Canola Oil Reduces Breast Cancer Risk: Synergistic Effect with an Anticancer Drug

Proposal No. 1 – Chung Park, $42,600

A. INTRODUCTION

(1) Long-term Goals and Supporting Objectives:

Rationale. Over the last three years, the North Central Region Canola Research Program (NCRCRP) has supported our studies on canola oil and breast cancer risk. Our first study was aimed at determining whether or not there is any correlation between canola oil supplementation and reduced breast cancer risk in vivo. Results from that study were encouraging as we found that canola oil may reduce breast cancer risk. The second year funding was requested to confirm the anticancer effects of canola oil using human breast cancer cells. Results from that study confirmed our earlier findings, as we found that canola oil reduces growth of MCF-7 and T47D human breast cancer cells.

Given the positive findings from the initial projects which dealt with the basic correlation between canola oil supplementation and breast cancer growth and development, as well as findings by others, we decided to expand the scope of the project to answer important questions in cancer prevention and treatment. In FY 2009, we proposed a new concept and received funding from the NCRCRP and Northern Canola Growers Association (NCGA). The idea was to study the mechanism responsible for the inhibitory effect of canola oil on breast cancer cell growth, focusing on the potential synergistic effects of canola oil and chemotherapeutic agents on drug resistant human breast cancer cells. We understand that this is a new concept, and addresses one of the main challenges in reducing breast cancer risk. This 2-year project is currently ongoing and is expected to be completed in 2011. Funding received last year (FY 2009) covered the first year of the project (Specific Aims 1 and 2) which encompasses the animal trial portion (begun this past October).

The rationale for this proposal is to request a second year (FY 2010) funding to enable us to carry out Specific Aim 3 of the project which involves tissue sample analysis as well as data analysis and report. To strengthen the project, we will collaborate with Dr. Steven Qian's laboratory at the Pharmaceutical Sciences at NDSU. The objective of this collaboration is to investigate the extent to which drug transport and metabolism (free radicals determination) are affected by dietary canola oil supplementation using an electron spin resonance experiment. The information from the proposed studies may aid pharmaceutical and food industries in the development of nutraceutical products that reduce breast cancer risk by reducing the formation of multidrug resistant tumors. We also hope that studying this essential aspect of cancer treatment may also help open up potential market avenues for canola growers, especially in nutrition and health.

Epidemiological studies have indicated that women consuming high-fat diets have a higher risk of breast cancer than women consuming low-fat diets (1). However, recent evidence suggests that it is not the quantity of lipid but the type of lipid intake that influences cancer risk (2). Current research on dietary oils and cancer prevention is focusing on vegetable and animal oils rich in monounsaturated fatty acids, particularly oleic acid, as well as long chain omega-3 polyunsaturated fatty acids, docosahexaenoic and eicosapentaenoic acids, which have been linked to the reduction in cancer risk (1, 3).

Although the specific mechanism through which omega-3 fatty acids and oleic acid reduce cancer risk is still not clear, evidence suggests that when incorporated into the cell membrane, omega-3 fatty acids and oleic acid may alter lipid membrane fluidity (3, 4, 5). This alteration of the cell membrane integrity may also coincide with an increased susceptibility of cancer cells to cytotoxicity by anticancer drugs, and subsequently, reduced tumor growth and development. Thus, our long-term goal is to establish a mechanistic link underlying the role of dietary canola oil (a major source of both omega-3 fatty acids and oleic acid) supplementation in cancer prevention and chemotherapy.

(2) Background:

Cancer is one of the main causes of mortality worldwide, with breast cancer constituting 10% of all cancer cases (6, 7). The United States currently has the highest breast cancer incidence (8). Breast cancer accounts for nearly 1 in 3 cancers diagnosed in American women (9). The geographical differences in incidence rates suggest a key effect of environmental factors, especially diet, in its etiology (7). The possibility that canola oil may be a useful tool in cancer prevention and therapy seems to have ignited a great deal of interest among scientists and consumers. We therefore hope to establish a mechanistic link through which canola oil reduces human breast cancer risk, information which may be of clinical significance to pharmaceutical companies.

Multidrug Resistance and Membrane Fluidity. Although breast cancer is often considered to be one of the more chemoresponsive tumors, the duration of response is relatively short, and most of the initially responsive breast tumors acquire a multidrug resistance (MDR) phenotype (10). This has been a major impediment and a primary cause of failure in cancer therapy (11, 12). The common cause of MDR is believed to be the enhanced expression of a transmembrane glycoprotein termed P-glycoprotein (Pgp) in cancer cells (12). Pgp is an efflux plasma membrane protein overexpressed in tumor cells, acting as a pump, and effluxing anticancer drugs from the cells (13). A possible route to overcome this is by identifying alternative mechanisms of MDR reversal, such as nonspecific inhibition by membrane fluidizers (11). Membrane fluidity is the extent of molecular disorder and motion within a lipid bilayer (14). It is regarded as a critical modulator of several cellular functions, including mitochondrial functioning, signal transduction, surface receptor binding, and membrane-protein interactions (15). Research on the properties of a tumor cell membrane suggests that alteration of the membrane fluidity affects a number of cellular functions, including chemotherapeutic

cytotoxicity (16). It is suggested that altering the membrane fluidity may interfere with Pgp activity and then modulate the Pgp-mediated accumulation of MDR-type drugs such as doxorubicin (Dox) (11, 17). However, reversal of multidrug resistance may also be due to an increased rate of passive transmembrane drug movement which is also correlated to altered tumor cell membrane fluidity (11). Evidence suggests that when incorporated into the cell membrane, omega-3 fatty acids and oleic acid may alter lipid membrane fluidity (3, 4, 5). This may result in enhanced accumulation of anti-cancer agents within the tumors (11).

Dox, an anthracycline antitumor antibiotic is one of the most useful antineoplastic agents, displaying its cytotoxic effects by, 1) initiation of DNA damage via inhibition of topoisomerase II, 2) DNA intercalation, 3) direct membrane effects, and 4) redox cycling leading to the generation of reactive oxygen species and subsequent lipid peroxidation and DNA damage (18). Evidence has been presented describing Dox-induced hydroxyl free radical formation as one of the most likely mechanisms for its antitumor activity (19). Low hydroxyl radical formation was previously reported in resistant MCF-7 breast cancer cells as compared to the parent MCF-7 cell line after treatment with Adriamycin, a difference attributed to the poor drug transport in the resistant cell line (19). We therefore wish to investigate the effect of dietary canola oil supplementation on drug resistant cancer cells and the mechanism involved.

(3) Preliminary Studies:

Completed Projects. We have studied the effect of canola oil on nitrosomethylurea-induced mammary carcinogenesis *in vivo*, and the proliferation of MCF-7 and T47D human breast cancer cells *in vitro*. *In vivo*, canola oil supplementation (10%) reduced tumor incidence by 20% when compared with the control (20). Tumor volume was significantly higher in the control group when compared with the canola oil group (2.32 ± 0.06 mm^3 and 1.61 ± 0.06 mm^3, respectively, $P = 0.01$) [Fig. 1A]. Survival of rats tended to be higher for the canola oil fed group when compared to the control group ($P = 0.10$) [Fig. 1B]. Immune cell proliferation was higher on the canola oil fed group as compared to the control group ($P = 0.01$) (20). *In vitro*, cell proliferation, caspase-3 activity, and p53 expression were measured (21). In T47D cells, 0.1 and 1 mmol/L doses of canola oil caused 7.3% and 25.2% growth inhibition (Figure 2A) respectively, and similar cell growth inhibitory effects were also evident in MCF-7 cells accounting for 13.4% and 19.4% growth inhibition (Figure 2B) respectively, following 72 hours of canola oil treatment. At 72 hours after canola oil treatment, the increase in caspase-3 of T47D and MCF-7 cells was 16.2% and 8.2%, and the increase in p53 was 8.4% and 14.8% respectively. Treatment with 1 mmol/L canola oil and 10 µmol/L Tamoxifen (a widely used cancer drug) resulted in 49.0% and 5.7% growth inhibition (as compared to Tamoxifen alone) in T47D and MCF-7 cells at 96 hours after treatment respectively. These results suggest that canola oil may have inhibitory effect on human breast cancer growth, and may also enhance therapeutic outcomes of Tamoxifen treatment.

Currently Ongoing Project. At present, we are investigating Specific Aims 1 and 2 of the FY 2009/10 project (phase 1). This project is at an early stage; thus we do not have any data to report at this point. Briefly, athymic nude mice were obtained from Harlan (Indianapolis, IN) and assigned to either the control diet or diet supplemented with canola oil. This was followed with tumor induction by MCF-7/adr to mice. Mice are currently being monitored for tumor development and growth. Although ongoing, the following sections will detail objectives, rationale, significance, and experimental protocol as previously proposed, with an addition of the electron spin resonance (ESR) experiment to be conducted at the Department of Pharmaceutical Sciences at NDSU.

B. OBJECTIVES

The role of omega-3 fatty acids and oleic acid in cancer prevention has received attention over the years in cancer research, with evidence linking them with reduced cancer risk. Although the mechanism through which these fatty acids reduce cancer risk is still not clear, alteration of the tumor cell membrane fluidity needs consideration. We hypothesize that canola oil, due to its fatty acid composition, may reverse multidrug resistance of human breast cancer cells by altering the tumor lipid cell membrane fluidity. The long-term goal is to establish a mechanistic link underlying the role of dietary canola oil supplementation in cancer prevention and chemotherapy. The specific aims of the project are in two phases: Phase 1 (Specific Aims 1 and 2) and Phase 2 (Specific Aim 3). The specific aims are to determine if dietary canola oil supplementation: 1) reduces growth of implanted MCF-7/adr human breast cancer cells (drug resistant cell line) in female athymic BALB/c nude mice, 2) reverses multidrug resistance of mammary tumor cells, and 3) alters tumor cell membrane fluidity, P-glycoprotein expression, and free radicals. Specific Aims 1 and 2 in phase 1 are already being investigated through the FY 2009 funding.

C. RATIONALE AND SIGNIFICANCE

(1) Rationale:

The consumption of canola oil has been increasing over the last few years in North America due to its many health benefits. Canola oil has the lowest concentration of saturated fatty acids (7%) of all eight major vegetable oils, it is high in monounsaturated fatty acids (61%, particularly oleic acid), and it has an omega-6 to omega-3 fatty acid ratio of 1.9:1, which falls within the recommended range of dietary strategies in cancer prevention (22). A recently published study by Wang et al. (23) suggests a correlation between canola oil and reduced cancer risk compared to the use of corn oil. Hardman (24) found that canola oil suppresses growth of implanted MDA-MB 231 human breast tumors in nude mice. The latter study also observed that the amount of omega-3 fatty acids in breast tumor cell membranes increased when mice received a diet supplemented with canola oil as compared to the corn oil supplemented diet (24). It is suggested that once incorporated into the cell membrane, oleic acid and omega-3 fatty acids may alter membrane fluidity, thereby rendering cancer cells more sensitive to cytotoxicity.

In FY 2009, we received funding from the NCRCRP/NCGA to study the mechanism responsible for the inhibitory effect of canola oil on breast cancer cell growth, entailing potential synergistic effects of canola oil and chemotherapeutic agents. This 2 year project is currently ongoing and is expected to be completed in 2011. Funding received from NCCRP/NCGA covered the first year (FY 2009) of the project (Specific Aims 1 and 2) which focused on the animal trial portion initiated recently. Specific Aim 3, which concentrates on detailed cell and molecular determinations for tumorigenesis, is the basis for the present research proposal. To strengthen the project, we will also collaborate with Dr. Steven Qian's laboratory at the Department of Pharmaceutical Sciences at NDSU. The objective of this collaboration is to investigate the extent to which drug transport and metabolism is affected by dietary canola oil supplementation using an ESR experiment.

(2) Significance:

The prevention and treatment of cancer is one of the most pressing challenges facing scientists and public health policy-makers (25). Although breast cancer is often considered to be one of the more chemoresponsive tumors, the duration of response is relatively short, and most of the initially responsive breast tumors acquire an MDR phenotype (10). This has been a major impediment and a primary cause of failure in cancer therapy (11, 12). The common cause of MDR is believed to be the enhanced expression of Pgp in cancer cells, which pumps anticancer drugs out of the cancer cells (12). Clinical studies have demonstrated that approximately 40–50% of primary carcinomas express Pgp (10, 26). Therefore, the altered tumor cell membrane fluidity, enhanced drug uptake (as a result of reduced Pgp activity), and reduced tumor growth due to canola oil supplementation may have potentially significant implications in breast cancer prevention and therapy. Data on free radicals is essential to determine the extent to which canola oil supplementation affects Dox transport and metabolism.

The implantation and growth of human breast cancer cells in athymic nude mice has been a very useful tool in prevention and treatment research for human cancers (27, 28). Therefore, the use of canola oil, athymic nude mice, Dox, and MCF-7/adr human breast cancer cells is vital in extrapolating the findings to the potential clinical application of breast cancer prevention and treatment. Although data on canola oil and breast cancer risk exists, the mechanism underlying this link is not yet understood, and this information may be important for dietary counseling of human breast cancer patients. If the proposed study demonstrates that dietary canola oil supplementation increases the sensitivity of the cancer cells to Dox, then there is a possibility of developing an effective nutritional regimen to aid in human breast cancer prevention and therapy.

The information from the proposed studies may aid pharmaceutical and food industries in the development of nutraceutical products that reduce breast cancer risk by reducing the formation of multidrug resistant tumors. Dissemination of findings through publication and by presenting at meetings of canola producers as well as national scientific meetings may increase awareness of the health benefits of canola oil

consumption. We therefore hope that studying this nutritional aspect of cancer treatment may help open up potential market avenues for canola growers, especially in nutrition and health. We understand that this concept has never been investigated.

D. APPROACH

(1) Description of the Proposed Activities:

At 42 days of age, female Balb/c nude mice will be assigned randomly to either the control diet or diet supplemented with canola oil. At 56 days of age, mice will receive a subcutaneous (s.c.) injection of the human breast adenocarcinoma MCF-7/adr cells using 1×10^7 cells/inoculation. Mice will be observed daily for tumor development and growth. At 65 days of age, mice will be assigned into four dietary groups: the control diet, canola oil supplemented diet, control diet plus Dox treatment, or canola supplemented diet plus Dox treatment. At 86 days of age, four randomly selected mice per group will be sacrificed for tumor tissue collection. Collected tissues will be used to determine mammary cancer cell membrane fatty acid composition and fluidity, and Pgp expression assays. MCF-7 and MCF-7/adr will also be incubated with canola oil, Dox, and DMPO to determine free radicals formation using an ESR experiment. The proposed research requires 2 years to complete.

(2) Experimental Protocol:

PHASE 1 (First Year Funding):

***Specific Aim 1*. To determine if dietary canola oil supplementation reduces growth of implanted MCF-7/adr human breast cancer cells in female athymic BALB/c nude mice.**

Animal and Diet. All animal procedures will be in accordance with institutional guidelines and approved by the University Institutional Animal Care and Use Committee. Forty female athymic BALB/c nude mice (approximately 5 weeks old) will be obtained from Harlan (Indianapolis, IN). All mice will be maintained in autoclaved microisolator cages housed in a positive pressure containment rack (Thoren Caging Systems, Inc., Hazelton, PA) and acclimated for 1 week to the experimental environment of approximately 25°C and 50% relative humidity. Mice will be provided ad libitum access to autoclaved water and control diet (AIN-93) [Table 1]. At 42 days of age, mice will be assigned randomly to either the control diet or diet supplemented with 10% canola oil, based on our previous study (20). The experimental diets will be formulated to be both isocaloric and isonitrogenous. Mice will have ad libitum access to respective autoclaved diets and water. The 2 week period in between the start of the dietary treatment and tumor injection is essential for dietary fatty acids to be incorporated into the lipid membrane phospholipids (24). Fig. 3 illustrates the experimental protocol starting from 42 days of age until the termination of the experiment at 120 days of age.

Tumor Cell Line. MCF-7/adr (with a specific degree of resistance to Dox treatment, with overexpression of Pgp being the main cause for the MDR) will be obtained from the National Cancer Institute (Bethesda, MD). Cells will be grown in Dulbecco's Modified Eagle Medium (Gibco Invitrogen, Carlsbad, CA), supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco Invitrogen) and 1% antibiotic-antimycotic solution (Gibco Invitrogen) as recommended by the supplier. Cells will be maintained in monolayer or suspension culture in 25 or 75 cm^2 tissue culture flasks (Falcon; BD Biosciences, Franklin Lakes, NJ) at 37°C in a 5% CO_2-humidified atmosphere during experimentation.

Mammary Tumor Implantation and Dox Treatment. At 56 days of age, mice will receive a *s.c*. injection of the human breast adenocarcinoma MCF-7/adr cells using 1×10^7 cells/inoculation (17, 29). Mice will be observed daily for tumor growth and progression, and tumor size will be measured with a Vernier caliper every other day as described previously (30). Tumor volumes will be calculated using the following formula: $[\text{length} \times (\text{width})^2]/2$.

Specific Aim 2. **To determine if dietary canola oil supplementation reverses multidrug resistance of MCF-7/adr human breast tumor cells.**

At 65 days of age, mice will be assigned into four dietary groups: control group (no Dox), canola oil group (no Dox), control group receiving Dox (4 mg/kg) intraperitoneally every 2 days, and canola oil group receiving Dox (4 mg/kg) intraperitoneally every 2 days (17, 29). At 86 days of age, four randomly selected mice per group will be sacrificed for tumor tissue collection (29). Remaining mice will be maintained for tumor measurement until 120 days of age, at which time, the experiment will be terminated. However, tumor size will not be allowed to exceed 2.5 cm each. In addition, if animals are seen to be in distress (e.g. body weight and breathing) they will be euthanized regardless of the size of the tumor. Collected tissues will be used to complete the major part of Specific Aim 3.

PHASE 2 (Second Year Funding):

***Specific Aim 3*. To determine if dietary canola oil supplementation alters tumor cell membrane fluidity, Pgp expression, and free radicals formation. This is aimed at elucidating the mechanism through which canola oil may reverse multidrug resistance.**

Fatty Acid Composition. Tumor cell membrane fatty acid composition will be determined by gas chromatography. Four mice per group will be sacrificed at 86 days of age and tumor tissues collected. Tissues from this experiment will also be used for membrane fluidity and Pgp expression assays. The fatty acid methyl esters will be prepared as described previously (24). Briefly, after the samples have been individually homogenized, membranes will be separated by ultracentrifugation. Lipids will be extracted by the use of Chloroform: methanol, methylated, and then esterified in acetyl chloride-methanol (31). Gas chromatography (6890N, Agilent Technologies, Sunnyvale,

CA) will be used as described in the manufacturer's manual. Peak identification will be done by the use of fatty acid methyl ester standards with a Chem Station (Agilent Technologies, Sunnyvale, CA) (32). The percent of the total methylated fatty acids represent the fatty acid methyl esters.

Tumor Cell Membrane Fluidity. Membrane fluidity will be determined by measuring fluorescence polarization as described by Tang et al. (33). Briefly, tumor tissue cells will be isolated from mice sacrificed for gas chromatography. The fluorescent dye 1,6-diphenyl-1,3,5-hexatriene (DPH) (Sigma Aldrich, St. Louis, MO) will be inserted into the lipid fraction of the plasma membranes. Prepared DPH stock solution will be diluted in PBS and vibrated to prepare the working solution of the probe. This working solution will be prepared immediately before use. Once prepared, 2 mL of the working solution will be added to the harvested cells. After the addition of the working solution, the cell suspension will be incubated at 37°C for 30 min before washing with PBS. After washing, the cells will be resuspended in PBS. To check light scattering, cell suspensions with no DPH will be assessed similarly. Analyses will be performed with a spectrometer (LS50B, Perkin-Elmer, USA). Vertically polarized light (357 nm) will excite the prepared samples, and emission (430 nm) measured through the polarizer both horizontal (H) and vertical (V) at four fluorescence intensities (I_{VV}, I_{HV}, I_{HH}, I_{VH}) to the excitation polarizer. The degree of polarization will then be calculated using a formula (Pr): $Pr = (I_{VV} - G \times I_{VH}) / (I_{VV} + G \times I_{VH})$, $G = I_{HV} / I_{HH}$. Membrane fluidity $= 0.5 - P / P^2$. Therefore, the lower the Pr, the more fluid the membrane is (34).

P-glycoprotein (Pgp) Expression. Pgp expression will be determined by immunohistochemistry. This analysis will be performed using the avidin-biotin-peroxidase complex method. Mammary tissues collected above will be formalin fixed, processed, and paraffin embedded as described previously (30, 35). Briefly, after deparaffinization and blocking endogenous peroxidase activity, the mammary tissue sections will be incubated with FBS. The sections will then be reacted at 4°C overnight with anti-pgp monoclonal antibodies (Sigma Aldrich, St. Louis, MO) and incubated with biotinylated secondary antibodies (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA). After washing with PBS, avidin-biotin-peroxidase complex solution (Vectastain Elite ABC Kit, Vector Laboratories) will be added and incubated. Bound antibodies will be visualized using 3-3'-diamino-benzidine-tetrahydrochloride substrate solution (Vectastain DAB Substrate Kit, Vector Laboratories). Microscopic analyses will be carried out on a Nikon Microphot-FX upright microscope (Nikon, Melville, NY) with Image Pro-Plus Software (Media Cybernetics Inc., Silver Springs, MD). Positive cells will be counted and results will be expressed as the median of positive cells per mm^2.

Free Radicals Measurements. MCF-7 and MCF-7/Adr cells incubated with/without canola oil will be harvested by trypsinizing, washed twice with ice cold phosphate-buffered saline, pH 7.4, and suspended in phosphate buffered saline at 2.5 x 10^6cells/ml (19). A typical incubation for the determination of hydroxyl radicals will contain in 1 mL: cells, DMPO (100 mM), and Dox. The mixture will be incubated in the presence of air for 10 min. ESR spectra will be recorded on a Bruker EMX (Billerica, MA, USA) ESR spectrometer equipped with a Super High Q cavity (36).

(3) Statistical Analyses:

The number of mice (n = 20, total 40) to be used is the number required to detect a statistical difference (P = 0.05; power = 0.75) of one-third of the standard deviation between treatments. Tumor volume will be analyzed with the nonparametric Kruskal-Wallis test. Data on tumor cell membrane fatty acid composition, membrane fluidity, free radicals, and Pgp expression will be analyzed by two-way analysis of variance (37).

(4) Potential Pitfalls (Feasibility):

We have experience in the experimental procedures described herein including the handling and propagation of human breast cancer cells (30, 35, 38). Fatty acid composition of the tumor cell membrane will be determined by gas chromatography, which is regarded as a precise and rapid method of choice in fatty acid analysis (39). Fluorescence polarization is regarded as the most trusted technique for monitoring membrane fluidity (14). We do not foresee any problems with the use of the technique. However, in case of any unexpected inaccuracy due to the restricted interactions between DPH and membrane lipids, flow cytometry will be used to determine membrane fluidity (40, 41). Immunohistochemistry will be used for determining Pgp expression. In case of any undesirable response, Western blot may be used (13). ESR is a reliable method for determining free radical formation. The Department of Pharmaceutical Sciences at NDSU has been using this experiment with satisfying results.

(5) Expected Outcomes, Uses for Results, and Future Studies:

Based on the results from our *in vivo* and *in vitro* studies, and results by others, we expect dietary canola oil to reduce tumor growth. We also expect that tumor growth will be slower on mice fed canola oil and Dox treatment as compared to the other three diet groups due to altered tumor cell lipid membrane fluidity and Pgp expression. Evidence suggests hydroxyl free radical formation as one of the most likely mechanisms for Dox's antitumor activity. We anticipate that canola oil will increase the formation of hydroxyl radicals. Altering membrane fluidity may affect Pgp expression, which in turn may affect Dox transport, and subsequently, free radical formations (which are responsible for Dox antitumor activity). If this study demonstrates an enhanced susceptibility of cancer cells to Dox due to canola oil supplementation, then there is a possibility of developing an effective nutritional regimen to aid in the efficacy of some chemotherapeutic and cancer prevention agents in human breast cancer. The goal is to investigate the extent to which canola oil affects the efflux and influx of cancer drugs in cancer cells. Therefore, results from the proposed study will allow further investigation of canola oil either singly or in combination with anticancer drugs to reduce breast cancer risk.

(6) Time Line:

The proposed study requires 2 years to complete. Animal trial and mammary tumorigenesis experiment as well as mammary tumor tissue collection will be conducted during the first 12 months (Phase 1). Tissue sample processing and analyses, and the electron spin resonance experiment will be performed during the next 9 months (Phase 2). Data analyses and report writing will be done during the remaining 3 months (Phase 2).

(7) Research Team:

1) Chung S. Park (Principal Investigator), As Professor and Lactation Biologist, he will be responsible for the overall administration of the project.

2) Dr. William D. Slanger, a biostatistician and Director of Institutional Research & Analysis, will assist on data analysis and interpretation.

3) Dr. Steven Y. Qian, Assistant Professor at Pharmaceutical Sciences, will direct the electron spin resonance experiment to determine free radicals formation.

4) Lawrence Mabasa, Ph.D. Student in Lactation Biology will devote 100% of his time to the proposed studies and will be responsible for most aspects of the proposed study.

5) Andrea Fowler, Research Technician in Dr. Park's laboratory will assist in most aspects of the proposed experiment.

E. OUTREACH/EXTENSION ACTIVITIES

Over the last three years, the NCRCRP/NCGA has supported our studies on canola oil and breast cancer risk. Data were presented at the 2008 (San Francisco) and 2009 (San Diego) Annual American Society for Cell Biology meetings and at the 2009 FASEB Experimental Biology meeting in New Orleans (20, 21, 42). Data will also be presented at the November 2010 Canola Research Conference in Long Beach, CA. Efforts to publish a manuscript of these data are currently underway. Our findings will be disseminated through publications such as scientific journal articles and NDSU extension bulletins. Results will also be presented at NCGA and appropriate scientific meetings (e.g., American Society for Nutrition, American Society for Cell biology, and American Cancer Society meetings). It is hoped that this research will increase the awareness of the health benefits (reduction in breast cancer) of canola oil as well as possibly change the public perception on vegetable oil and carcinogenesis, which subsequently may increase consumer demand/market thereby benefiting the canola industry.

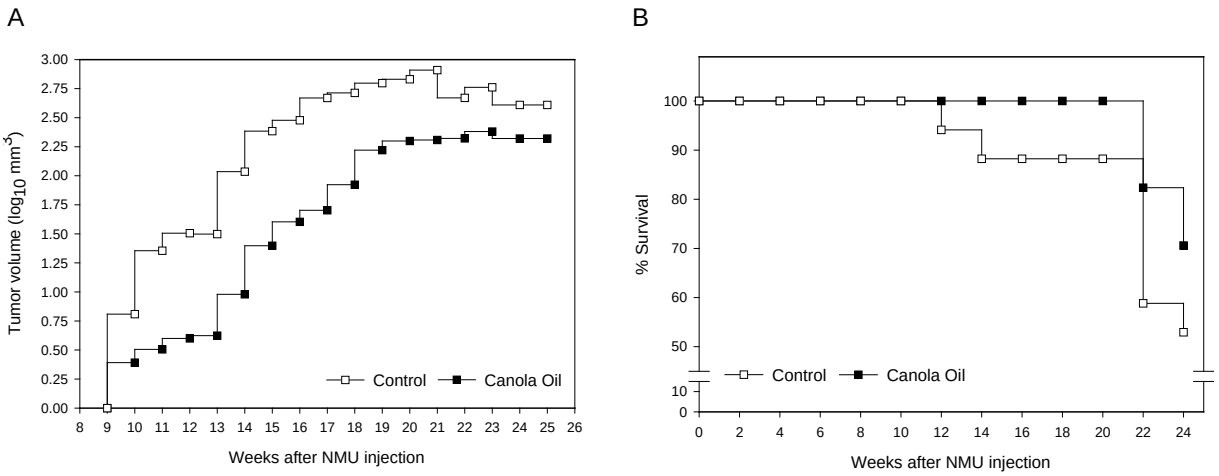

Figure 1. (a) Tumor volumes (mm^3) of rats fed either the control diet or diet supplemented with canola oil beginning 10 wks after NMU injection. (b) Survival (%) of rats fed either the control diet or diet supplemented with canola oil after NMU injection.

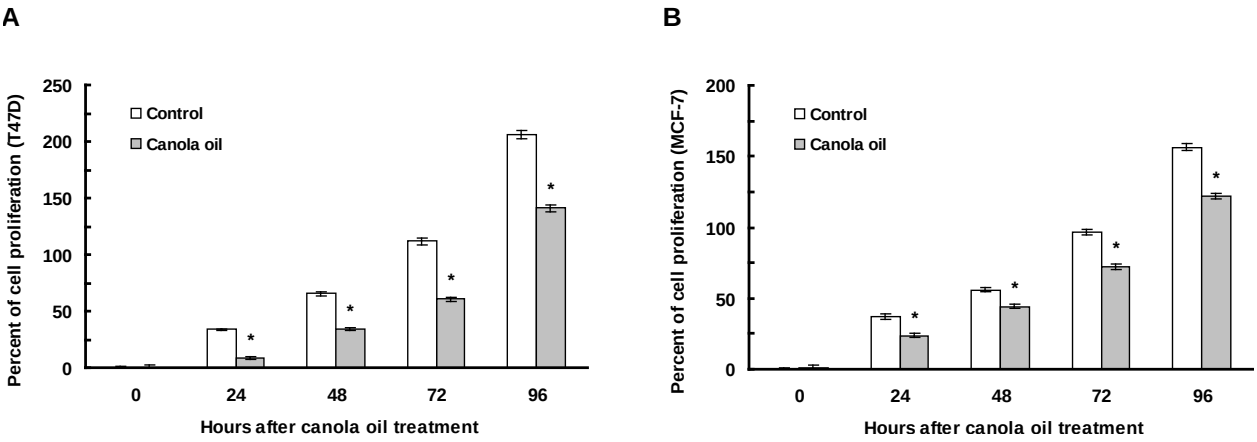

FIGURE 2. Effects of canola oil on time-dependent cell growth. (*a*) T47D cells were treated with 1 mmol/L of canola oil. (*b*) MCF-7 cells were treated with 1 mmol/L of canola oil. Data in *a* and *b* are means ± SEM (*n* = 12) and expressed as percentages of cell proliferation ([treated cell absorbance - initial (seeding) cell absorbance] / initial (seeding) cell absorbance) X 100.

Table 1. Composition of the experimental diets

Item	g/kg Diet
Cornstarch	397.5
Casein	200.0
Dextrinized cornstarch	102.0
Sucrose	80.0
Soybean oil (control)	100.0
Canola oil (treatment)	100.0
Alphacel, non-nutritive bulk	70.0
Mineral mix (AIN-93M-MX)	35.0
Vitamin mix (AIN-93-VX)	10.0
L-Cystine	3.0
Choline bitartrate	2.5
Tert-Butylhydroquinone	0.014
Crude protein (g/kg)	177.0
Gross energy (MJ/kg)	17.8

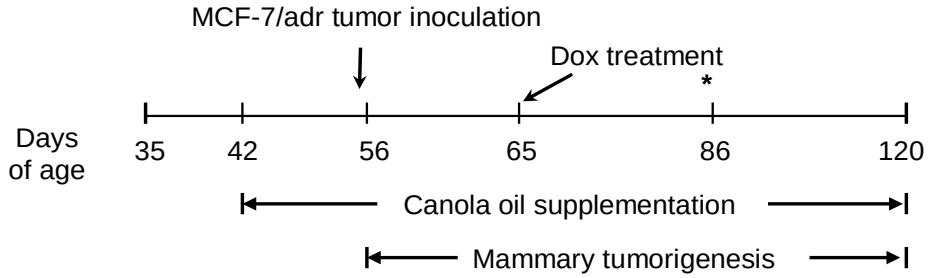

Figure 3. At 42 days of age, female BALB/c nude mice will be assigned randomly to either the control diet or diet supplemented with canola oil (20 mice/treatment). At 56 days of age, mice will receive a *s.c*. injection of the human breast adenocarcinoma MCF-7/adr cells using 1×10^7 cells/inoculation. Mice will be observed daily for tumor growth, and tumor size measured. At 65 days of age, all mice will be assigned into four dietary groups: the control diet, canola oil supplemented diet, control diet plus Dox treatment, or canola supplemented diet plus Dox treatment. At 86 days of age, four randomly selected mice per group will be sacrificed for tumor tissue collection. Remaining mice will be maintained for tumor measurement until 120 days of age, at which time, the experiment will be terminated. Collected tissues will be used for cell membrane fatty acid composition and fluidity, and P-glycoprotein expression assays after the animal trial is terminated. Asterisk represents days in which randomly selected mice will be sacrificed for tissue collection.

BUDGET JUSTIFICATION

Sections A & B ─ Personnel:

Chung Park, Ph.D., Principal Investigator will be responsible for overall administration and direction of the project; however, his time will not be charged to this grant.

Lawrence Mabasa, Ph.D. Graduate Student (effort = 12.0 calendar months; $15,300/year) will develop protocols, perform experiments, analyze data, report/present results, and prepare a journal article. This research will be assigned as his dissertation research.

Fringe Benefits. As requested by North Dakota State University, fringe benefits will be paid as part of direct costs (2% of salary for students).

Section C ─ Equipment:

Purchase of an ultra-low temperature freezer ($7,500). An existing ultra-low freezer in the laboratory is old (21 years) and has reached its maximum functional life and is in disrepair.

Section D ─ Travel:

Funding for domestic travel is requested for the Principal Investigator and graduate student to attend the National Canola Research Conference ($3,000).

Section F ─ Other Direct Costs:

Materials and Supplies (Consumables)

Materials and supply costs represent a reasonable estimate of what is required to carry out the proposed experiment ($16,000).

Chemicals and Supplies: Cell line; magnetic cell sorter; cell culture media and components such as fetal bovine serum, and antibiotics; reagents or kits for gas chromatography and membrane fluidity, and immunohistochemistry assays; reagents for electron spin resonance spectrometer measurement; chemical and fixative kits for histology; Doxorubicin and miscellaneous chemicals; and general laboratory supplies.

Funding is requested for publication in a refereed scientific journal ($800).

Section H ─ Indirect Costs:

Not allowed.