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13. SUPPLEMENTARY NOTES					
14. ABSTRACT This project is a traineeship for the Primary Investigator's doctoral training and encompasses a training and research plan in breast cancer research. The PI is being mentored by three prominent researchers with expertise in multiple areas of breast cancer research: coordination and execution a highly-controlled breast cancer prevention feeding trial by Susan Raatz, PhD, R.D.; exposure to clinical oncology and clinical application of the results of the proposed research project by Douglas Yee, M.D.; and laboratory analysis of sex hormones and dietary prevention of breast cancer by Mindy Kurzer, PhD. All required course work for the PI was completed in December of 2007. The PI passed the oral preliminary examination and the written preliminary examinations prior to September 15, 2007. The purpose of the proposed dietary intervention trial is to determine the effects of type and amount of dietary fat on sex hormone metabolism, eicosanoid balance, and breast cancer risk in postmenopausal women. The study objectives are to: 1) evaluate the effects of total fat and omega-3 fatty acid intake on plasma and urinary sex hormone and urinary eicosanoid levels; 2) determine the relationships among plasma fatty acids, urinary prostaglandin E₂, plasma and urinary sex hormones, and plasma insulin, insulin-like growth factor, and insulin-like growth factor binding proteins. The controlled feeding trial portion of the study is currently proceeding. To date 142 subjects have been screened via telephone, 32 subjects have been screened in the clinic, and 24 subjects have been enrolled in the study. Sixteen subjects have completed all parts of the feeding trial. Plasma sex hormones were analyzed by radio-immuno assay for the first 10 subjects to complete the trial. Preliminary data from this analysis was used to generated two abstracts that were presented at the DOD 2008 Era of Hope meeting. No manuscripts have been generated to date.					
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Introduction

The training program funded by this proposal is an innovative combination of mentoring in execution of human clinical trials, training in nutritional sciences research and dietary prevention of breast cancer, and clinical mentoring by an oncologist. These aims support the PI's goal to become a clinical researcher in breast cancer prevention. The human feeding trial in this project, "Dietary Fat, Eicosanoids, and Breast Cancer Risk", is a dietary intervention aimed at evaluating the effects of total fat intake and omega-3 fatty acids on breast cancer risk markers in postmenopausal women. This will study take place at the General Clinical Research Center (GCRC) at the University of Minnesota. Under the guidance of Susan Raatz, PhD, RD, the PI will recruit and screen subjects, manage data, and attend subject visits to the clinic. The PI will assist in the analysis of biological samples with trained technicians at the GCRC core laboratory and in the lab of Mindy Kurzer, PhD. The data resulting from this project will be the basis of the PI's PhD dissertation.

Body

This project is proceeding along the timeline outlined in the approved Statement of Work. The PI has been coordinating the proposed study by advertising for and screening subjects, managing data, ensuring proper study meal preparation, communicating with subjects and ensuring subject compliance. Advertisement for the study is extensive and includes print flyers and email announcements distributed on the University of Minnesota campus and print ads in local newspapers including the "Minnesota Women's Press" and the "Star Tribune". Word of mouth was also useful for recruitment. Subjects enrolled on the study have been quite compliant with picking up and consuming study meals for the dietary intervention. However, some potential participants found the meal pick-up to be too large of a commitment because of the time and expense involved in picking up the meals and parking on campus, which hindered recruitment of study subjects. In future studies it would be advisable to alter the protocol to reduce the number of days subjects need to pick up meals. Also, another restriction that made recruitment difficult was exclusion of post menopausal women on hormone replacement therapy or women who have had a bilateral oophorectomy, but these exclusion criteria were unavoidable because of the sex hormone endpoints for the study. Also, the exclusion criteria of women on anti-inflammatory medications or lipid altering drugs made recruitment more challenging, but again these were necessary criteria to measure eicosanoid balance and plasma fatty acids in response to the diets. The diets were largely well-accepted by the participants, however some small substitutions in the diets were made on a case-by-case basis to increase compliance. An example would be substituting canned peaches for fruit cocktail, which the subject preferred and as long as there was minimal difference in dietary composition of the foods. Many of the subjects appreciated the high quality of the meals and the convenience of having meals prepared for them. Subjects were largely compliant with the protocol. Pitfalls in data collection include adequately informing subjects regarding the 48-hour urine collection for sex hormone metabolite measurements. Otherwise, data collection proceeded smoothly. To date 142 subjects have been screened via telephone, 32 subjects have been screened in the clinical research center, and 24 subjects have been enrolled in the study. Sixteen subjects have completed all aspects of the feeding trial to date. The final subject in the study is anticipated to complete the study by January of 2009. Analysis of biological samples will follow in January/February of 2009.

The PI has shadowed Douglas Yee, M.D., on clinical rounds and attended weekly discussion sessions with oncologists, radiologists, surgeons, and pharmacists to discuss breast cancer case studies. The PI largely observes the multidisciplinary meeting, but it has been a good avenue to become familiar with many doctors and researchers from the Cancer Center at the University of Minnesota and the PI could potentially conduct post-doctoral research in one of their labs.

The PI has assisted in organizing and executing the analysis of plasma sex hormones by radioimmunoassay for the first 10 subjects to complete the study. Analysis took place in the lab of Mindy Kurzer, PhD, with the assistance of trained technicians. Appendix 1 and 2 include abstracts that outline these research findings. Briefly, plasma estradiol (E₂), estrone (E₁), estrone sulfate (E₁-S), testosterone (T), androstenedione (AS), sex hormone binding globulin (SHBG), follicle stimulating hormone (FSH), dehydroepiandrosterone (DHEA), and

dehydroepiandrosterone sulfate (DHEAS) were analyzed by radio-immunoassay for 10 participants. SHBG levels were significantly increased at 8 weeks with low fat high omega-3 diet (LFn3) compared to low fat diet (LF) ($p < 0.05$), and there was a trend for decreased DHEAS level at 8 weeks with LFn3 compared to LF ($p < 0.15$). A trend for increased E_2 was observed with high fat diet (HF) compared to both LF and LFn3 at 8 weeks ($p < 0.15$). A trend for decreased E_1 and FSH was observed from baseline to 8 weeks with LFn3 ($p < 0.15$). No statistically significant differences were observed between treatments for AS, T, E_1 -S, or DHEA. These preliminary results suggest that LFn3 alters estrogen metabolism in a direction associated with reducing breast cancer risk in postmenopausal women. These results are preliminary, but if they hold up in the final analysis, recommendations could be made to reduce dietary fat and increase consumption of omega-3 fatty acids in order to reduce one's risk of breast cancer.

Urinary hormones have not yet been analyzed. All remaining biological samples will be analyzed beginning in January/February of 2009, directly after the final subject completes the study.

Plasma fatty acids and urinary prostaglandin E_2 and thromboxane B_2 have not yet been analyzed. These samples will be analyzed when the final subject completes the study.

No manuscripts have been generated to date.

Key Research/Training Accomplishments

- PI passed preliminary written and oral examinations, leading to doctoral candidate status.
- PI completed the remainder of necessary coursework in December of 2008.
- Recruitment for the study closed in March of 2008. The final subject is anticipated to complete the study in January of 2009.
- Analysis of plasma sex hormones has been completed for the first 10 subjects to complete the study. These data showed that a low fat high omega-3 fatty acid diet alters sex hormone metabolism in a direction of reduced breast cancer risk after 8 weeks consumption.

Reportable Outcomes

Data presentation

- Abstract (see Appendix 1) from The Era of Hope Department of Defense Breast Cancer Research Program Meeting, June 25-28, 2008 in Baltimore Maryland. Presenter: Lindsay Orr
- Abstract (see Appendix 2) from The Era of Hope Department of Defense Breast Cancer Research Program Meeting, June 25-28, 2008 in Baltimore Maryland. Presenter: Susan Raatz, PhD

Training

- The PI is being trained for a doctoral degree with this project.

Conclusion

This study is proceeding as put forth in the "Statement of Work". The feeding trial portion of the study is continuing. Some preliminary data from plasma sex hormone analysis for the first 10 participants indicates that a low fat, high omega-3 fatty acid diet reduces the risk of breast cancer. The final subjects are currently participating in the study and are expected to finish by January 2009. All analytes mentioned in the Statement of Work will be analyzed in batches when the final subjects complete the study. The PI is proceeding towards the goal of attaining a PhD and receiving training to become a clinical researcher in the field of nutrition and breast cancer prevention.

References

None

Effect of High Omega-3 Fatty Acid Diet on Markers of Breast Cancer Risk in Postmenopausal Women

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Sex hormone mediated cancers, such as breast cancer, present a significant problem in the United States. It is important to develop safe and effective preventative strategies for these diseases. Epidemiological evidence and animal studies show that dietary fat is associated with risk of development of sex hormone mediated cancer. Specifically that a high intake of omega-6 fatty acids increases risk while omega-3 fatty acids are associated with risk reduction. Although the associations between dietary fat and sex hormone mediated cancers is unclear, it is likely due to mechanisms of endocrine balance, eicosanoid production, or immune function.

The primary objective of this investigation is to determine whether diets designed to increase plasma omega-3 fatty acid concentrations (a low fat diet, with or without omega-3 fatty acid enrichment), will favorably affect sex hormone distribution in postmenopausal women in a direction associated with reduced risk of sex hormone-mediated cancer development. The specific aims of this study are to evaluate the effects of total fat and omega-3 fatty acid intake on plasma sex hormone levels in postmenopausal women.

In order to evaluate these relationships we are conducting a well-controlled feeding study to evaluate dietary fat and fatty acid effects. The diets being tested in 8 week feeding periods include a “high risk” American diet (40% fat; HF), a low fat diet (20% fat; LF) and a low fat diet with supplemental omega-3 fatty acids (23% fat; LFn3). Endpoint measures of plasma sex hormones were obtained at baseline and 8 weeks of each dietary treatment.

Plasma estradiol (E₂), estrone (E₁), estrone sulfate (E₁-S), testosterone (T), androstenedione (AS), sex hormone binding globulin (SHBG), follicle stimulating hormone (FSH), dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS) were analyzed by radio-immunoassay for 10 participants. SHBG levels were significantly increased at 8 weeks with LFn3 compared to LF ($p < 0.05$), and there was a trend for decreased DHEAS level at 8 weeks with LFn3 compared to LF ($p < 0.15$). A trend for increased E₂ was observed with HF compared to both LF and LFn3 at 8 weeks ($p < 0.15$). A trend for decreased E₁ and FSH was observed from baseline to 8 weeks with LFn3 ($p < 0.15$). No statistically significant differences were observed between treatments for A, T, E₁-S, or DHEA.

Preliminary results suggest that LFn3 alters estrogen metabolism in a direction associated with reducing breast cancer risk in postmenopausal women. LFn3 significantly increased plasma SHBG and decreased DHEAS concentrations in postmenopausal women compared to LF at 8 weeks. Within the LFn3 group, trends were observed for decreased E₁ and FSH from baseline to 8 weeks. A trend for elevated E₂ level was observed with HF relative to LF and LFn3 at 8 weeks. The full effects of the three diets on plasma sex hormone profile will be further elucidated as more subjects complete the study.

Breast cancer risk reduction: Effect of dietary fat and fatty acids on plasma estrogen and testosterone indices in postmenopausal women

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Breast cancer, a sex hormone mediated cancer, presents a significant problem in the United States. It is important to develop safe and effective preventative strategies for this disease. Epidemiological evidence and animal studies show that dietary fat is associated with risk of development of sex hormone mediated cancer. Specifically that a high intake of omega-6 fatty acids increases risk while omega-3 fatty acids are associated with risk reduction. Although the associations between dietary fat and sex hormone mediated cancers is unclear, it is likely due to mechanisms of endocrine balance.

The primary objective of this investigation was to determine whether diets of varied fat and fatty acid content would favorably affect sex hormone distribution in postmenopausal women in a direction associated with reduced risk of sex hormone-mediated cancer development. The specific aims of this study are to evaluate the effects of total fat and omega-3 fatty acid intake on androgen and estrogen indices.

In order to evaluate these relationships we are conducting a well-controlled feeding study to evaluate dietary fat and fatty acid effects. The diets being tested in 8 week feeding periods include a “high risk” American diet (40% fat; HF), a low fat diet (20% fat; LF) and a low fat diet with supplemental omega-3 fatty acids (23% fat; ω 3). Endpoint measures of plasma sex hormones were obtained at baseline (BL), 4, and 8 weeks of each dietary treatment. Plasma estradiol (E_2), testosterone (T) and sex hormone binding globulin (SHBG) were analyzed by radio-immunoassay for 10 participants. The Estrogen and Androgen Indices (EI, AI) were calculated as the ratio of E_2 :SHBG and T:SHBG, respectively.

A trend for decreased concentrations from baseline to 8 weeks was detected for E_2 with the LF and ω 3 diets. Consumption of the ω 3 increased SHBG concentrations significantly from baseline to 8 weeks ($p = 0.04$). The EI showed a trend for increase from baseline to 8 weeks in the HF compared to the ω 3 diet at week 8 ($p = .08$). The AI was significantly reduced following the HF compared to LF and ω 3 ($p = .006$ and $.002$, respectively).

Preliminary data shows that within subjects, 8 weeks consumption of a ω 3 diet significantly decreased plasma E_2 and increased SHBG concentrations in postmenopausal women. Compared to HF, consumption of the ω 3 and LF diets significantly decreased E_2 concentrations between groups at 8 weeks. The HF diet resulted in a decreased AI and increased EI. The LF and ω 3 diets are associated with reduced free estrogen levels which may reduce risk for breast cancer.