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PRINCIPAL INVESTIGATOR: Ruth K. Peters, Sc.D.

CONTRACTING ORGANIZATION: University of Southern California
Los Angeles, California  90033

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# The Effect of Moderate Aerobic Exercise Training Program on Ovarian Function

There is substantial evidence to suggest that estrogens play a key role in the etiology of breast cancer. Both cross-sectional studies of highly trained athletes and prospective studies of high intensity exercise training programs have found a higher frequency of anovulation, lower levels of estradiol and in some cases a shortened luteal phase length with associated lower estradiol levels among these women. However, little is known about the effects of moderate intensity exercise on ovarian function. The aim of this study was to investigate the relationship between a moderate intensity exercise training program and ovarian function. Specifically, we aimed: 1) to determine whether changes occurred in frequency of ovulation as a result of a 6 month exercise training program, 2) to determine whether changes occurred in serum E2 levels in ovulatory and anovulatory cycles in these women, and 3) to determine the luteal phase menstrual cycle lengths of these women as a result of the training program. We plan to have completed data collection on 72 women by February 1, 2000. The preliminary analysis for this study is currently underway.
FOREWORD

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Ruth K. Peters  10/12/99

PY - Signature  Date

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The Effect of a Moderate Aerobic Exercise Training Program on Ovarian Function (Year 3 Annual Report)

Section 5: INTRODUCTION

Breast cancer is the most common serious cancer occurring in American women. As a cause of death among women, breast cancer ranks second only to lung cancer [1]. On the basis of current incidence rates, one in nine women will be diagnosed with breast cancer in her lifetime [1].

There is substantial experimental, clinical and epidemiological evidence that ovarian hormones, particularly estrogens, play a major role in breast cancer risk. Studies have shown that lower levels of estrogen are associated with a reduced risk of disease [2,3].

The study described here will generate new information about the influence of exercise on ovarian function in non-athletes. By beginning the process of establishing how much exercise is needed to reduce estrogen levels, we hope to be able to provide practical advice to women on how to reduce their breast cancer risk.

Hormones and breast cancer risk:

A great deal of evidence exists demonstrating that ovarian hormones, in particular estrogens, play a major role in breast cancer risk [2,3]. The age-incidence relationship of the common non-hormone related cancers such as stomach and bladder shows a continuous steady increase with age. In contrast, breast cancer incidence increases steadily and rapidly with age until about age 50 (average age at menopause) at which time the rate of increase slows dramatically [2]. Direct epidemiological study of the effect of age at menopause shows that for each year a woman's ovaries continue to function there is a 10% increase in her subsequent breast cancer risk [1,2,4]; this is true whether the menopause is natural or artificial (bilateral oophorectomy). The decline in the rate of increase in incidence around age 50 is thus directly correlated with the markedly reduced serum levels of estrogen (and progesterone) after menopause.

Ovulating women in low breast cancer risk Asian countries have been shown to have lower levels of circulating estrogens than women in the US and the UK, both high risk countries [5]. Postmenopausal breast cancer cases have been found to have higher serum estrogen levels than controls [3]. Studies, which paid strict attention to factors which may influence hormone levels in cases, found statistically significant elevated serum levels of estradiol in premenopausal breast cases compared to controls [5].

Estrogen is presumed to increase risk of breast cancer through its known action as a breast cell mitogen [2]. Higher levels of endogenous estrogen would be expected to increase mitotic activity. Both follicular and luteal phase estradiol (E2) are of interest; the breast cell proliferation rate in the follicular phase is some 50% that in the luteal phase and so E2 levels in both phases are important [2]. Progesterone (Prg) also acts to increase breast cell proliferation. We are making measurements of both E2 and Prg.

Exercise and breast cancer risk:

A survey of surviving Harvard female college athletes found that the athletes had a 46% reduction in prevalence of breast cancer compared to non-athletes (24/2622 vs. 45/2776; 2 sided \(P=0.05\)) [6]. A Finnish cohort study showed that physical education teachers had a 19% lower risk of breast cancer than language teachers, but the results were not statistically significant (22/924 vs. 106/3239; 2-sided \(P=0.21\)) [7]. The NHANES I cohort was reported as showing no overall relationship between exercise level and breast cancer risk, but the questions asking about exercise activity had no duration component and the study has to be considered non-informative [8].

We completed a case-control study of 545 young (age 40 or younger) breast cancer cases and 545 control women matched for age, race, parity and neighborhood of residence [9]. The
daily average lifetime (post menarche) number of hours spent in exercise activities was a significant predictor of reduced breast cancer risk (2-sided P<0.0001). Compared to inactive women, risk of breast cancer was reduced by 27% in women who exercised on average 2.5 hours per week, and was reduced by 58% in women who exercising 4 or more hours per week (average approximately 60 mins/day).

**Exercise and reproductive function:**
We believe, based on our understanding of the relation of ovarian hormones to breast cancer risk [2,3], that the observed protective effect of exercise against breast cancer is likely to be due to a reduction in exposure to serum estrogen. Reduced serum estrogen levels may be due to an increased frequency of anovulatory cycles and/or to decreased circulating levels of estrogen in ovulatory cycles. E2 is the most important estrogen and we have concentrated our attention on E2 in this study [2,3]. Cycles with long follicular phase are associated with lower than average cumulative E2 exposure since such cycles have an increased number of days with early follicular phase low E2 levels. Cycles with short luteal phases have also been found to be associated with low E2 values [10].

**Training studies:**
The studies discussed above compared groups of women who were self selected on exercise level. Some or all of the effects may, therefore, be due to other aspects of their lifestyles or to genetic factors that are strongly correlated with exercise activity. Furthermore, the validity of self-reported responses in cross-sectional studies are of concern. The strength of this prospective training study lies in the ability to structure and monitor the type, intensity and duration of exercise without having to rely on second-hand reports.

Except for a study by Shangold et al. [11] of a single individual, we have identified only four exercise training studies of exercise and ovarian function under a controlled protocol which included pre- and post-training testing, all were conducted using very few subjects. The first of these studies was conducted by Boyden et al. [12] among 19 women who had previously engaged in informal running (average, 15.1 mi/wk) and were trained rigorously to run a full marathon over a 14 to 15 month period. Each subject had blood samples taken at baseline, after their weekly mileage had increased by 30 mi/wk and again when weekly mileage had increased by 50 mi/wk. The mean plasma mid-follicular E2 values were 76% of the baseline values after a weekly mileage increase of 30 mi/wk and 48% at 50 mi/wk (2-sided P=0.03). However, Prg values were not obtained during the test cycles, and it is, therefore, unclear as to whether the E2 values reported occurred within ovulatory cycles.

In a study by Bullen et al. [13], 7 young women with prior athletic experience were trained at a high-intensity. The training protocol consisted of cycle ergometry 2 days/wk and running 4 days/wk at exercise intensities eliciting 85% of maximum heart rate. The duration of high-intensity activity was increased from 20 mins/session to 45 mins/session over a 4 week period. Subjects trained at 45 mins/session for the remaining 4 weeks of the study. All of the cycles appeared to have been ovulatory, as evidenced by midcycle surges of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Diminished urinary estriol (E3) levels were observed in 4 of the 7 subjects. Serum E2 levels were reported to have not changed appreciably, however no quantitative values were offered in the published report.

In a subsequent study of Bullen et al. [14], 28 initially untrained women with documented ovulation (urinary LH surge) were studied to determine whether strenuous exercise spanning two menstrual cycles would induce menstrual disorders. Initially, subjects ran 4 mi/day and increased their training regimen to 10 mi/day by the end of week 5 and continued to run 10 mi/day for the remaining 5 weeks of the study. Subjects ran at 75% to 80% of maximum heart rate. Only 4 of 28 subjects (14%) had a normal cycle during one or both periods of exercise training. The criterion for normalcy included “a biphasic temperature curve, an
ovulatory pattern of changes in gonadotropin and sex steroid excretion, and normal luteal function, defined as excretion of free Prg in a characteristic parabolic curve between the LH surge and beginning of the following menses”. Keizer et al [15] assessed the effect of a 12 week endurance training program on plasma hormone responses among 8 previously untrained women. The training program consisted of running (2-3 times/week) and cycling (once/week). The training duration and intensity was progressively increased from a mean running speed of 9 km/hr (approx. 60% VO₂ max, equivalent to 65% of maximum heart rate [16]) and 20 min/day to 11-12 km/hr (approx. 80-85% of maximum heart rate) and 50-75 min/day. After training, follicular E2 values were 58% higher. However, the mean E2 value in the luteal phase was 57% (2-sided P<0.01) of the pre-training value. All subjects were reported to have ovulated in the pre-training test cycle. Based on pre- and post-training levels of Prg in the luteal phase (22.3 +/- 4.9 nmol/l, 20.8 +/- 0.4 nmol, respectively), all subjects were reported to have ovulated post-training, however, individual values were not reported and the data remain inconclusive. It is unclear when post-training measurements were obtained.

In summary, there is some evidence to suggest that high intensity exercise training programs alter ovarian function and are in agreement with cross-sectional studies. There have been no reported studies which assess the effect of a moderate exercise training program on ovarian function in previously sedentary women [16].

In this proposal, we are conducting a modified version of the above described studies by integrating elements of each study to meet our standards of a moderate intensity exercise training program in which previously inactive women can reasonably be expected to partake in. We are enrolling each subject in a 6 month moderate exercise training program. Developing a long-term exercise training program allows us to assess the chronic effects of a moderate intensity exercise program on ovarian function. Furthermore, we will have a substantially larger sample size than in other training studies. We have restricted the protocol to aerobic exercise training. This restriction allows us to standardize the form and intensity of exercise for all subjects. We are able to monitor each participant and adjust their workload to maintain a previously set training level (see Methods). We will be able to assess the specific effects of aerobic activity with changes in E2 and Prg. In addition to determining changes in ovarian hormone levels, we will assess changes in luteal phase length and frequency of anovulation. These latter parameters have not been assessed in a prospective training study and will add significantly to our understanding of the effects of moderate exercise on ovarian function.

Section 6: BODY
Hypotheses
The hypotheses of this study are:
1) Frequency of ovulation will be reduced as a result of a 6 month aerobic exercise training program of moderate intensity.
2) Serum estradiol (E2) levels will be lower as a result of a 6 month aerobic exercise training program of moderate intensity.
3) Luteal phase menstrual cycle lengths will be shorter as a result of a 6 month aerobic exercise training program of moderate intensity.

Procedures
A prospective study has been undertaken to assess the effect of a 6 month moderate intensity exercise training program on basal hormonal levels among previously sedentary premenopausal women. In this study, we are collecting blood and urine specimens (baseline, after 14 weeks on the training program and near the end of the 6 month training program) and questionnaire data from women who agree to participate in a 6 month exercise training program.
Subject Selection

Interested female participants are asked to complete a brief screening survey. The screening survey is intended to identify subjects who meet our criteria of inclusion. All study participants must meet the following criteria:

* nulliparous
* 18 to 35 years of age
* free of underlying diseases or conditions that may interfere with the measurement of hormone levels and/or the interpretation of hormone data
* have not used hormonal contraceptives over the past six months and not planning to over the course of participation in the study
* average menstrual cycle length between 15 and 45 days
* no regular exercise over the past 6 months
* BMI value between 20 and 30 kg/m²
* no dieting over the past 6 months
* no smoking over the past 6 months

These criteria are set to reduce the impact of confounding variables which may be associated with altered ovarian function. We contact all interested women and review the responses provided on the screening survey to confirm eligibility. We then ask selected participants to notify us of the first day of their next menstrual cycle. At that time, subjects are asked to meet with us at a specified location, at a mutually convenient time to sign to an Informed Consent, to pick up a study kit, and to receive the questionnaire (described below).

Training Protocol:

A 6 month endurance training program (see Table I) at the Spectrum Club Manhattan Beach and the downtown YMCA has been undertaken. We have chosen to conduct this training program over an extended period of time in an effort to determine the effects of a long-term exercise training program of moderate intensity on ovarian function, the effects of which are presently unknown. Subjects report to the study site and engage in a monitored aerobic exercise training program (weight lifting or cross training are not incorporated into the exercise prescription) for a total of 3 hours per week. The participants begin their training at 50% of their maximum heart rate for 20 minutes per session. Their training regimen is increased gradually to 60 minutes per session while they are at 65% of their maximum heart rate (see Table II).

<table>
<thead>
<tr>
<th>Table I - Protocol for Individual Participation</th>
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<td>Study Month</td>
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<td>2-3</td>
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<td>4</td>
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<tr>
<td>4-7</td>
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<tr>
<td>7</td>
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</table>
Table II - Training Protocol

<table>
<thead>
<tr>
<th>Week</th>
<th>Time (min)</th>
<th>% max. heart rate</th>
<th>% VO2 max.</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>40</td>
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<td>8</td>
<td>60</td>
<td>65</td>
<td>50</td>
</tr>
</tbody>
</table>

Over the last 4 months of the study, each subject exercises for an hour each session at about 65% of her maximum heart rate. Initially, this was to be determined by individualized VO2 max testing [16]. Corresponding heart rates were to be calculated at the time of the VO2 max test. The only machine available to determine VO2 max was located at the Spectrum Club headquarters in West Los Angeles and the availability of this machine was limited for research purposes. We therefore defaulted to a more general approximation for determining VO2 max using a standard equation to determine maximal oxygen uptake. This equation, the Karvonen equation, is maximum heart rate = [220-age]. Using this equation we were able to establish a starting point. Since training effects are expected to occur over the 6 month time period, staff assist participants in adjusting their exercise protocol to coincide with 65% of their maximum heart rate.

Data collection:

Height and weight and percent body fat (months 1, 4 and 7): Subjects are weighed without shoes or over-sweaters. A Gopher G82-419 weigh balance scale is used to weigh each subject. The scale is re-calibrated daily. Measurement of height is performed immediately after weighing, and is done without shoes. Body fat is measured using skin calipers at hip, waist and arm. These measurements are repeated and must come within 5% of the comparison measure or a third set of measurements are made. A member of the study team or a personal trainer from the health club performs the measurements.

Questionnaire (month 1): Each participant completes a structured questionnaire. This questionnaire was designed by combining other questionnaires developed by Dr. Bernstein in her previous studies of exercise activity and risk of breast cancer, and of the effect of exercise activity on menstrual patterns in adolescents, as well as questionnaires developed by Dr. Paffenbarger and his colleagues on physical activity [17-19], and Dr. Willett and his colleagues on diet [20-22]. It is important to collect detailed information on past exercise and dietary patterns since these may potentially influence the outcome measurements of this study. The main study questionnaire includes basic demographic questions (age, race and socio-economic class based on education of parents), age at menarche, family history of cancer, and use of tobacco and alcohol. We are collecting this additional information to be used as covariates in analyses regarding the effects of an exercise training program on ovarian function (see Statistical Analysis).

Diet is assessed with a slightly modified version of the Semi-quantitative Food
Frequency Questionnaire (SFFQ) developed and validated by Dr. Willett and his colleagues [20-22]. The SFFQ was designed with the objective of categorizing individuals by their intake of nutrients hypothesized to affect the occurrences of cancer and heart disease. In its original form, the SFFQ consists of a 4-page printed Diet Assessment which can be mailed to subjects, and, when coded in pencil, provides machine-readable data. This form asks respondents how often they usually consumed a specified portion of 116 foods and drinks (over the previous year) with 9 response categories ranging from less than once a month to 6 or more servings per day. Additional items not presented in the frequency format include the types of fat used for frying and cooking, the type of margarine used, the amounts of bran and sugar added to food, usual brands of cold breakfast cereal, frequency and brand of multiple vitamin supplements and the doses of iron, zinc, and calcium. We have modified the original questionnaire in consultation with Dr. Willett by adding a list of 24 additional foods commonly consumed in Southern California. The main aim of collecting these data will be to investigate whether diet is a confounder of any effects found with exercise.

Daily records and menstrual calendars (months 1-7): Sedentary subjects are recruited and it is required that they avoid making any substantial changes in lifestyle habits (e.g. diet, smoking, drinking and exercise -- aside from the training study). However, some occasional recreational or other activity may occur. For this reason, subjects were originally requested to record all physical activities over the previous 24 hours not included in the training program (i.e., recreational activity, occupational activity and daily routine) in daily logs over the entire study – this proved difficult for participants to accomplish and we subsequently restricted our efforts to ensuring that this was done in hormone collection months. Additionally, participants are asked to maintain menstrual cycle calendars over the duration of the study. These calendars are included in the study kit. On the calendar, each participant records each day of menstrual bleeding for each menstrual cycle that occurs by circling the appropriate dates. These calendars are used to determine menstrual cycle lengths and to monitor menstrual cycle frequency over the duration of the study. Attendance and activity at the gym of each participant is monitored with a membership ID at the front desk and recorded by a member of our research team.

Heart rates: During the course of the 6 months training program, heart rates are self-monitored using an electrode chest belt with a corresponding wrist display to allow continuous monitoring of heart rate. Research assistants are present at both sites. The wrist alarms alert the assistants to necessary changes in workloads so that subjects may maintain their exercise level at 65% of their maximum heart rate.

Biological collections: Biological specimens (urine and blood) are collected at baseline (month 1), at midpoint (month 4) and during the final month of training (month 7).

Urine collection: During the collection cycles, daily urine samples beginning on cycle day 10 and continuing until the first day of the next cycle are collected from each participant. Plastic bottles for urine collection are provided. Accompanying the bottles is a list of directions instructing the subject to collect a 30 ml sample of first morning urine and specifying procedures to follow. Each participant's progress is monitored and reminder phone calls are made to check on the study protocol and answer any questions that may have arisen. Subjects notify us as to the start of their next menstrual cycle. Each participant is telephoned the night prior to the first scheduled urine collection as a reminder, and to answer any questions they may have regarding urine collection. At least once a week, subjects are required to deposit their daily urine specimens at the health club for processing. Samples are picked up daily by Dr. Shames or a research assistant and stored on the medical campus at -20°C until analyzed.
Each day's collection for each subject is identified by a 8 digit alpha-numeric ID (Axxxx-xx-x) and date of urine collection. The first 5 digits of the ID represent the participants unique ID number (A1001-....) and is assigned chronologically at the time of enrollment. Digits 6 and 7 represent the day of the menstrual cycle for the particular sample. The final digit is the cycle number.

Serum collection: During the collection cycles, subjects are asked to provide from 2 to 5 15 ml blood samples (depending on the length of their cycle). Blood is taken at the health club on cycle days 11 (±1) and 22 (±1) [and subsequently on days 29 (±1), 36 (±1) and 43 (±1), in the event menses has not occurred]. Most subjects need to provide only 2 or 3 samples. Allowance is made for a one day variation to account for samples which may fall on a weekend and for unavoidable conflicts. Subjects must report to the gym between 7:30 AM and 9:30 AM (on the day of their scheduled appointment) in a fasting state and have refrained from exercise activities for at least 5 hours. Participants are phoned the evening prior to their appointments as a reminder. Blood specimens are processed into serum and stored at -20°C for analysis. Sterile 2 ml polypropylene low temperature freezer vials are coded with the same coding system as described above for urine, plus an additional digit for aliquot number.

Progress (Year 1):
We devoted an enormous amount of time and effort in year 1 to the advertisement and recruitment for this study. As indicated above, our study criteria are quite rigorous and only very select women meet these criteria.

We began our recruitment efforts by placing fliers within a one mile radius of the designated study sites. We placed fliers at grocery stores, video stores, movie theatres, shopping malls and various other local shops. Additionally, we attended local health fairs and posted signs in several of the larger engineering and computer firms (e.g., Xerox, TRW, Hughes Aircraft, Mattel, Aerospace).

As a study incentive, we arranged to provide a 6 month health club membership at the health clubs at no cost to the subject. At the end of the training study, each participant who had fully completed the study, receives a reduced membership rate to continue use of these facilities and is compensated with $160.00 for their time, effort and transportation costs.

During this year 59 participants met our criteria and were enrolled in the study. At the end of Year 1, 6 participants had completed the study, 26 were currently exercising, and 7 were in the baseline phase. The remaining 20 participants had dropped out of the study.

Progress (Year 2):
We enhanced our strategy for recruitment during the second year of this study. We began placing ads in the health and calendar sections of local newspapers and cable television stations to increase our range of exposure. We secured spots on 4 area cable stations and 9 newspapers covering a range from as far east as Pasadena traveling west through Santa Monica and downward into the South bay. We were featured in the downtown news, participated in the downtown health fair and spoke to several of the large businesses in the area. We also found it useful to set-up display tables at local health fairs, shopping malls, and other highly visited sites. Several participating subjects suggested this study to friends and colleagues.

Progress (Year 3):
The original protocol called for stationary bicycling only. Due to complaints of boredom from many of the participants, we extended the type of activity to include other forms of aerobic activity such as the treadmill, stairmaster and aerobic classes. We implemented this change with the first exercising subject and have continued to utilize this slightly modified protocol.
Frequency, intensity and duration of these activities has not been altered and participants are required to wear a heart monitor to record intensity and duration of activity. To account for vacation or sick leave, we added additional weeks to the program, accordingly, to ensure each subject has participated for 24 weeks.

As outlined in our year 2 progress report, we indicated an underestimation of the difficulty in recruiting for a 6 month exercise training study in part, due to our rigorous inclusion criteria. Additionally, we reported a 50% drop out rate - higher than the 30% we had expected. We found that a number of subjects continued to participate through the 3 or 4 month mark and were lost to the study shortly thereafter. We requested IRB approval to conduct a midpoint measurement (defined as the next menstrual cycle beginning after the completion of 14 weeks of exercise). We received IRB approval for this addendum on October 12, 1998 and incorporated this additional 4 month blood draw and urine collection shortly thereafter. The addendum has provided useful information on subjects lost to follow-up over the originally planned 24 weeks.

4 month measurement statistics
21/40 completed mid point measurement.
19/40 pending.

Data management:

The day-to-day tracking of participants has been managed on an IBM compatible PC using EXCEL. Using this database, we can effectively monitor participants accrual, assessment responses, gym attendance and track all data collection throughout the study (i.e., questionnaire data, physical measurements, daily physical activity logs, urine and serum collection appointments, etc.).

Both the initial screening questionnaire and the study questionnaire have been coded and entered into an EXCEL file. Height, weight, and body fat measurements have been coded and entered into an EXCEL file as well. The completed Diet Assessments are checked for stray marks and completeness of coding and will be sent to Dr. Willett for analysis [34-36].

We strictly maintain the confidentiality of data through use of locked cabinets accessible only to employees directly involved in the study who have signed an employee confidentiality form. Computer-stored information has only the study identification number to ensure security. We will publish results from the study in tabular descriptions of groups or in a form which precludes identification of specific individuals.
## Participation statistics as of 10/11/99:

<table>
<thead>
<tr>
<th>Total screened</th>
<th>Total eligible/accepted AND began biological Collections</th>
<th>Total drop out</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1,500</td>
<td>138</td>
<td>66</td>
</tr>
</tbody>
</table>

(responded to ads, fliers, lectures, word-of-mouth, health fairs)

Total completed to date 35  
Pending completion 37  
Estimated completed by February 1, 2000 72  

**Enrollment statistics for Year 1**

Completed study 6  
Currently exercising 26  
Baseline Phase 7  

**Enrollment statistics for Year 2**

Completed study 20  
Currently exercising 20  
Baseline Phase 0  

**Enrollment statistics for Year 3**

Completed study 9  
Currently exercising 37  
Baseline Phase 0
Section 7: Key Research Accomplishments  
Pending analyses.

Section 8: Reportable Outcomes  
Pending final report.

Section 9: Conclusions  
Pending analyses.

Section 10: References  
See attached.

Sections 11-13: Appendices, Binding and Final Report  
Not applicable at this time
BIBLIOGRAPHY