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TITLE: The Use of Exercise to Increase CD4(+) T Lymphocytes Following Chemotherapy Treatment for Breast Cancer

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The Use of Exercise to Increase CD4(+) T Lymphocytes Following Chemotherapy Treatment for Breast Cancer

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T helper, CD4+ lymphocytes, recover slowly following chemotherapy. In healthy females. Exercise increased CD4+ cells. Our goal was to determine if exercise helped CD4+ cells recover following chemotherapy. Blood lymphocytes, before and after chemotherapy/radiation and 3 and 6 months of exercise were assayed for proliferation and for expression of leukocyte antigens. Following fitness testing, the subjects exercised with a trainer. Questionnaires (quality of life, diet, and physical activity) were administered 5 times. Thus far, we have recruited 44 women (29 exercisers and 15 controls). Seventeen women have finished 6 months of exercise and 11 have finished 3 months of exercise. After 6 months of exercise, there were significant increases in the number of lymphocytes (p<0.01), CD8 cells (p<0.05), and CD4/CD45RA (p<0.05) and no change in the control group. There were also significant changes in quality of life (p<0.01), physical well-being (p<0.05) and functional well-being (p<0.01). The members of the exercise group demonstrated significant increases in physical function parameters: biceps strength (p<0.05), triceps strength (p<0.05) and time spent on the bike (p<0.005). Thus far, the results suggest that an appropriately designed exercise training helps women recover from chemotherapy.
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INTRODUCTION
Breast cancer patients commonly receive chemotherapy as part of their treatment. Along with the tumor cells, the normal, continually renewing cells of the hematopoietic system are targets for the cytotoxic drugs. Hematopoietic stimulating factors can correct certain other blood cell populations but the loss of blood lymphocytes remains uncorrected. One population of lymphocytes, CD4+ T cells (T helper cells), major regulators of the immune system, are particularly susceptible to chemotherapy-induced depletion. CD4+ T cells levels in the blood frequently fall to those seen in AIDS patients (Kilmas et al., 1991) and remain low for many months after chemotherapy ceases (Greenberg & Riddell, 1999; Hakim et al., 1997). Nevertheless, we have measured an increase in CD4+ T cells in normal, healthy females after 3 months of resistance exercise training. There are similar reports of increases in CD4+ T cells in HIV seropositive individuals following training (La Pissiere et al., 1991). Therefore, we hypothesized that an appropriately designed exercise training program will help in the recovery of CD4+ T lymphocyte levels following chemotherapy. We have proposed to determine the total numbers of CD4+ T lymphocytes in the blood before and after chemotherapy/radiation and after three and six months of exercise training. We plan to determine underlying cellular mechanisms by assaying lymphocyte sub-populations, especially CD4+ T cells, sub-population differentiation, activation, and apoptosis, and to demonstrate that the exercise program designed for these individuals is sufficient to improve physical fitness, and quality of life and to decrease fatigue (Mock et al., 1994).

BODY: Research Accomplishments Associated with Statement of Work (Approved 4/01)
*Please note that this study has been extended for one year with additional funding in order to allow completion of recruitment process and intervention

Task 1: Procedures to coordinate with clinicians and cancer program coordinator to begin to enroll subjects

A collaboration with the associate investigators, Dr. Aaron Bleznak, Dr. Richard Dixon, and Judy Underwood, was established early on in the study (months 1-3). The procedures for recruitment in the Centre County area are well established

Task 1a: To continue to periodically meet with clinicians to assure that recruitment is effective.

We continue to be in contact with the physicians in the local area via phone calls, e-mails, and personal visits. They have been provided with copies of abstracts submitted to organizations for annual meetings as well as updates on the current numbers in the study. In addition, they have been given "bookmarks" describing the study to keep in their coat
pockets that they can provide to possible study participants. It has been helpful to be able to establish a relationship with the chemotherapy nurses at the physician offices as they are easier to contact and have become an important source of referrals.

After receiving official approval form Hershey Medical Center to conduct the study at that location, attempts have been made to update the physicians and staff. In October, 2000, a presentation by the PI and Co-PI was given at Hershey Medical Center to the staff of the General Clinical Research Center (GCRC), physicians, and chemotherapy coordinator at Hershey. Two nurses at the Hershey GCRC agreed to help with the recruitment process and to serve as the initial contacts for those in the Hershey area. Follow-up visits have been made by the PI to the Hershey Medical Center staff. In addition, the GCRC at Hershey Medical Center has been trained by the Co-PI, Nancy Williams, and is now conducting the fitness testing and blood draws at their facility.

Task 1b: To periodically advertise and promote the program to the medical and lay community.

The breast cancer exercise program is continually advertised and promoted in the Centre County area. The study has been listed the local newspaper (Centre Daily Times) each Monday in the health calendar section. In addition, flyers have been placed in public places (i.e. library, grocery stores) in the Centre County region. The study has also been promoted at the local American Cancer Society level at both the fundraiser, Relay for Life (June, 2001) by placement of an advertisement in the program and at the local office with visible brochures. A summary of the advertising efforts to the general public is listed below:

3. Announcement on the University sponsored Health Web, “Health Matters”
4. Advertisement on C-NET (public service television station)
5. Announcement in the health calendar section of the Centre Daily Times each Monday.
6. Advertisement at the Relay for Life. Former study participant passing out brochures at the event

The study has also been promoted to the medical community in the Centre County region. Brochures have been placed in the physician offices as well as in the chemotherapy and radiation suites in the area. In addition, brochures have been sent to other local hospitals in an attempt to recruit participants from those areas. Pocket bookmarks have been distributed to local physicians. Contacts were made with the local “Reach for Recovery Group” and they have agreed to provide brochures to women treated surgically for breast cancer. Reach for Recovery is a group of breast cancer
survivors who visit women in their homes shortly after surgery to provide emotional and medical support.

There has also been promotion of the program in the Hershey, Pennsylvania area through advertising in local newspapers and distribution of flyers and brochures. This effort has been coordinated by two of the staff at the GCRC at Hershey Medical Center. A summary of the efforts is as follows:

3. Distribution of brochures and flyers in the Hershey Medical Center facility.
4. Distribution of brochures to the local physicians in the Hershey area.
5. Distribution of brochures at local health fairs in which Hershey Medical Center participates.
6. Distribution of brochures at senior citizens centers, and church fairs.
7. Advertisement of study on a local radio station.

Task 2: Recruit possible subjects.

Recruitment is an ongoing process. Successful recruitment will occur through tasks 1, 1a, and 1b. We will continue to recruit until we have 35 exercisers and 35 controls enrolled into the study. Thus far we have enrolled 44 women, 29 exercisers and 15 controls, into the study. 12 have finished 6 months of exercise and 8 have finished 3 months of exercise. In addition we have 3 controls who have been followed for 3 months and 5 who have been followed for 6 months. The remainder subjects are either currently in treatment (6) or have just started into the exercise timepoint (10).

Task 3: Meet with potential subjects and go through the informed consent process prior to obtaining baseline measures of CD4+ levels.

As mentioned in Task 2, we have enrolled 44 women into the study. In Centre County, Drs. Dixon and Bleznak meet most of the women who undergo chemotherapy for breast cancer. If a potential subject is to receive chemotherapy, one of the physicians or a staff member briefly explains the study to the individual. The name and contact number is then passed on to the study coordinator so that the study can be explained in more detail. With the subject's consent, they are sent a packet of information, including an informed consent, which they are asked to read. The potential subject is then met by either Dr. Andrea Mastro, Dr. Nancy Williams, or Dr. Beth Orsenga-Smith during which any additional questions are answered and the informed consent is signed. The subject is then asked to provide a blood sample and complete baseline questionnaires.
Oftentimes, the informed consent is signed either at the physician’s office or at the GCRC at Penn State University. The blood is drawn at either the physician’s office or at the GCRC. If a subject has a port, the blood is drawn under sterile conditions at the physician’s office and brought back to our own laboratory for analyses.

When a subject is recruited from the Hershey area, the collaborators at Hershey Medical Center meet with the subject to review the informed consent. The blood is drawn at the GCRC and is sent overnight to University Park for further analyses.

Although the ideal point for entry into the study is prior to chemotherapy, sometimes this is not possible. Oftentimes when a potential subject is provided information about this research study, she may still be thinking about the ramifications of being diagnosed with breast cancer and participation in a study is not a priority. If they so choose to enroll following completion of their treatment, we have allowed them to participate. Following chemotherapy or chemotherapy/radiation treatment, the CD4+ T cells are usually at the nadir level which we can use as the baseline level. This can allow us to compare the post-exercise values to the nadir CD4+ T cells.

**Task 4:** Set up established protocols for immunological, biochemical, flow cytometric and cell assays.

The protocols for these assays have been well-established for this project. Please refer to Task 4 in the progress report from June 1, 1999 to June 30, 2000.

**Task 5:** Acquaint personnel with the specific of the exercise program (e.g. exercise techniques, exercise prescription procedures, nutritional assessments) and pilot all procedures.

Dr. Nancy Williams, Assistant Professor of Kinesiology, continues to coordinate the exercise program. All of the exercise testing and training sessions follow the protocols as outlined in progress report submitted June, 2000.

**Task 6:** Enroll potential subjects

Thus far we have enrolled 44 women, 29 exercisers and 15 controls, into the study. 12 have finished 6 months of exercise and 8 have finished 3 months of exercise. In addition, we have 3 controls who have been followed for 3 months and 5 who have been followed for 6 months. The remainder subjects are either currently in treatment (6) or have just started into the exercise timepoint (10). The timecourse for completion of the study may take as long as 14 months due in part to the lengthy chemotherapy treatments. There are several types of chemotherapy treatments that the women may undergo and they take anywhere between 4 to 6 months to complete. In addition, some of the women may undergo additional surgical treatment that may prolong their treatment. As described in Task 2, we will continue to recruit these women to have a total of 35 exercisers and 35 controls.
A timeline of the potential events during the study for an individual is commonly as follows:
1. Diagnosis of breast cancer and lumpectomy or mastectomy is performed
2. Pre-chemotherapy blood draw and completion of questionnaires
3. Participant undergoes chemotherapy (4-6 weeks dependent upon type of therapy)
4. Post-chemotherapy blood draw and completion of questionnaires
5. Participant undergoes 4-7 weeks of radiation therapy (most of the women do have radiation therapy)
6. Pre-exercise evaluation that occurs after completion of radiation therapy (fitness testing, blood draw, and completion of questionnaires)
7. Participant begins exercise training as soon after fitness testing as possible
8. Participant finishes 3 months of exercise and undergoes fitness testing, blood draw, and completion of questionnaires
9. Participant finishes 6 months of exercise an undergoes fitness testing, blood draw, and completion of questionnaires

In the present sample of women enrolled in the study, the age range is from 29-71 with a mean age of 47 years of age. The ethnicity of all of the subjects is Caucasian with the exception of one Hispanic woman. We are currently collecting additional demographic information from these women including education level. The baseline fitness level is influenced by age, course of treatment, and individual differences and varies within this population. The type of treatment varies within this population of women. Approximately 2/3 have had a lumpectomy while approximately 1/3 have undergone a mastectomy. Most of the women (70%) have had 4 cycles of Adriamycin/Cytoxan as their chemotherapy treatment. This regimen takes approximately 4 months to complete. However, others have been treated with a cocktail of CMF (Cytoxan, Methotrexanate, 5-Flourauricil) that takes about 6 months to complete.

Tasks 7, 8, 9, 10, 10a, 11, 12

These tasks are repeated for each of the subjects while in the study. The protocols have been established as outlined previously and are followed for each subject enrolled in the study. It is continuous enrollment so that potential subjects can continue to participate until we have the needed number of subjects as indicated by a power analyses.

Task 9a: Perform fitness evaluations for the non-exercising controls.

After concentrated recruitment in the Hershey area, several subjects were enrolled from that geographical location. Approval was granted to perform the exercise testing at the Hershey Medical Center GCRC. Dr. Nancy Williams supervised the training of the personnel at Hershey. They followed the exact testing protocol that was implemented at the University Park GCRC for testing of the subjects. Piloting of the fitness testing with the personnel at Hershey occurred over a 1 month period from mid December 2000 to mid

**Task 13:** Develop statistical database, enter data, and statistically analyze data when appropriate

Dr. Beth Orsega-Smith was hired as a post-doctoral scholar and coordinator on this project in August 2000. At that time, she created statistical databases to be used for the study. In addition, a coding scheme was written to analyze the physical activity questionnaire as well as the FACT-An (Functional Assessment of Cancer Therapy-Anemia) as suggested by the authors of the questionnaires. In addition, several students have been trained to enter the data therefore allowing statistical analyses to be completed on the data. At this time, paired t-tests are being used to examine differences in immune function, quality of life, physical activity, and physical function in each group over time. In addition, the nutritional data has been analyzed using Nutritionist Pro and the results are being entered into a database.

**Task 13a:** To write reports, abstracts, and papers for publication in referred journals.

Over the past year, three presentations have been made at national conferences. The abstracts have been peer reviewed for acceptance to present the data from this study. In March 2001, a poster was presented at the American Association of Cancer Research Meeting held in New Orleans. The focus of this was to examine immune changes in the exercisers. In May 2001 a poster emphasizing the physical function changes was presented at the American College of Sports Medicine conference. In addition, a paper was presented at the same meeting that described the changes in quality of life and physical activity that was found in these women over three months of the exercise intervention.

Although the study has not been completed, a manuscript is currently in progress that will provide a general overview of the study and describe the preliminary 3 month changes in immune function, quality of life, and physical function.

**Key Research Accomplishments**

- Established recruitment system
- Emphasized recruitment in Hershey
- Recruitment of 44 subjects, 15 completed the program
- Established exercise protocol
- Established functional and phenotypic assays
- Established intercellular cytokine assay
- Established soluble cytokine assays
- Received approval to recruit from Hershey Medical Center
- Training of personnel at Hershey medical Center's GCRC
• Testing of subjects at Hershey Medical Center’s GCRC
• Establishment of a workable database
• Established a data entry system
• Preliminary statistical analyses leading to the presentation of 3 papers at national conferences

Reportable Outcomes

March 2001 AACR Annual Meeting Poster Presentation

May 2001 ACSM Annual Meeting Poster Presentation

May 2001 ACSM Annual Meeting Paper Presentation

Post-doctoral experience for Beth Orsega-Smith
Practicum work for Beth Baker MS degree from Indiana University of Pennsylvania Masters thesis data for Mike Perry
Independent study credits for students working with the data under Dr. Beth Orsega-Smith
  Marie St.Pierre
  Syreeta Cherry
Independent study credits for Kinesiology students working as personal trainers:
  (approximately 15)
Training of Keira Fuerner as a lab technician
Applied for Susan Koman Post-doctoral Award for Dr. Beth Orsega-Smith to expand upon this research
Conclusions:
Listed below are some of the preliminary findings from this study.

Immune Parameters
Comparison of the data between pre-exercise and 6 months into exercise reveals some significant findings:
- After 6 months of exercise, there were significant increases in the number of lymphocytes (p<0.01), CD8 (p<0.05), and CD4/CD45 RA (p<0.05) cells in women who were in the exercise group with non statistically significant changes in the control group.
- In addition, the change in the number of CD4 (p<0.09) and CD3 (p<0.06) approached statistical significance in the exercise group.
- The number of naïve CD4T lymphocytes (CD4/CD45RA) increased in the exercise group but declined in the control group over the same time period.

Quality of Life
- There were significant changes over time between pre-exercise and 6 months into exercise in overall health-related quality of life (p<0.01), self-reported physical well-being (p<0.05) and functional well-being (p<0.01) in members of the exercise group.
- There were non-statically significant changes in the control group.

Physical Fitness Data
- There were significant changes from pre-exercise to 6 months into exercise in biceps strength (p<0.05), triceps strength (p<0.05) and aerobic time spent on the bike (p<0.005) for those in the exercise group.
- Too few in the control group to calculate at this time.

Why this study is important
This study addresses an important health-related immune problem that has not been examined previously by others in breast cancer research. It tests an intervention for women with breast cancer that is carried out following chemotherapy treatment. A search of the literature from 1990-2001 revealed no other comparable studies examining exercise interventions as a way to increase immune cell recovery following chemotherapy. This study examines the mechanism of the recovery of the immune cells after depletion by chemotherapy including the source of the cells (naïve or memory), state of activation, cycling or apoptotic, and the role of cytokines. It also examines the quality of life over the time from pre-chemotherapy through completion of exercise intervention following chemotherapy for breast cancer. This exercise intervention is longer than most studies that have examined physical activity and quality of life in cancer patients.
There are several possible outcomes from this study. The results from this study may help to develop a non-invasive, low cost therapeutic intervention for women recovering from breast cancer. This intervention can be carried out in a variety of settings therefore allowing replication in both the community and clinical settings. It is hoped that this intervention may help to provide scientific clues to the mechanism by which exercise affects the immune system as well as enhanced quality of life.

References:
Appendices:

DAMD 17-98-814210
Progress Report 6/30/00 - 6/3/01
Exercise, Quality of Life, and the Recovery of CD4 Lymphocytes Following Chemotherapy for Breast Cancer

Andrea M. Mastro, Nancy I. Williams, William J. Kraemer, Elizabeth M. Orsega-Smith, Michael Perry, Richard H. Dixon, Aaron D. Bleznak, Judy Underwood, Penn State University, University Park, PA; Ball State University, Muncie, IN; Centre Medical and Surgical Associates, State College, PA; Penn State Geisinger, State College, PA; Centre Community Hospital, State College, PA.

Breast cancer currently affects over 250,000 women each year. Therapies such as chemotherapy and radiation, can lead to a prolonged decrease in the number of blood lymphocytes particularly the CD4+ T helper cells with a negative impact on immune function. In addition, cancer therapies may affect quality of life. We are currently conducting an exercise intervention study to determine if physical activity can stimulate the recovery of the CD4+ and other lymphocytes and positively affect the quality of life in breast cancer survivors. This is a longitudinal study in which women have blood drawn for analysis of lymphocyte population subsets and for mitogen responsiveness, complete questionnaires on diet, physical activity, and quality of life; and undergo a fitness evaluation pre-chemotherapy, post-chemotherapy, post-radiation, 3 months post-radiation, and 6 months post-radiation. Following chemotherapy/radiation, the women undergo an exercise regimen of cardiovascular and muscular strength exercise for 6 months. Currently 12 women (ages 32-69, mean = 49.2 yrs) have completed 3 months of exercise. We analyzed pre-exercise and 3 months post-exercise data for lymphocyte function, physical fitness, and quality of life data. There was a significant increase in total lymphocytes (p<.001), and the CD4+ T lymphocytes(p<.05) as well as total T lymphocytes, CD3(p<.01), and B lymphocytes CD-19 (p<.001), over time. In addition, there was a significant increase in overall cancer related quality of life (p<.005). Fitness testing data indicated an overall increase in muscular strength and cardiovascular fitness; specifically the women had a significant increase in their VO2 (p<.05) and time on an exercise bike (p<.0001) and near significant increase in triceps strength (p<.056). There were no significant increases for those in a non-exercise control status. We are following these women through the next training period. Future directions include examination of mechanisms relating physical activity and recovery of lymphocytes following chemotherapy. Research supported by the US Army Medical Research and Material Command under DAMD 17-98-1-8142.
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This research has been approved by the Institutional Review Board, under FDA regulations, at Penn State Milton S. Hershey Medical Center

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DO YOU HAVE BREAST CANCER?

Researchers at PENN STATE UNIVERSITY are studying the effect of exercise on the immune system and the overall well being of breast cancer patients following chemotherapy. To learn more about this study, please call Shirlynn Mottillo at 1-800-583-9585. This research has been approved by the Institutional Review Board, under FDA regulations, at Hershey Medical Center.

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- Fight Fatigue and Depression
- Improve Quality of Life
- Help the Immune System Recover

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Dr. Andrea Mastro (814) 863-0512
Dr. Beth Orsega-Smith (814) 863-2434
Dr. Nancy Williams (814) 865-1346
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Dr. Andrea Mastro,
Professor of Cell Biology and Immunology at
Penn State University.

814-863-0512 or 814-863-2434

This study is approved by the Institutional Review Board at Penn State University.
Penn State University
Exercise and
Breast Cancer Study

Physical Activity and Breast Cancer?

Can physical activity accelerate the recovery of the immune system of breast cancer patients who have undergone chemotherapy?

Exercise and Immune Function

Researchers and local physicians are seeking participants for a project to determine if physical activity can help in the recovery of a certain class of white blood cells (T-lymphocytes) following chemotherapy. These white blood cells are important in fighting off bacteria, viruses and tumor cells. However, chemotherapy severely decreases the number of these cells. The researchers are asking if exercise can stimulate an increase in these cells and therefore aid in the restoration of good health.

Participation

The researchers are seeking women who will be or are currently undergoing chemotherapy/radiation for breast cancer. Participants will be asked to give a blood sample and fill in brief questionnaires about physical activity, diet and quality of life several times: during and following treatment. Participants will also be given fitness evaluations on 3 occasions.

To Be Eligible to Participate
You Must:

- Be diagnosed with breast cancer
- Be between the ages of 25 and 80
- Be able to carry out normal daily activities
- Have undergone or will undergo chemotherapy

Participates Benefits:

- Information about your fitness and body composition.
- Detailed information about the status of cells of your immune system.

Joining The Project:

For more details, contact Dr. Andrea Mastro (Univ. Park) (814-863-0152) or (814-863-2454) or Shirllyn Mottilla (Hershey) (717-551-5154).
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