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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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Effects of moderate aerobic exercise combined with caloric restriction on circulating estrogens and IGF-I in premenopausal women

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This study will test whether a program of moderate aerobic exercise that is combined with a moderate level of dietary restriction will result in significant decreases in two biomarkers of breast cancer, circulating estrogens and IGF-I. We will also test whether this type of diet/exercise program will produce significant reductions in the peripheral production of estrone by adipose tissue through its impact on the reduction of fat mass. Lastly, validation studies for a novel method of assessing energy status will be performed. To date we have completed our first year of the three year study protocol. Our cohort for Year 1 consisted of 20 women (25-35 yrs) divided into high (26-30 kg/m²) and low BMI (21-25 kg/m²) exercise groups. Aerobic exercise training was 4 times per week for four consecutive menstrual cycles at 77±3 % of maximum heart rate for 40-60 minutes, resulting in an average of 22% increase in aerobic capacity as defined by VO₂ max (P < 0.05 pre vs post). Dietary intake was successfully reduced using the food exchange system (Low BMI = 1889 ± 354 to 1214 ± 239 kcales; High BMI = 2125 ± 287 to 1450 ± 264 kcales). The combination of moderate exercise and diet produced significant weight loss in both groups (Low BMI –X%; High BMI –X% P < 0.05). Significant changes in body composition occurred in both groups (Low BMI 32 ± 4 % to 25 ± 8 %; High BMI 39 ± 5 to 32 ± 6 %; P < 0.05). As expected, menstrual cycle length in these reproductively mature women did not change significantly with training and weight loss. We are currently conducting biochemical determinations of urinary estrone-3 glucuronide and pregnanediol-3 glucuronide, LH, and FSH, and serum IGF-I, Insulin, T3, Leptin, IGFbp-1, estrone and estradiol. Data reduction and analyses are being completed for dietary intake, energy expenditure, resting metabolic rate. We will begin recruiting for our Year 2 cohort in December, 2002.
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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" will provide important scientific contributions with respect to the primary prevention of breast cancer in women. Specifically, this study will examine potential mechanisms relating to the role of physical activity in the reduction of the risk of breast cancer by 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 may modulate circulating levels, we wish to extend previous findings from epidemiological and cross-sectional studies by performing a tightly controlled, prospective clinical study that addresses 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 therefore 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 reduced 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 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 centers on the introduction of a novel approach to estimating energy status by measuring metabolic hormones in plasma, insulin, IGF-I, IGFBP-1 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 insulin, IGF-I, IGFBP-1 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 utilizes a prospective, randomized design that tests 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%-30% kcals (Figure 1). Previously sedentary, eumenorrheic women aged 25-35 years will be assigned to exercise or control groups. Both normal weight (BMI 21-25 kg/m²) and overweight (BMI 26-30 kg/m²) will be assigned to either exercise or control (no exercise, no dietary restriction) groups; 4 groups, n=15
Subjects will be 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:**

*Note: Human Subjects Approval obtained from DOD October, 2001*

**Proposed Month 1**
1. Recruitment of subjects for year 1 of study (n=5 in each of 4 groups)
   a. Place ads in local newspapers, on tv, radio, announce study on email lists, post signs
   b. Train research staff to take phone interviews
2. Get organized
   a. Make up subject notebooks with instructions for all aspects of study
   b. Prepare individual subject files and labels and storage for urine collection devices
   c. Meet with GCRC staff and review study procedures

**Actual Month 1: September, 2001**

Recruitment was delayed due to delay in human subjects approval. We prepared ads, trained staff to take phone interviews, drew up data forms, made subject notebooks, prepared subject files, and met with GCRC nurses, physicians, and dietary staff to set up procedures.

**Proposed Month 2**
1. Ongoing recruitment
2. Begin subject information sessions
3. Begin subject screening/initial testing

**Actual Month 2: October, 2001**

The subjects were recruited from the local Centre County, PA area using multiple advertising methods. Newspaper ads ran for one week in the local newspaper. Additionally, advertising incorporated flyers and ads on a local rolling news station. As a result, approximately 55 phone and e-mail contacts were made. Four one-hour “Informational Sessions” were held for the subjects to learn more about the study.

**Proposed Month 3**
1. Ongoing recruitment/information sessions
2. Continue subject screening/initial testing

**Actual Month 3; November, 2001**
Due to limitations in laboratory personnel, recruiting and sign-up was delayed and a “second phase” of recruiting was planned. Contact was maintained with potential participants through phone and email.

**Proposed Months 4-12**
1. Continue recruitment efforts only if necessary
2. Continue subject screening/initial testing
3. Complete subject exercise training/experimental testing

**Actual Month 4; December, 2001**

Due to limitations in laboratory personnel, recruiting and sign-up was delayed and a “second phase” of recruiting was planned. Contact was maintained with potential participants through phone and email.

**Actual Months 5 -6; January and February, 2002**

Second phase of recruitment began. We re-contacted our pool of initial contacts, and placed ads in the local newspaper again. We held two more information sessions, and then began study sign-ups. We signed up 20 individuals who were willing to complete the study during Year 1. We had difficulty signing up subjects who would serve as controls, i.e., subjects who would go through all the procedures in the study except receiving dietary counseling to have their food intake reduced and participating in the exercise sessions. Many individuals indicated upon phone screening that they would not participate if they weren’t going to lose weight and get in shape. Therefore, we decided to postpone the recruitment of control subjects until our Year 2 cohort. We plan to recruit subjects during Year 2 differently, so that can successfully sign-up women who will not exercise or have their calories reduced, and women who will participate in the diet and exercise groups. We will do this by increasing our recruitment efforts over a longer time period with more ads, and by placing ads that emphasize the other health benefits of the study besides exercise and calorie reduction.

**Actual Months 7-11; March-July, 2002**

We began exercise training and dietary counseling for several subjects in late February and March, after screening procedures had been completed, and documentation of normal menstrual cyclicity for two complete menstrual cycles with daily urine samples and corroborative testing for ovulation and adequate progesterone levels had occurred. The last woman completed exercise training and post-testing in July, 2002.

**Actual Month 12, August, 2002**

In August we began aliquotting urine samples from the subjects’ collections, preserving the urine samples, and measuring specific gravity. We also began data entry for initial survey and demographic data, daily training, weekly food exchanges, body weight, body composition, resting metabolic rate and diet and physical activity logs.

**Actual Month 13, September, 2002**

We continued aliquotting and processing urine samples and data entry.

**Actual Month 14, October, 2002**

We have completed data entry and are beginning preliminary data analysis. We began assaying urine for E1G and PDG, and are beginning to assay metabolic hormones, i.e., insulin, leptin, T3, and IGF-I. We are also preparing data reports for subjects that completed the study, and getting ready to recruit for Year 2.
Preliminary Results From Year 1:

Subjects: Our subjects were previously sedentary, eumenorrheic women (ovulatory menstrual cycles with circulating mid-luteal phase progesterone levels>5 ng/ml) 25-35 years old. They had the following characteristics: non-smoking; not using hormonal contraceptives for at least 6 months prior to the study; gynecological age greater than or equal to 13 years of age; no history of depression, disordered eating, or other affective disorders; no history of weight loss; no apparent disease; not aerobically trained (less than 1 hour a week of aerobic activity); weight stable (less than or equal to 2.3 pound change) in the last year; no medication incompatible with hormonal analyses, exercise or caloric restriction. Out of 30 subjects that signed informed consents, 20 subjects made it through the screening procedures and began the control phase of the study. The twenty women described their ethnicity as the following: 14 Caucasian, 3 Asian, 2 African-American and 1 Other.

Subject Attrition and Compliance: Nine women dropped out at various times during the study for the following reasons: 1 for menstrual abnormality, 5 medical (2 were exercise-related injuries), 3 self (time, intervention, etc.), and 2 for noncompliance. Compliance in the study was excellent, with women completing 3.9 out of 4 workouts per week, and over 85% of their scheduled visits (every other week) with the dietician. Subjects followed the diet as is evidenced by the significant weight loss achieved. Adherence to other testing procedures was excellent, and urine collections were completed with less than 3% of samples missing.

Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yrs)</th>
<th>Weight (kg)</th>
<th>Height (kg)</th>
<th>BMI (kg/m²)</th>
<th>% Fat</th>
<th>VO₂ max (ml/kg/min)</th>
</tr>
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<tbody>
<tr>
<td>Low BMI</td>
<td>31 ± 3</td>
<td>63 ± 6</td>
<td>165 ± 6</td>
<td>23 ± 2</td>
<td>32 ± 4</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>High BMI</td>
<td>32 ± 2</td>
<td>75 ± 8</td>
<td>165 ± 6</td>
<td>28 ± 2</td>
<td>39 ± 5</td>
<td>32 ± 6</td>
</tr>
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</table>

Values are mean ± SD

Subjects met our initial targets for weight, age, BMI and fitness levels. Average menstrual cycle length was 29.7 ± 5 days, and did not change significantly in either Low or High BMI group. Aerobic exercise training was 4 times per week for four consecutive menstrual cycles at 77 ± 3 % of maximum heart rate for 40-60 minutes, resulting in an average of 22% increase in aerobic capacity as defined by VO₂ max (32.6 ± 4.7 to 40.0 ± 10 ml/kg/min; P < 0.05 pre vs post, Figure 2). Dietary intake was successfully reduced using the food exchange system (Low BMI = 1889 ± 354 to 1214 ± 239 kcals; High BMI = 2125 ± 287 to 1450 ± 264 kcals; P< 0.05 pre vs post in both groups). The combination of moderate exercise and diet produced significant weight loss in both groups (Low BMI -3.3 %; High BMI -7.6% P< 0.05, Figure 3). Significant changes in percent body fat occurred in both groups (Low BMI 32 ±4 % to 25 ± 8 %; High BMI 39 ± 5 to 32 ± 6 %: P < 0.05, Figure 4).

![Changes in VO₂ Max](image)

Figure 2. Changes with exercise training in VO₂ max (ml/kg/min)
Figure 3. Weight loss with diet and exercise over four menstrual cycles

Figure 4. Percent body fat loss with diet and exercise over four menstrual cycles

Preliminary Analysis of Urinary Steroid Metabolites

Figure 5 shows the profiles generated by our assays for E1G and PDG, urinary metabolites for estrogen and progesterone, respectively. We should complete these assays for our Year 1 subjects, in addition to those for metabolic hormones insulin, T3, leptin, IGF-I, and IGFBP-1 in the next two-three months.
Figure 5. Urinary steroid metabolites for an individual subject during a control cycle and then four menstrual cycles thereafter where caloric restriction and exercise training occurred.

Overall Results from Year 1:

We achieved excellent results from our dietary and exercise intervention. Our only anticipated changes for Year 2 include an expanded recruitment period, and a change in recruitment strategy to attract women who will agree to participate without the diet and exercise intervention. To do this we will structure the advertisements to highlight other aspects of the study from which subjects will benefit. These include diet counseling for non-weight loss purposes. We have developed several educational modules on nutrition topics of interest that do not advocate weight loss. Additionally, we will draw attention to the benefits of learning about one’s body composition, fitness level, and reproductive status.

Key Research Accomplishments:

-not applicable at this point in time

Reportable Outcomes:

Presentations:
The following presentations have resulted from work in N. Williams’ laboratory, since funding from DOD. Note: Funding from DAMD17-01-1-0361 was used to directly support only presentation #5.


2. 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


Publications:
The following publications have resulted from work in N. Williams' laboratory, since funding from DOD. Note: Funding from DAMD17-01-1-0361 has not directly supported these projects.


**MANUSCRIPTS IN PRESS**


Whipple TJ, Petit Moira, Sharkey N, Demers L, Williams NI. Leptin and the skeleton. (*Accepted, Clinical Endocrinology for publication in November, 2002 issue*)

**MANUSCRIPTS IN REVIEW**


Conclusions:

See Overall Results from Year 1.

References - none

Appendices - none