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Award Number: W81XWH-04-1-0296

TITLE: Fish Oil Supplementation and Fatty Acid Synthase Expression in the Prostate: A Randomized Controlled Trial

PRINCIPAL INVESTIGATOR: Jackilen Shannon, Ph.D.

CONTRACTING ORGANIZATION: Oregon Health and Science University
Portland, OR 97239-0398

REPORT DATE: March 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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One in seven men over the age of 60 will be diagnosed with prostate cancer. Elucidation of early cellular changes that may predict progression to prostate cancer and the identification of factors that may inhibit or reverse these cellular changes would be of great clinical significance. Alteration of the fatty acid synthase (FAS) pathway is an early cellular change that has recently come under investigation. Overexpression of the lipogenic enzyme FAS has been noted in several tumor and pre-cancerous tissue types, including prostatic intraepithelial neoplasia (PIN) and prostate cancer and has been suggested as an independent predictor of disease stage. Additionally, inhibition of FAS has been demonstrated to induce apoptosis and reduce cell proliferation in cancer cells. Fatty acid synthase expression in cancer and normal cells is regulated by the transcription factor sterol regulatory element binding protein 1c (SREBP-1). The up-regulation of SREBP-1 in tumor cells results in increased FAS expression and fatty acid synthesis. Research in normal cells has demonstrated that dietary supplementation with polyunsaturated fatty acids (PUFA), particularly omega-3 fatty acids, inhibits SREBP-1 activation, resulting in a decreased transcription of FAS.
INTRODUCTION

SPECIFIC AIMS:
The proposal aims have been modified to reflect our decision to drop the statin arm of this trial. Please see attached DOD Statement of Work (last year’s, Appendix 1) and DOD Revised Statement of Work (Appendix 2). We propose to conduct a double-blind, placebo-controlled, randomized intervention study to evaluate the effects of Fish Oil (FO) supplementation use on markers of lipid metabolism in prostate tissue samples. The primary endpoints of this trial are fatty acid synthase expression, caveolin-1 expression, changes in lipid raft fractions in the plasma membrane and cell proliferation (Ki-67 expression) in benign, pre-neoplastic and neoplastic prostate tissue. The secondary endpoints include measuring the expression of SREBP-1, a transcription factor for fatty acid synthase, cell death (apoptotic fraction using TUNEL), red blood cell (RBC) fatty acid concentration and change in PSA. Subjects will be men from the Portland VA Medical Center (PVAMC) and Oregon Health and Science University (OHSU) and Kaiser Permanente Northwest (KPNW) urology clinics who are scheduled for a repeat biopsy. These men will have had an initial negative biopsy yet are still considered at high risk due to continued elevated prostatic specific antigen (PSA >4\(\mu\)g/dl), are positive for PIN and/or have suspicious findings by DRE or TRUS. Approximately 5 men per month over 24 months will be recruited and randomized to receive three months of either fish oil capsules (treatment 1) or olive oil (placebo) capsules (treatment 2). Potential confounding variables will be assessed through completion of a comprehensive diet history questionnaire and risk factor questionnaire, assessment of pre and post-treatment PSA and surveillance of medication and supplement use. Compliance will be assessed using pill count and evaluation of RBC fatty acid concentrations. While this study population is limited to men at high risk of disease, the results may be more broadly generalizable to any man considered at risk of prostate cancer due to standard clinical indicators such as a PSA >4\(\mu\)g/ml.

BODY

During FY02 this award has supported study coordination, local and federal human subjects review, subject recruitment and data collection to address our primary aims. Human Subjects Review: We obtained final DOD HSSRD review and approval to add a statin arm to the originally funded trial of FO on 7/7/05. This action moved this study into the ‘greater than minimal risk’ category. Early in August 2005, we listed our protocol on the National Cancer Institute’s (NCI) on the PDQ cancer.gov website that links to www.clinicaltrials.gov, per their request. Following negotiations with Dr. Julie Wilberding, we received additional funding on 8/15/05 to provide travel reimbursement to our participants and therefore help us more successfully recruit subjects. On 8/27/05, Ms Caryn Duchesneau provided approval of five amendments that were approved by the PVAMC (see Appendix 3). The DOD approved the addition of OHSU as a second recruitment site on 1/29/05. DOD approval to add KPNW was received on 3/28/06. In order to more accurately track approvals for each separate site, the DOD assigned the HSRRB Log number A-12538.a to PVAMC, Log number A-12538.b to OHSU and Log number A-12538.c to KPNW.

The DOD approved other protocol modifications throughout the year. An authorized prescriber was added to the study, we met PVAMC’s continuing review requirements (DOD approval for both: 10/22/05); the eligibility criteria ‘heart disease’ (a very vague and broad term) was deleted from our screening lists (DOD approval for PVAMC and OHSU approvals: 1/26/06 and 1/29/06, respectively); a protocol deviation notification was accepted (DOD approval 2/14/06) and minor revisions for consistency were made to study documents at both PVAMC and OHSU (DOD approval 2/16/06).
Finally, DOD approval to add KPNW as a third site as well as to drop the statin study arm was received on 03/28/2006.

Additionally, despite receiving approval to add a statin arm to this study earlier in the year, we requested approval to drop the statin arm of the study. This decision was not made lightly. An increasing number of men are being prescribed statins for their comorbid conditions; this has made it exceedingly difficult to accrue subjects. After accrual of only three men to the FO+statin study over a six month period, conferring with our co-investigators and Dr. Wilberding, it was decided that dropping the statin arm of the study would encourage the success of the study. The DOD HSRRD approved this change on 2/16/06. The study remains Greater Than Minimal Risk, as we will still collect two extra biopsy cores for research purposes. However, the number of men we intend to recruit changes from 144 to 88.

**STUDY COORDINATION:** Ms. Paige Farris continues to function as study coordinator and has maintained ongoing contact with Dr. Donna Ferrandino (CDMRC), the collaborating investigators and Dr. Garzotto and Ms. Peters, RN. The primary changes with regard to study coordination are with regards to dropping the statin arm of the trial. The decision to drop this arm was made after substantial discussions with Drs Garzotto and Beer and Dr. Wilberding, the Project Officer for the study. In the month between dropping the statin arm and submission of this annual report, we have successfully scheduled 2 men for their initial visits. Research assistants, Amy Palma and Gretchen Luhr, and study coordinator, Paige Farris, are able to make recruitment phone calls to potential subjects; conduct visits, record data and complete all paperwork on each participant. All study staff have been trained on how to collect prostate biopsy cores from subjects, which are stored in a -80°C freezer.

**PROGRESS TO DATE:** Despite a slow start up we are optimistic that we will reach our recruitment goal of 88 subjects into the FO trial within the next 18 months. To date, of a total of 32 potential repeat biopsy subjects, 22 (69%) met our fish oil/ statin inclusion/ exclusion criteria (70% of whom were excluded due to statin use and would now be eligible for a fish oil only trial). Of those eligible, 45% (n=10) were successfully recruited, 7 of whom have completed the trial and no participant has dropped out of the trial post-randomization.

With the recent DOD human subjects approval of new participating sites, we will expand our recruitment to include patients scheduled for re-biopsy from the OHSU, KPNW and the PVAMC urology clinics. Urologists (Garzotto, Sokoloff and Lieberman) at each facility work together closely and follow similar guidelines for determining need for repeat biopsy. Using our experience from the previous year, we are better able to identify potential pitfalls and estimate realistic accrual statistics. As demonstrated in Table 1, the recruitment rate for the fish oil trial has been 45%. However, 20% of men refusing to join the study noted that they would gladly participate if they were either a) reimbursed for the travel or b) could have their first visit by telephone. With this knowledge we have

<table>
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<tr>
<th>FO+Statin Trial Recruitment: January 2005–February 2006</th>
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<tbody>
<tr>
<td>FO+Statin Participant Status</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>Total patients eligible: 22</td>
</tr>
<tr>
<td>Enrolled in trial</td>
</tr>
<tr>
<td>Refused: gas/ travel distance</td>
</tr>
<tr>
<td>Refused: other reasons</td>
</tr>
<tr>
<td>Total patients ineligible: 10</td>
</tr>
<tr>
<td>Current statin use</td>
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<td>Fish oil or warfarin use</td>
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</table>
made two adjustments to the current protocol (as described above); first we will open enrollment to OHSU and KPNW patients, the majority of whom live locally, and second we have requested and received funds to cover travel costs for subjects. Further, this trial is also listed for consideration as part of the DOD Prostate Cancer Consortium. Discussions are currently underway between Dr. Tomasz Beer (OHSU Consortium PI) and at least one consortium center that is considering participation in this trial.

Thus, estimating that between 22 to 26% of all men with a negative biopsy undergo re-biopsy each month at each of the participating institutions, we can expect an estimated number of potential subjects from each clinic of: KPNW, 10-15 men; PVAMC, 4 men; OHSU 3 men. Conservatively assuming a 40% response rate, we estimate successful recruitment of 6 men per month overall for a total of as many as 108 men over the next 18 months. Please note, due to slow start up in year 01, we requested an extension without funds through February 2008 (see attached e-mail communication dated 03/03/2005, Appendix 4). This change will assure us the time necessary to accrue an adequate number of study subjects and complete data analyses.

**KEY RESEARCH ACCOMPLISHMENTS:** Because adequate subject recruitment was not reached, immunohistochemistry for fatty acid synthase (FAS) and sterol regulatory element binding protein (SREBP-1) in the biopsy samples did not begin this year. However, methods for quantitation of the cholesterol component of lipid rafts have been fully developed and tested in existing non-study tissue specimens.

**REPORTABLE OUTCOMES:** None to date

**CONCLUSIONS:** The primary outcome of the second year was our success in gaining the interest of two other urology clinic sites, in order to supplement the slow recruitment at the PVAMC, as well as receiving another supplemental award to reimburse subjects for traveling to the first visit. In preparation for dropping the statin arm, there were very few changes to be made to the infrastructure of this study. All questionnaires but two (Fish Oil Adverse Events and Post-Intervention) remained the same. We fully anticipate reaching our proposed study accrual in the next 18 months and completion of all laboratory and statistical analyses within the next 24 months.
C. Revised Statement of Work

Fish Oil Supplementation and Fatty Acid Synthase Expression in the Prostate:
A Randomized Controlled Trial

Task 1. Finalize clinical protocol and training:  Months 1-6

a. Develop tracking system for recording patient recruitment, contact and consent information.

b. Obtain IRB approval from both Portland VA Medical Center and Oregon Health and Sciences University.

c. Finalize encapsulation procedure and obtain treatment and placebo capsules.

d. Finalize and review clinical protocol with GCRC nursing staff.

e. Review and optimize blood processing procedures with laboratory staff.

f. Review procedures for patient contact and recruitment with Mark Garzotto, MD, Laura Peters, RN and study coordinator, Amy Palma.

g. Modify tracking system, protocol, and consent form to allow for the addition of a statin arm and a statin+fish oil arm.

h. Modify tracking system, protocol, and consent form to allow for the collection of an additional prostate biopsy core to be cryopreserved.

Expected Product: Tracking system, IRB approval, IRB approval of amendment.

Task 2. Subject recruitment and data collection  Months 6 – 28

a. Patients who have had a negative prostate biopsy in the previous two weeks or patients who have had a positive biopsy but who have chosen no therapy (watchful waiting) and who have been scheduled by Dr. Garzotto to undergo a repeat biopsy will be recruited for our proposed trial.

b. Initial telephone contact and schedule appointment.

c. 1st visit – at the OHSU GCRC
   • Review the study purpose, protocol, exclusion criteria and consent documents.
   • Complete the diet history questionnaire, risk factor questionnaire.

d. Randomization

e. 2nd visit -- at the OHSU GCRC
   (note: this visit may be combined with visit 1 to reduce subject burden)
   • Complete history and physical exam, 10 ml blood specimen.
   • Four week supply of placebo or treatment capsules distributed.

f. 3rd visit – at the OHSU GCRC (for subjects residing outside the Portland area, this visit may be replaced by a telephone contact and mailed supplements)
   • Four week supply of placebo or treatment capsules distributed.
   • Complete side-effects and adverse events reporting form.

g. 4th visit – at the OHSU GCRC (for subjects residing outside the Portland area, this visit may be replaced by a telephone contact and mailed supplements)
   • Four week supply of placebo or treatment capsules distributed.
   • Complete side-effects and adverse events reporting form.

h. 5th visit – at PVAMC clinic area E
   • Return unused capsules.
   • Obtain 10 ml blood specimen
   • Repeat biopsy conducted per standard clinical procedure (this is not a study linked event)
   • Obtain one additional biopsy core for cryopreservation and analyses of lipid raft fractions. In men with known prostate cancer this core will be taken, if possible, from
the quadrant farthest from the known tumor. Fresh tissue collected at surgery by study RA and delivered immediately to the OHSU Pharmacokinetics Core for cryopreservation.

Expected Product: Questionnaire, blood specimen and biopsy (frozen and paraffin embedded) data for 120 patients.

Task 3. Preparation for Immunohistochemistry (IHC) / Data Entry. Months 13-24

a. Optimize IHC for fatty acid synthase (FAS) and sterol regulatory element binding protein (SREBP-1)
b. Optimize protocol for lipid raft extraction from tissue specimens (using stored non-study tissue).
c. Develop database for tracking specimen receipt and analysis
d. Begin data entry of questionnaire forms and event reporting forms

Expected Product: High functioning antibodies and procedures for FAS and SREBP-1 IHC, final procedures for lipid extraction and raft associated protein analyses, laboratory database, complete questionnaire data entry.

Task 4. Laboratory / Dietary Analyses. Months 24-32

a. Blood specimens shipped to Seattle for red blood cell fatty acid analyses.
b. Initial and repeat biopsy specimens obtained from PVAMC pathology.
c. Perform IHC for FAS and SREBP-1 on pre and post intervention tissue specimens.
d. Perform IHC for Ki-67 and TUNEL assay on post-intervention tissue specimens.
e. Perform lipid extraction and mass spectrometry for identification of raft associated proteins and lipids.
f. Run nutrient analysis program on diet history questionnaire data.
g. Data cleaning.

Expected Product: Complete data on FAS and SREBP-1 expression in 240 tissue specimens from 120 patients. Complete data on Ki-67 expression and TUNEL for 120 tissue specimens from 120 patients. Complete data on quantity of raft associated long chain fatty acids and caveolin-1 from 120 frozen biopsy specimens. Red blood cell fatty acid concentrations from 240 blood specimens from 120 patients. Nutrient intake data from 80 patients.

Task 5. Final Analyses and Report Writing Months 32-36

a. Final analysis of data from questionnaires, blood specimens and tissue specimens will be performed
b. Prepare final report and initial manuscripts.

Expected Product: Completed and submitted final report a minimum of 1 submitted manuscript.
C. Revised Statement of Work, version 2

Fish Oil Supplementation and Fatty Acid Synthase Expression in the Prostate:
A Randomized Controlled Trial

Task 1. Finalize clinical protocol and training: Months 1-6

a. Develop tracking system for recording patient recruitment, contact and consent information.
b. Obtain IRB approval from Portland VA Medical Center (PVAMC), Oregon Health and Sciences University (OHSU) and Kaiser Permanente Northwest (KPNW).
c. Finalize encapsulation procedure and obtain treatment and placebo capsules.
d. Finalize and review clinical protocol with GCRC nursing staff.
e. Review and optimize blood processing procedures with laboratory staff.
f. Review procedures for patient contact and recruitment with (PVAMC) Mark Garzotto, MD, Laura Peters, RN and study coordinator, Amy Palma.
g. Modify tracking system, protocol, and consent form to allow for the addition of a statin arm and a statin+fish oil arm.
h. Modify tracking system, protocol, and consent form to allow for the collection of an additional prostate biopsy core to be cryopreserved.

Expected Product: Tracking system, IRB approval, IRB approval of amendment.

Task 2. Subject recruitment and data collection Months 6 – 28

a. With the addition of two study sites (IRB and DOD approved); review procedures for patient contact and recruitment with (OHSU) Mitchell Sokoloff, MD and Mark Johnson, RN; (KPNW) Stephen Lieberman, MD.
b. Patients who have had a negative prostate biopsy in the previous two weeks or patients who have had a positive biopsy but who have chosen no therapy (watchful waiting) and who have been scheduled by Drs. Garzotto, Sokoloff or Lieberman to undergo a repeat biopsy will be recruited for our proposed trial.
c. Initial telephone contact and schedule appointment.
d. 1st visit – at the OHSU GCRC
   • Review the study purpose, protocol, exclusion criteria and consent documents.
   • Complete the diet history questionnaire, risk factor questionnaire.
e. Randomization
f. 2nd visit – at the OHSU GCRC
   (note: this visit may be combined with visit 1 to reduce subject burden)
   • Complete history and physical exam, 20 ml blood specimen.
   • Four week supply of placebo or treatment capsules distributed.
g. 3rd visit – at the OHSU GCRC (for subjects residing outside the Portland area, this visit may be replaced by a telephone contact and mailed supplements)
   • Four week supply of placebo or treatment capsules distributed.
   • Complete side-effects and adverse events reporting form.
h. 4th visit – at the OHSU GCRC (for subjects residing outside the Portland area, this visit may be replaced by a telephone contact and mailed supplements)
   • Four week supply of placebo or treatment capsules distributed.
   • Complete side-effects and adverse events reporting form.
i. 5th visit – at PVAMC clinic area E or OHSU or KPNW Urology clinics
   • Return unused capsules.
   • Obtain 20 ml blood specimen
• Repeat biopsy conducted per standard clinical procedure (this is not a study linked event)
• Obtain one additional biopsy core for cryopreservation and analyses of lipid raft fractions. In men with known prostate cancer this core will be taken, if possible, from the quadrant farthest from the known tumor. Fresh tissue collected at surgery by study RA and delivered immediately to the OHSU Pharmacokinetics Core for cryopreservation.

Expected Product: Questionnaire, blood specimen and biopsy (frozen and paraffin embedded) data for 88 patients.

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Expected Product: High functioning antibodies and procedures for FAS and SREBP-1 IHC, final procedures for lipid extraction and raft associated protein analyses, laboratory database, complete questionnaire data entry.

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a. Blood specimens shipped to Seattle for red blood cell fatty acid analyses.
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Task 5. Final Analyses and Report Writing Months 32-36

a. Final analysis of data from questionnaires, blood specimens and tissue specimens will be performed
b. Prepare final report and initial manuscripts.

Expected Product: Completed and submitted final report a minimum of 1 submitted manuscript.
SUBJECT: Amendment #2, Continuing Review, and Deviation to Protocol, “Fish Oil Supplementation and Fatty Acid Synthase Expression in the Prostate: A Randomized Controlled Trial,” Submitted by Jackilen Shannon, PhD, Oregon Health and Science University, Portland, OR, Proposal No. PC030386, Award No. W81XWH-04-1-0296, HSRRB Log No. A-12538

Chronological List of Protocol Actions since March 2005

a. Amendment: 28 March 2005; expedited review and approval. This amendment requests the use of the older PVAMC IRB approved consent form to consent subjects until the DOD has approved amendment #1 and the more recent consent form. No change in risk level.

b. Amendment and Deviation: 11 May 2005; full board review and approval. This amendment again requests approval to use the previously approved consent form pending DOD approval of the more recent form. The deviation involved the use of this consent form. No change in risk level.

c. Amendment: 16 June 2005; expedited review and approval. This amendment requests the use of an initial letter to mail to potential subjects who have no telephone. A copy of the approved telephone script would be sent also; a copy of the letter is provided. No change in risk level.

d. Amendment: 12 July 2005; expedited review and approval. This amendment requests that subjects be reimbursed for travel costs to visit the PVAMC for the first study visit. Rate would be 40.5 ¢ per mile; study documents were revised accordingly. No change in risk level.

e. Amendment: 19 July 2005; expedited review and approval. This amendment requests that the PVAMC IRB grant approval of the study documents that were revised in response to the recommendations of the HSRRB. No change in risk level.

f. Amendment: 21 July 2005; full board review and disapproval. This amendment requested approval to consent potential subjects and complete the first visit questionnaires by mail and over the telephone instead of in person, to save travel costs for potential subjects. Also, the amendment requested approval to have initial blood and urine collected at the local VA clinic, and shipped to the PI for analysis. The HSRRB will not accept this amendment since the local IRB did not grant approval.
Continuing Review: 25 July 2005; annual review completed by PVAMC IRB.

(1) Number of subjects enrolled as of 22 July 2005: 4 of 144

(2) Amendments: as of 22 July 2005, all of the above amendments had already been submitted and approved or disapproved by the local IRB. An additional amendment (22 July 2005) was included in the Continuing Review Report. See below.

(3) Deviations: as of 22 July 2005, one deviation (11 May 2005) had already been submitted. See above.

(4) Injuries, withdrawals, or complaints from subjects: None

(5) Literature search: see amendment (22 July 2005) listed below; appropriate changes were made to study documents to reflect this information.

(6) Adverse Events: Yes; however, the adverse events referred to in the continuing review report are those listed in the JAMA article, and are not related to this protocol.

(7) Unanticipated problems involving risks to subjects: None

(8) Additional amendment included in Continuing Review Report: 22 July 2005; approval from PVAMC IRB is pending. The PI submitted a Safety Supplement, in response to an article that appeared in JAMA. The amendment requests approval to change the exclusion criteria to exclude patients who have an implantable cardioverter defibrillator (ICD) or who have a history of sustained ventricular tachycardia (VT) or ventricular fibrillation (VF). This part of the amendment would require changes to the protocol, consent form, and inclusion table. This amendment also requests changing the consent form to better reflect the fact that no genetic testing will be done in this study. No change in risk level.

(9) Status of study: Recruitment is ongoing; no results to report at this time.

Notification: 28 July 2005; no approval required from local IRB or HSRRB. During telephone recruitment, two potential subjects were informed solely about the fish oil study, and not about the fish oil/statin study. Thus, the most recent version (stamped 12 July 2005) of the Fish Oil Only Version of the consent form was used with these two subjects. No change in risk level.
Hi Jackilen, no problem on an extension without funds, let me forward this to the contracting office to issue the modification. You can be assured it is a done deal.

-----Original Message-----
From: Jackie Shannon [mailto:shannoja@ohsu.edu]
Sent: Thursday, March 03, 2005 11:37 AM
To: Wilberding, Julie A Dr USAMRMC
Cc: Paige Farris
Subject: question regarding extensions - PC030386

Hello Julie -
We talked quite some time ago about the slow start-up of my project due to delayed DOD human subjects reviews. We have finally been able to open enrollment just into the fish oil portion of the trial, but even thought we have had VA approval for the statin arm, this paperwork (submitted several months ago) has not been reviewed yet by HSSRD. We are in regular contact with Donna Ferrandino, but are still quite behind on planned enrollment.

I have carefully reviewed my budget and the amount spent in year 01, and would have adequate funds to extend the project for an additional year. Is this something I can receive permission to do right now or do I have to wait until the end of year 03 and then ask for an extension of the project period? I would rather have the project period extended now so I could budget for this and not create confusion for the university grants people.

Please let me know.
Thanks,
Jackilen

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