio of 2/16 . Because this direction of change is associated with a reduced risk of breast cancer, this finding indicates a potential for a beneficial effect of exercise training in breast cancer risk in some subjects when risk is assessed using these estrogen metabolites. Current studies are planned to examine the response of these metabolites in younger women age 18-25 yrs to exercise training associated with weight loss.

Data for the dried blood spot indicate promise with respect to the potential use of dried blood spot assays and collection procedures to assess metabolic hormones and changes in these hormones with weight loss. We found significant correlations between leptin in serum and leptin in blood spots, and between IGF-1 in serum and IGF-1 measured in blood spots. Reasons for the lack of a significant correlation between serum and blood spot T3 are unknown but could be due to problems with the assay or blood collection techniques using filter paper. Future studies might yield a more positive result if trouble-shooting were performed. These results suggest that for IGF-1 and for leptin, field testing for assessment of energy balance is possible using dried blood spot techniques.

KEY RESEARCH ACCOMPLISHMENTS

Manuscripts from DAMD Funding:

1) Effect of Exercise Training on Estrogen Metabolism in Premenopausal Women. Kim C. Westerlind, Jennifer Bell, Nancy I. Williams

This manuscript in Review, Medicine, Science, Sports and Exercise. AMC Cancer Research Center, Denver, CO and Noll Laboratory, Penn State University, University Park, PA 16802

2) Susceptibility to energy-related menstrual disturbances is reduced with increased age. Nancy I. Williams, Kelly Dougherty, Brandy Weller, Ann Albert. Penn State University, University Park, PA 16802

This manuscript is drafted and is in Review.

3) Effects of Exercise Combined with Caloric Restriction on Circulating Estradiol and Menstrual Cyclicity in Premenopausal Women. Nancy I. Williams, Brian Frye, Kelly Dougherty

Methods and Results have been written.

4) Effects of Exercise Combined with Caloric Restriction on Circulating Biomarkers of Breast Cancer

This manuscript is in progress, and will be completed when results for insulin, IGFBP-3, and SHBG are obtained from assays planned for Spring '07.

REPORTABLE OUTCOMES (2001-2005)

Funding Applied for and Received Based on Work Supported by this Award:

1. PR054531 10/05-10/09
United States Department of Defense CDMRP Program

"Increased Caloric Intake to Reverse Energy Deficiency in Exercising Women: Impact on Bone & Menstrual Cyclicity"

This study investigates the effects of increasing caloric intake in exercising women with reduced bone mass and amenorrhea on menstrual and bone health status.

Role: Co-Principal Investigator (With MJ De Souza)

2. Cancer Research and Prevention Foundation 1/04-12/05
0%

"Exercise and Estrogen Metabolism: Implications for Breast Cancer Prevention"

The major goal is to test the hypothesis that a diet and exercise intervention produces favorable alterations in the ratio of key catechol estrogens that are associated with a reduced risk of breast cancer.

Role: Co-Investigator (PI is Kim Westerlind, AMC Cancer Research Center, Denver, CO)

Pending Funding Applied for and Received Based on Work Supported by this Award:

1. National Institutes of Health (NIH) 4/1/05- 3/31/09
15%

"Antioxidant Status, Diet and Early Pregnancy"

Role: Co-Investigator (PI is Terryl Hartmen, Dept. Nutrition, Penn State)

UPDATE: November 20, 2006 Status: FUNDED

2. **National Institutes of Health (NIH)** 1 RO1 HD39245-01 7/1/05-6/30/09 20%

"Bioenergetics of Exercise-Induced Menstrual Disturbances"

Role: Principal Investigator

UPDATE: November 20, 2006 Status: NOT FUNDED

3. National Athletic Training Association Research Grant 1/1/06-12/31/06

"Reversing Energy Deficiency in Amenorrheic Athletes: Effects on Bone Turnover and Physical Performance"

Role: Principal Investigator

UPDATE: November 20, 2006 Status: FUNDED

Student Training:

The following individuals have been supported in Dr. Williams' Laboratory during the funding period covered by DAMD17-01-1-0361:

Masters Students

2002 Megan Senior "Screening for Subclinical Eating Disorders in Female Athletes: The Use of an Indirect Interview Technique " (Nutrition)
2004 Michael Perry " Effects of chemotherapy followed by exercise training on reproductive status and stress hormones in breast cancer patients" (Kinesiology)
2004 Kelly Dougherty "No relation between leptin and exercise-associated reproductive disturbances in healthy normal weight young women" (Kinesiology)
2004 Brian Frye "Predictors of weight loss in a diet and exercise intervention in young women" (Kinesiology)
2005 Sarah Giambuzzi (Kinesiology – In Progress)
2005 Jennifer Ward (Physiology- In Progress)

Doctoral Students

2004 Heather Leidy "Role of ghrelin in energy homeostasis"(Physiology)
2005 Jennifer Bell (In progress, Physiology)
2005 Brandy Weller (In progress, Kinesiology)

MANUSCRIPTS DURING THE ENTIRE PERIOD OF SUPPORT BY DAMD17-01-1-0361 (2001-2006)

UPDATED NOVEMBER 20, 2006

(TOTAL 15 PUBLISHED, 3 IN REVIEW, 5 IN PROGRESS)

Williams, N.I., Caston-Balderrama, A.L. Helmreich, D.L., Parfitt, D.B., Nosbisch C, Cameron, J.L. Longitudinal changes in reproductive hormones and menstrual cyclicity in cynomolgus monkeys during strenuous exercise training: rapid transition to exercise-induced amenorrhea *Endocrinology* 142: 2381-2389, 2001

Williams NI, DL Helmreich DL, DB Parfitt, Caston-Balderrama AL, JL Cameron. Evidence for a causal role of low energy availability in the induction of menstrual cycle disturbances during strenuous exercise training. *J Clin Endocrinol Metab* 86: 5184-5193, 2001

Miles MP, Mackinnon LT, Grove DS, **Williams NI**, Bush JA, Marx JO, Kraemer WJ, Mastro AM. The relationship of natural killer cell counts, perforin mRNA and CD2 expression to post-exercise natural killer cell activity in humans. *Acta Physiol Scand* 174: 1-9, 2002.

McConnell HJ, KA O'Connor, E Brindle, and **NI Williams**. Validity of methods for analyzing urinary steroid data to detect ovulation in athletes. *Med. Sci. Sports Exerc*, 34(11): 1836-1844, 2002

Whipple TJ, Petit Moira, Sharkey N, Demers L, **Williams NI**. Leptin and the skeleton. *Clin. Endocrinol*. 57: 701-711, 2002.

Williams, NI. Experimental disruptions of the menstrual cycle: Lessons from long-term prospective studies. *Med Sci Sports Exerc* 35 (8): 1564-1572, 2003.

De Souza, M.J., H. McConnell, E. O'Donnell, B. Lasley, and **Williams NI**. Fasting Ghrelin Levels in Physically Active Women: Relationship with Menstrual Disturbances and Metabolic Status. (*J Clin Endocrinol Metab*. Jul;89(7):3536-42, 2004.

Leidy HJ, Gardner JK, Frye BR, Snook ML, Schuchert MK, Richard EL, and **Williams NI.** Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal weight young women (Special edition: *J Clin Endocrinol Metab*. Jun;89(6):2659-64, 2004.

De Souza, M.J., and **N.I. Williams**. Physiological Aspects and Clinical Sequelae of Energy Deficiency and Hypoestrogenism in Exercising Women (Hum Reprod Update. Sep-Oct;10(5):433-48, 2004

Whipple TJ, Le BH, Demers LM, Chinchilli VM, Petit MA, Sharkey N, **Williams NI** Acute effects of moderate intensity resistance exercise on bone cell activity *Int J Sports Med*. Oct;25(7):496-501, 2004.

De Souza, MJ, **Williams NI**. Beyond Hypoestrogenism in Amenorrheic Athletes: Energy Deficiency As a Contributing Factor for Bone Loss. *Curr Sports Med Rep*. 2005 Feb;4(1):38-44.

Williams, NI and De Souza, MJ. "Exercise-associated menstrual cycle disturbances: practical and clinical considerations", in "The Endocrine System in Sports and Exercise" as part of the series "The Encyclopedia of Sports Medicine" an International Olympic Committee Medical Commission Publication in collaboration with the International Federation of Sports Medicine. Kraemer WJ, Rogol AD, eds. Blackwell Publishing, Malden, MA, Chp 20, pp 261-278, 2005

Hutnick NA, **Williams NI**, Kraemer WJ, Orsega- Smith E, Dixon RH, Bleznak AD, Mastro AM. Exercise and Lymphocyte Activation following Chemotherapy for Breast Cancer *Med Sci Sports Exerc*. Nov;37(11):1827-1835, 2005.

Hertel J, Williams NI, Olmsted-Kramer LC, Leidy HJ, Putukian M. Neuromuscular performance and knee laxity do not change across the menstrual cycle in female athletes. *Knee Surg Sports Traumatol Arthrosc*. 2006; 14(9):817-22.

Williams NI, Leidy HJ. Food Attitudes in Female Athletes: Association with Menstrual Cycle Length *Journal of Sport Sciences,* 2006 24(9): 979-86.

Williams, NI, De Souza MJ**.** Letter to the Editor: Female Athlete Triad Errors and Misunderstandings *Med Sci Sports Exer* May;38(5):1021), 2006.

Leidy HJ, **Williams NI.** Meal Energy Content is Related to Features of Meal-related Ghrelin Profiles Across a Typical Day of Eating in Non-obese Premenopausal Women. *Horm Metab Res*. 2006 May;38(5):317-22.

Leidy HJ, Dougherty KA, Frye BR, Duke KM, **Williams NI**. 24 hr ghrelin is elevated following calorie restriction and exercise training in non-obese women. (*Accepted, Obesity*).

De Souza MJ, **Williams NI**, Alleyne J, Vescovi J, Van Heest J. Letter to the Editor: Correction of Misinterpretations and Misrepresentations of the Female Athlete Triad. (*Accepted, British Journal of Sports Medicine*)

RS Legro, R Zaino, L Demers, AR Kunselman, CC. Gnatuk, **NI Williams**, WC Dodson. The Effects of Metformin and Rosiglitazone, Alone and in Combination, on the Ovary and Endometrium in PCOS. (In Press: *American Journal of Obstetrics and Gynecology*)

De Souza MJ, Hontscharuk R, Olmsted M, Burke T, Kerr G, and **NI Williams**. Drive for thinness score is a proxy indicator of energy deficiency in exercising women. (*Accepted, Appetite*)

MANUSCRIPTS IN REVIEW (All 2006)

1. **Williams, N.I.** , Berga S.L., and Cameron, J.L. Synergistic effects of multiple sub-threshold stressors on menstrual cyclicity in cynomolgus monkeys. (In Review: American Journal of Physiology)

2. Orsega-Smith, E. , Mastro, A.M., **Williams, N.I**., Perry, M., Kraemer, W., Bleznak, A., & Dixon, R. The impact of a combined program of strength training and aerobic exercise on quality of life and physical function in breast cancer survivors. (In Review: Journal of Women's Health).

3. Westerlind KC, **Williams, NI**. Effect of Exercise Training on Estrogen Metabolism in Premenopausal Women. (In Review: Medicine and Science in Sports and Exercise)

4. De Souza, MJ, Lee D, Van Heest J, Scheid J, **Williams NI**. Severity of energy-related menstrual disturbances increases in proportion to indices of energy conservation in exercising women (In Review: Journal of Applied Physiology)

5. DiNallo JM, Le Masurier GC, **Williams NI** , Downs DS. Walking for Health During Pregnancy: How Much is Enough? (In Review: Research Quarterly).

6. Loucks AB, De Souza MJ, **Williams NI.** (Letter to the Editor in Response to: Effects of lifetime exercise on the outcome of in vitro fertilization) (In Review, Obstetrics and Gynecology, November, 2006)

7. De Souza MJ, West SL, Jamal SA, Hawker GA, Gundberg CM, **Williams NI**. Bone Turnover in Premenopausal Exercising Women: Effects of Energy Deficiency and Estrogen Deficiency. (In Review, Journal of Bone and Mineral Research)

8. Susceptibility to energy-related menstrual disturbances is reduced with increased age**. Williams NI**, Kelly Dougherty, Brandy Weller, Ann Albert. Penn State University, University Park, PA 16802

ABSTRACTS DURING THE ENTIRE PERIOD OF SUPPORT BY DAMD17-01-1-0361 (2001-2005)

(TOTAL = 27)

Mastro AM, **Williams NI**, Kraemer WJ, Orsega-Smith EM, Perry MD, Dixon RH, Bleznak AD, Underwood J. Exercise, quality of life, and the recovery of CD4 (+) lymphocytes following chemotherapy for breast cancer *Proceedings of the American Association for Cancer Research 92nd Annual Meeting,* New Orleans, LA , 42 : 331, March 24-28, 2001

Perry MD, Mastro AM, Orsega-Smith E, Miles MP, Kraemer WJ, **Williams NI**. Exercise training and immune function following chemotherapy for breast cancer. *Proceedings of the American College of Sports Medicine Annual Meeting,* Baltimore, MD, June 2-6, 2001

Orsega-Smith E, **Williams NI** (FACSM), Perry MD, Mastro AM, Kraemer WJ, Bleznak A, Dixon R, Underwood J. Fatigue, quality of life and physical function after chemotherapy for breast cancer. *Proceedings of the American College of Sports Medicine Annual Meeting*, Baltimore, MD, June 2-6, 2001

Galucci, AN, **Williams NI**. Physiological indicators of psychological stress prior to competitive exercise. *Proceedings of the American College of Sports Medicine Annual Meeting*, Baltimore, MD, June 2-6, 2001

McConnell HJ, O'Connor KA, Brindle E, **Williams, NI**. Assessing reproductive function in exercising women: validity of ovulation detection algorithms. *Proceedings of the Endocrine Society Annual Meeting, Abstract* #P2-408, 2001

Senior MK, **Williams NI**, McConnell HJ, Clark KC. Screening for subclinical eating disorders in female athletes: validation of an indirect interview technique. *(Presented at the 24th Annual meeting of the Mid-Atlantic Regional Chapter of the American College of Sports Medicine, Bushkill, PA, November 2-3, 2001).*

McConnell HJ, **Williams NI**, O'Connor KA, Clark KL, Putukian M. Menstrual irregularities and disordered eating in female athletes: survey vs follow-up clinical and physiological studies. *(Presented at the 24th Annual meeting of the Mid-Atlantic Regional Chapter of the American College of Sports Medicine, Bushkill, PA, November 2-3, 2001).*

Mastro AM, **Williams NI**, Ford J, Fuener K, Orsega-Smith E, Kraemer WJ, Bleznak AD, Dixon RH, Underwood J, Miles M, Wagner K. IL-6 and interferon-gamma levels following chemotherapy for breast cancer. *Proceedings of the American Association for Cancer Research Annual Meeting*, San Francisco, CA, April 6-10, 2002

Hertel J, **Williams NI**, Gribble PA, McConnell HJ, DiPasquale AA, Putukian M. Changes in risk factors of ACL injuries across the menstrual cycle: A pilot study. *Proceedings of the American College of Sports Medicine Annual Meeting*, St. Louis, MO, May 29-June 1, 2002

Williams NI, McConnell HM, Gardner JK, Albert AC, Cameron JL. Lifestyle factors such as exercise, caloris intake, and psychological stress: relationship to reproductive hormones and possibly the risk of breast cancer. *Era of Hope* meeting, Department of Defense Breast Cancer Research Program, Orlando, FL, September 25-28, 2002

Dougherty, K., Galucci AN, McConnell HJ, **Williams NI.** Cortisol and testosterone levels prior to competitive exercise. *Proceedings of the American College of Sports Medicine Annual Meeting* , San Francisco, May 28-June 1, 2003, *Med. Sci. Sports Exerc.* 35 (5), p. S1837, 2003.

Williams NI, McConnell HJ, Gardner JK, Cameron JL, Schuchert MK, Richard EL, Snook ML. Susceptibility of menstrual cycle to various stressors: correlation with baseline luteal progesterone levels. *Proceedings of the American College of Sports Medicine Annual Meeting* , San Francisco, May 28-June 1, 2003, *Med. Sci. Sports Exerc.* 35 (5), p. S12, 2003.

McConnell HJ, Schuchert MK, Gardner JK, Frye BR, **Williams NI**. Basal Ghrelin is sensitive to changes in body weight during a controlled diet and exercise program in normal weight young women. (Presentated at the 2003 Endocrine Society Meeting, Philadelphia, PA, June 2003).

Whipple TJ, Le, B., Demers, L., Petit M.A., Sharkey N. **Williams, NI**. Acute Effects of Moderate Intensity Resistance Exercise on Bone Cell Activity. (*Presented at Association for Bone and Mineral Research Meeting, 2003*).

J K Gardner, H J McConnell, B R Frye, K A Dougherty, T S Parrott, E L Richard, M L Snook, M Schukert, **Williams, NI**. Validation of an Improved Method to Estimate Energy Requirements in College-aged Women: The PERK method (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S79, 2004)

K Dougherty , H J McConnell, J Gardner, B Frye, E Richard, M Snook, M Schucert, A Albert, T Parrott and **Williams, NI.** Effects of Diet and Exercise on Leptin Levels In Women: Dependence on Body Composition Changes (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S80, 2004)

Williams, NI, HJ McConnell, JK Gardner, BR Frye, EL Richard, ML Snook, KL Dougherty, TS Parrott, A Albert, M. Schukert. Exercise-associated menstrual disturbances: dependence on daily energy deficit, not body composition or body weight changes. (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S280, 2004.

De Souza, M.J., E. O'Donnell, R. Hontscharuk, T. Burke, J. Goodman, **Williams, NI**.

Diagnosis of Osteopenia May Indicate the Presence of Increased Cardiovascular Risk in Female Athletes. (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S280, 2004.

R. Hontscharuk, E. O'Donnell, **Williams, NI** T. Burke, M.J. De Souza**.** Dietary Cognitive Restraint: A Marker for Altered Energy Homeostasis and Menstrual Disturbances in Athletic Women (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S33, 2004.

McConnell, H., **Williams, NI**, E. O'Donnell, B. Lasley, M.J. De Souza. Fasting Ghrelin Levels in Physically Active Women: Relationship with Menstrual Disturbances and Metabolic Status. (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S280, 2004)

De Souza MJ, Lee D, Burke T, Hontscharuk R, O'Donnell E, Williams NI. Metabolic Adjustments to Maintain Energy Balance in Amenorrheic Exercising Women Indicate Chronic Energy Deficiency. (Abstract # P3-568, Proceedings of Endocrine Society, San Diego, CA, 2005) p. 684.

Williams NI. Leidy HJ, Legro R, Demers L, Gardner J, Frye B, Dougherty K, Albert A, Parrott T, De Souza MJ. Predictors of Menstrual Disturbances in Exercising Women. (Abstract # P3-569, Proceedings of Endocrine Society, San Diego, CA, 2005) p. 684.

Williams NI, Albert AC, Parrott TS, Leidy HJ, Frye B, Snook M, Duke, KA, Richard EL, Gardner JK. Effects of caloric restriction combined with moderate aerobic exercise on circulating estrogens and IGF-I in premenopausal women. (Abstract presented at 2005 Era of Hope Breast Cancer Research Meeting, Phiadelphia, PA, June, 2005)

A.M. Mastro, N.A. Hutnick, **N.I. Williams**, W.J. Kraemer, E. Orsega-Smith, R.H.Dixon, A.D. Bleznak**.** The use of exercise to increase lymphocyte activation following chemotherapy for breast cancer. (Abstract presented at 2005 Era of Hope Breast Cancer Research Meeting, Phiadelphia, PA, June, 2005)

Kim C. Westerlind, Jennifer Bell, and **Nancy I. Williams**, Estrogen Metabolism is Altered with Exercise Training (Submitted for presentation at 2006 American College of Sports Medicine Meeting, Denver, Colorado, 2006)

Brandy Weller, Ann Albert, Matthew Johnson, and **Nancy I. Williams** Susceptibility to energy-related menstrual disturbances is reduced with increased age**.** (Submitted for presentation at 2006 American College of Sports Medicine Meeting, Denver, Colorado, 2006)

Jennifer L. Scheid, **Nancy I. Williams**, Sarah L. West, Tanya G. Burke, Mary Jane De Souza. Bone Resorption Is Increased In Premenopausal Exercising Women With Osteopenia (Submitted for presentation at 2006 International Osteoporosis Federation, June 2006, Toronto, Ontario, Canada)

INVITED PRESENTATIONS DURING THE ENTIRE PERIOD OF SUPPORT BY DAMD17-01-1-0361 (2001-2005)

(TOTAL = 17)

“Low Energy Availability and the Menstrual Cycle: Clinical and Physiological Implications” *Society for the Study of the Menstrual Cycle, Bi-Annual meeting,* June, 2001, Hartford Connecticut

"Physiological Connections Between Factors of the Female Athlete Triad" *Penn State Athletic Training Conference*", April 12, 2002, Penn State University, University Park, PA

"Exercise and Women's Health: Lessons from the Female Athlete Triad", Department of Health and Exercise Science, April 25, 2002, *Wake Forest University,* Winston-Salem, NC

"Subclinical Eating Disorders and Menstrual Cycle Irregularities in Female Athletes" *Eating Disorders on Campus, The Institutional Response*, June 7, 2002, Eighth Annual Conference, Penn Stater Conference Center Hotel, Penn State University, University Park, PA

“Effects of Exercise on the Menstrual Cycle: Physiological mechanisms and practical considerations” February, 2003, *School of Kinesiology and Health Science,* York University, Ontario, Canada.

“Effects of Estrogen on Vascular Function”, February, 2003, *School of Kinesiology and Health Science*, York University, Ontario, Canada.

“Exercise and the Menstrual Cycle: Psychological, sociological, and physiological factors. June, 5, 2003, Keynote for *Society for Menstrual Cycle Research* meeting, Pittsburgh, PA

“Exercise-associated Menstrual Disturbances: Role of Energy Availability”, October 10, 2003. *Faculty of Health and Physical Education*, University of Toronto, Toronto, Ontario, Canada.

“Exercise-associated menstrual disturbances: Physiological mechanisms and role of caloric restriction”, November 24, 2003, *Department of Health and Kinesiology, Texas A and M University*, College Station, TX

“Exercise-associated menstrual disturbances: Physiological mechanisms and role of caloric restriction”, November 25, 2003, *Department of Nutrition, Texas A and M University*, College Station, TX

“Exercise-associated menstrual disturbances: Physiological mechanisms and role of caloric restriction”, December 4, 2003, *Department of Endocrinology, Endocrine Research Conference*, Hershey Medical Center, Penn State University, Hershey, PA

“Exercise-associated menstrual disturbances: Phyiological mechanisms and clinical sequelae”, March 2, 2004, Department of Exercise Science, University of Massachusetts, Amherst, MA)

“Exercise-associated menstrual disturbances: Phyiological mechanisms and clinical sequelae”, April 30, 2004, Department of Physical Therapy, Faculty of Medicine, University of Toronto, Toronto, Ontario)

“Exercise-associated menstrual cycle disturbances: Physiological and Clinical Considerations”, December, 2004. Department of Exercise Science, University of Connecticut, Storrs, Ct. December, 2004.

Exercise, Estrogen, and Women’s Health: What’s the Connection? Keynote Speaker- Penn State Nutrition Conference, May 13, 2005.

“Estrogen and Cardiovascular Disease”. Univerisity of Toronto, Faculty of Physical Education and Health, November 21, 2005.

"The effects of caloric restriction on the menstrual cycle". Keynote Speaker, Mid-Atlantic American College of Sports Medicine Meeting, Harrisburg, PA November, 2005

SYMPOSIUM PRESENTATIONS DURING THE ENTIRE PERIOD OF SUPPORT BY DAMD17-01-1-0361 (2001-2005)

(TOTAL = 3)

"Menstrual Disturbances in Athletes: Lessons from Prospective Experiments on Animals and Humans (Chairperson Anne B. Loucks, PhD) Lessons from Experimental Disruptions of the Menstrual Cycle in Primates and Humans, N. Williams (*American College of Sports Medicine Annual Meeting*, St. Louis, MO, May 30, 2002)

Chairperson, Symposium on "Current Practices for Screening and Prevention of the Female Athlete Triad" (*American College of Sports Medicine Annual Meeting*, San Francisco, CA, May 31, 2003).

Co-Chairperson, Symposium on "Assessing Endocrine Alterations in Exercising Women". Weight loss and changes in circulating metabolic hormones and metabolic rate as biomarkers for exercise-associated menstrual disturbances: evidence from prospective studies. Proceedings of the *American College of Sports Medicine Annual Meeting*, Indianapolis, IN, 2004)

CONCLUSIONS:

- Caloric restriction combined with an amount of moderate aerobic exercise shown to be associated with reduced breast cancer risk produces significant weight loss and fat loss in healthy premenopausal women age 25-40 yrs over four menstrual cycles.

- Although previous prospective studies have shown that weight loss can significantly impact menstrual cyclicity and ovarian steroid excretion of estrogen metabolites that reflect circulating estradiol concentrations in young premenopausal women (age 18-25) (Bullen, et al. 1985), an exercise and diet stimulus that promotes a similar magnitude of weight loss does not perturb menstrual cyclicity, circulating serum estradiol, estrone, or urinary excretion of estrone -1 -glucuronide (E1G) in premenopausal women that are older, i.e., 25-40 years. This novel finding suggests an age-related decrease in susceptibility to energy deficiency of the reproductive axis, but also suggests that the mechanism for decreased risk of breast cancer in premenopausal women of this age range is not related to declines in the body's exposure to circulating estrogens despite significant weight loss.

- In contrast, when the impact of exercise that promotes an increase in fitness and decrease in body weight on estrogen metabolites 2 hydroxy estrone and 16 alpha hydroxyl estrone, and on the ratio between the two is assessed, the data suggest that women who have low 2/16 ratios may respond positively to exercise training with an increase in 2/16. This result may suggest that those women incur a lower risk for breast cancer since higher levels of 2OHE1 have been associated with decreased breast cancer risk. In animal tumor models, increased 20HE1 results in reduced tumorigenesis. In contrast, 16OHE1 has potent estrogenic effects and has been reported to be elevated in normal and malignant tissue from

women with breast cancer. This finding suggests one mechanism whereby physical activity that promotes weight loss may reduce breast cancer risk.

- Although IGF-1 levels can be nutritionally modulated and have been shown to decrease with weight loss or energy deprivation, when weight loss is combined with exercise training, no changes in circulating IGF-1 result. This may represent the result of competing influences of energy deprivation and exercise training. These findings suggest that the observation of reduced risk of breast cancer due to physical activity with weight loss is likely not due to declines in this circulating biomarker.

- The Dried Blood Spot technique represents a viable alternative to determining metabolic status in humans.

- Although a moderate exercise and diet program promoting significant weight loss may alter breast cancer risk for some premenopausal individuals through its effects on estrogen metabolites that have been associated with risk, these positive lifestyle changes do not appear to reduce breast cancer risk in premenopausal women age 25-40 yrs as it is indicated by circulating IGF-1, estradiol, or estrone.

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APPENDICES: None