UNIVERSITY OF CALGARY

The Relation Between Dietary and Serum Cholesterol and Mammographic Density as a Risk Factor for Breast Cancer

by

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Abstract

High mammographic density is a well-established risk factor for breast cancer in postmenopausal women, yet little is known about its etiology. Cholesterol has been associated with breast cancer and is linked to endogenous estrogen formation which is independently related to mammographic density. This cross-sectional study examined the association between serum and dietary cholesterol and mammographic density. The sample consisted of 302 healthy, sedentary postmenopausal women, aged 50-74 years, enrolled in the Alberta Physical Activity and Breast Cancer Prevention Trial. In multiple linear regression analysis, no significant associations were observed between total cholesterol, high- and low-density lipoprotein and percent density or dense tissue area. Alcohol consumption modified the association between triglycerides and percent density. There was no evidence of an association between dietary cholesterol and mammographic density. Cholesterol was not a good predictor of mammographic density in this study, however, cholesterol may still be important in breast cancer etiology.

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Dedication

To my parents, Lois, Zen and Ezio

and to my partner, Andrew C. Betik

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List of Symbols, Abbreviations and Nomenclature

ABSP	Alberta Breast Screening Program
AFPRN	Alberta Family Physicians Research Practice Network
ALPHA Trial	Alberta Physical Activity and Breast Cancer Prevention Trial
ANOVA	Analysis of variance
BMI	Body mass index
CI	Confidence Interval
CT	Computed Tomography
DHEAS	Dehydroepiandrosterone sulphate
DXA	Dual x-ray absorptiometry
EPIC	European Prospective Investigation into Cancer and Nutrition
HDL-C	High-density lipoprotein
HR	Hazard ratio
HRT	Hormone replacement therapy
ICC	Intra-class correlation
LDL-C	Low-density lipoprotein
MDA	Mutagen malondialdehyde
r	Pearson correlation coefficient
RR	Relative risk
SD	Standard deviation
SE	Standard error
SHBG	Sex hormone binding globulin
TG	Triglycerides
WHR	Waist-hip ratio

Chapter One: INTRODUCTION

1.1 Rationale

Breast cancer is the second leading cause of cancer mortality among women [Canadian Cancer Society and National Cancer Institute of Canada, 2008], yet the etiology of this disease remains unclear. Known breast cancer risk factors include age, family history of breast cancer, parity, endogenous and exogenous hormone exposure as well as many lifestyle factors (alcohol intake, physical inactivity, obesity and diet) [Dumitrescu and Cotarla, 2005]. Besides these personal and lifestyle risk factors, attention has been given to examining physiologic, biologic and physical changes to the breast that could provide clues regarding the natural history and etiology of breast cancer. Of these factors, one of most interest has been the proportion of dense tissue (stromal and epithelial cells) in the breast, also known as mammographic density, which was first recognized in 1967 by John Wolfe to be of relevance to breast cancer etiology. Wolfe identified that breast tissue patterns were associated with carcinoma in the breast tissue [Wolfe, 1967]. Later, in 1976 he published the results of two retrospective cohort studies in which he categorized women according to four risk patterns and compared breast cancer incidence over time [Wolfe, 1976]. Wolfe noted a gradual increase in breast cancer incidence from the lowest risk pattern to the highest risk pattern. Based on this research, he recommended that routine population-based screening programs with mammography be implemented [Wolfe, 1976]. These screening programs are partly responsible for the reduction in breast cancer death rates that have been observed over the past decade [Canadian Cancer Society and National Cancer Institute of Canada, 2008]. Although mammographic density is now recognized as a good predictor of breast cancer

risk, there is limited understanding on the exact role of increased mammographic density in breast cancer etiology and specifically which factors are involved in the biologic pathway between lifestyle factors, mammographic density and breast cancer risk.

Serum cholesterol and dietary fat have long been hypothesized to be associated with breast cancer. These hypotheses were generated from population-based ecological studies demonstrating that cancer rates and dietary consumption of fat and cholesterol varied across countries in similar patterns [Armstrong and Doll, 1975;McMichael et al., 1984b]. Studies showed that consumption of dietary fat and meat were positively correlated with breast cancer rates in Western populations [Howe et al., 1990]. These studies indicated that cholesterol may be associated with breast cancer as cholesterol is highly associated with the amount of saturated fat and total fat in the diet [Hu et al., 2001]. More recently, investigation of the association between cholesterol and mammographic density has been undertaken. In studying this relation, researchers are attempting to elucidate the etiologic risk factors and biologic mechanisms that link dietary factors, such as cholesterol intake, with increased mammographic density as well as with breast cancer risk. Four studies have examined the relation between serum cholesterol and triglycerides and mammographic density [Aiello et al., 2005;Boyd et al., 1989;Boyd et al., 1995b;Maskarinec et al., 2001], and four studies have examined dietary cholesterol in relation to mammographic density [Boyd et al., 1989;Brisson et al., 1989;Knight et al., 1999;Vachon et al., 2000]. These studies have all produced

inconsistent results. The inconsistency may be attributable to sample size, population characteristic differences, or measurement error of dietary factors.

The motivation for the current research study was to provide new empirical evidence on the associations between dietary and serum cholesterol levels and mammographic density as a means of clarifying these putative associations. By examining this relation in postmenopausal women, this study addresses a specific gap in breast cancer prevention research, namely to increase understanding of the underlying biologic mechanisms operative in breast cancer etiology in the subgroup of women most at risk for this disease. By examining how cholesterol, a modifiable lifestyle risk factor is associated with mammographic density, a known risk factor for breast cancer, this study aims to provide more clarity regarding the potential mechanisms associated with mammographic density and address a new and emerging hypothesis in the etiology of breast cancer.

Besides adding new empirical evidence to the scientific literature on this relation among postmenopausal women, this research study will provide evidence that could directly impact dietary recommendations used as a strategy to reduce breast cancer risk. Since this study examines cholesterol, a modifiable lifestyle risk factor for breast cancer [Hegsted et al., 1993], it has implications for dietary interventions in a population not only at high risk for breast cancer but also at increased risk for other chronic diseases that are associated with cholesterol . Finally, if a relation is found between serum cholesterol and mammographic density and the results are consistent with previous studies,

cholesterol levels could be used as a biomarker of increased risk for breast cancer. This finding, along with future studies confirming the relation, could alter screening modalities for breast cancer.

1.2 Aims and objectives

The aim of this cross-sectional study is to understand the nature of the association between cholesterol and mammographic density, a known risk factor for breast cancer, among sedentary, postmenopausal women. The specific primary study objectives are:

1) to determine if serum cholesterol and triglycerides are associated with mammographic density;

2) to determine if dietary cholesterol is associated with mammographic density.

The secondary objective is:

1) to determine if dietary cholesterol is related to serum cholesterol in postmenopausal sedentary women.

Chapter Two: LITERATURE REVIEW

2.1 Descriptive epidemiology of breast cancer

Breast cancer is the most common cancer among Canadian women. In 2008, it is estimated that 22,400 women will be diagnosed with breast cancer, and approximately 5,300 women will die as a result of this disease.[Canadian Cancer Society and National Cancer Institute of Canada, 2008]. Women in Alberta account for 2,100 of those estimated new incident cases. Incidence rates in Alberta rank second to Quebec nationally with 108 and 109 new cases per 100,000 people expected, respectively. Prevalence estimates made in 2004 stated that 166,000 women in Canada were living with breast cancer [Canadian Cancer Society and National Cancer Institute of Canada, 2008]. Breast cancer is the second leading cause of death due to cancer among women in Canada [Canadian Cancer Society and National Cancer Institute of Canada, 2008] with one in 28 women dying from this disease. Despite these staggering rates, deaths due to breast cancer have been decreasing by 1.7% per year since 1999 [Canadian Cancer Society and National Cancer Institute of Canada, 2008]. This decrease in mortality rates is thought to be attributable to earlier detection because of increased uptake in screening mammography as well as advances in breast cancer treatment.

Incidence rates of breast cancer increase steadily with increasing age with women over 50 years experiencing the highest incidence rates [Canadian Cancer Society and National Cancer Institute of Canada, 2008]. This trend is common in most populations up to age 45, around the perimenopausal period. After this age, the incidence trends tend

to differ by continent. In the US and Sweden, the risk of breast cancer increases up to age 75 [Dumitrescu and Cotarla, 2005], whereas in Japan, incidence rates level off and then slowly decrease [Hulka and Moorman, 2001]. In Canada, the absolute number of new breast cancer cases in 2008 was the greatest in the 50-59 age group, followed by the 60-69 and 70-74 age groups, accounting for 68% of all new breast cancer cases in total [Canadian Cancer Society and National Cancer Institute of Canada, 2008].

As mentioned above, breast cancer incidence and prevalence varies by country of residence. Breast cancer rates have been shown to be high in North America and Northern Europe and low in Asia, Africa and South America [Parkin, 2004]. These differences have led to hypotheses that the environmental factors could be important in breast cancer etiology. Of particular relevance to this theory are the results of migrant studies that have shown that populations that migrate from a low risk country (Italy, Poland for example) to a high risk country (Australia for example) have increased breast cancer rates overtime [Parkin, 2004]. Also, researchers have documented that breast cancer risk increases substantially from first to second and to third generation migrants whose families moved from a low-risk country to a high-risk country (from Japan to the USA for example) [Parkin, 2004]. This observation supports the hypothesis that breast cancer is influenced by environmental factors. Further to this argument, it is also well established that women of higher socioeconomic status and who reside in urban versus rural settings are also at increased risk of breast cancer [Trichopoulos et al., 2008]. These observations have inspired many researchers to examine the role of lifestyle factors,

particularly diet, in breast cancer etiology. These studies will be addressed later in the Literature Review.

Despite tremendous research efforts, the etiology of breast cancer has not been fully elucidated. Hence, examining breast cancer etiology remains extremely important in order to increase scientific understanding that could be used to address approaches to reduce breast cancer. The next section presents an overview of the analytic epidemiology of breast cancer.

2.2 Epidemiology of breast cancer risk factors

2.2.1 Endogenous estrogen exposure

Estrogen exposure is the leading hypothesized cause of breast cancer, especially in postmenopausal women [Muti et al., 2006;Trichopoulos et al., 2008]. Although the results of case-control, cross-sectional and retrospective cohorts have been inconsistent [Muti et al., 2006], a meta-analysis of nine cohort studies observed a dose-response relation between hormone serum concentrations and breast cancer risk [Key et al., 2002]. For example, the relative risk of developing breast cancer for women in the second-lowest quartile of circulating estradiol concentrations compared to the lowest quartile was 1.42 (95% CI: 1.04 to 1.95). The relative risk for the highest quartile compared to the lowest quartile for estradiol concentrations was 2.00 (95% CI: 1.47 to 2.71).

The biological rationale for an etiologic role of estrogen in breast cancer development is also strong. In rodent studies, estrogens have been shown to promote

tumour development in the breast tissue and indirectly promote proliferative activity in in-vitro human breast cancer cells [Clemons and Goss, 2001]. Pike and colleagues in 1983 developed a model that predicts breast cancer incidence by taking into consideration estrogen exposure at time periods of life, that are associated with breast cancer risk [Pike et al., 1983]. The model suggests that breast tissue ageing begins at menarche, when estrogens and prolactin increase, and then decreases with each birth and again after age 40 throughout the perimenopausal period. This “ageing” is representative of the mitotic activity of the epithelial cells in the breast which has been shown to increase and decrease, respectively during these periods. The mitotic activity represents a period of proliferative activity, increasing the number of cells in the breast as well as the potential for genetic damage. Therefore, the more “aged” the breast tissue is, the more estrogen exposure the breast tissue has accumulated and the greater the risk of developing breast cancer [Pike et al., 1983]. Hence, this model provides rationale for the etiologic role of estrogen in breast cancer development.

Another factor that contributes to this association with estrogen is that obese postmenopausal women are at increased risk of breast cancer, while obese premenopausal women are at decreased risk. In postmenopausal women, adipose tissue is the main source of endogenous estrogens [Bernstein, 2002]. Estrone is produced through the aromatization of androstenedione, an adrenal androgen found in adipose tissue. Estrone is converted to estradiol, a more biologically active estrogen. Portions of these estrogens bind specifically to sex-hormone binding globulin (SHBG) which is produced by the liver. The remaining portion of unbound bioavailable estrogens are then free to diffuse to

target cells in the breast and bind to estrogen receptors. The effect of these estrogens binding to receptors in the breast epithelium tissue is increased cell proliferation and inhibition of apoptosis which enhances cancer development (Figure 1). Obesity is also inversely related to SHBG [Stephenson and Rose, 2003]. SHBG binds 30-50% of free circulating estradiol, rendering it biologically inactive. Lower levels of SHBG allow more estradiol to remain free in circulation to bind to estrogen receptors in the breast tissue.

From this brief overview, it is evident that estrogen plays a central role in breast cancer etiology. The following paragraphs explain in more depth, the biological rationale underlying specific risk factors for breast cancer, most of which are associated with estrogens.

2.2.2 Menstrual and reproductive factors

Early age at menarche (<12 yrs), late first full-term pregnancy (>35 yrs), and late menopause (>55 yrs) have all been shown to be associated with an increase in breast cancer risk [Pike et al., 1983]. Early onset of regular menstrual cycles has been found to increase risk by 10-20% compared to those women who experienced later menarche [Dumitrescu and Cotarla, 2005]. The increase in risk is thought to be caused by an extended estrogen exposure in the breast epithelium tissue [Bernstein, 2002]. During the luteal phase of the menstrual cycle, estradiol and progesterone levels are substantially higher than in the follicular phase. Cell proliferation rates in the breast mirror these fluctuations in hormone levels, with high proliferation occurring during the luteal phase

and lower proliferation occurring in the follicular phase [Bernstein, 2002]. A key finding in this research is that the effect of early menarche on estrogen exposure is long lasting. Studies have shown that girls who experience early menarche, also achieve regular menstrual cycles more quickly and therefore have a higher frequency of ovulatory cycles, compared to those who reach menarche later [Bernstein, 2002]. One study found that the majority (80%) of girls who reached menarche before the age of 12 years had ovulatory cycles within 2.5 years, whereas only 60% of girls who reached menarche after age 13, were ovulatory 6.5 years after their first menstrual cycle [Apter and Vihko, 1983]. Having regular ovulatory cycles increases the frequency of high circulating estradiol and progesterone, and therefore increases the proliferative activity in the breast. This observation could explain why late age at menarche is protective against breast cancer.

The above rationale also explains why early age of menopause also decreases breast cancer risk. Women who experience menopause at 55 years or later have a two-fold increased risk of breast cancer compared to women who reach menopause at 45 years or younger [Apter and Vihko, 1983]. This difference in risk is thought to be attributable to the lower lifetime exposure to endogenous estrogens because of the decreased number of regular ovulatory cycles. Women who have had a bilateral oophorectomy have a greater decrease in the risk of breast cancer than those women who achieve early menopause naturally [Bernstein, 2002]. Removal of the ovaries results in immediate cessation of ovarian function, whereas natural menopause occurs gradually, resulting in a longer duration of sex steroid hormone exposure [Apter and Vihko, 1983].

This observed decrease in risk provides strength to the argument that breast cancer is highly associated with life-time estrogen exposure.

Age at first full term pregnancy is also associated with breast cancer risk, however the relation is complex. Higher parity decreases breast cancer risk to half that of nulliparous women, and having a first full term pregnancy before age 20, compared to after age 30 also decreases breast cancer risk by half that of nulliparous women [Dumitrescu and Cotarla, 2005]. However, women having their first full term pregnancy after age 35 have a greater risk of breast cancer than nulliparous women. Hence, a late age at first birth is a risk factor for breast cancer. Although Pike's aforementioned model of breast tissue ageing incorporated first full term pregnancy, it does not fully explain the biological mechanisms associated with the effect on breast cancer risk observed with late first full term pregnancy [Pike et al., 1983]. It has been observed that five to seven years after pregnancy, women, especially older women, are at increased risk of breast cancer. This increase in risk is thought to be attributable to the increase in gestational hormone levels that occur during the first pregnancy [Dumitrescu and Cotarla, 2005;Bernstein, 2002]. It is possible that the high sex hormone exposure so late in the premenopausal period increases the proliferative activity in the breast, thereby increasing breast cancer risk. These mechanisms still need to be elucidated, but it is clear that sex hormones have a central role in breast cancer etiology.

2.2.3 Exogenous Hormone Exposure

Exogenous hormones have also been shown to increase breast cancer risk. The main study that demonstrated this association was the Women's Health Initiative Randomized Controlled Trial [Heiss et al., 2008]. This study was examining the effects of hormone replacement therapy on cardiovascular and breast cancer risk in postmenopausal women when they stopped the trial prematurely because of an observed increase in breast cancer in the group taking combined estrogen-progestin therapy [Heiss et al., 2008]. Notably, the estrogen only group did not demonstrate an increase in risk. The results of four large studies [Heiss et al., 2008;Magnusson et al., 1999;Ross et al., 2000;Schairer et al., 2000] indicate that combined progestin and estrogen therapy increases breast cancer risk from 10% with estrogen only therapy, to 30% with combined therapy, after 5 years of use. It is hypothesized that the addition of progestin increases the proliferative effects of estrogen on the breast tissue [Bernstein, 2002].

2.2.4 Dietary factors

Alcohol intake is associated with increased breast cancer risk and is the only dietary factor that is consistently shown to be associated with breast cancer risk [Michels et al., 2007]. A meta-analysis of six cohort studies concluded that an addition of 10g/day (approximately 1 drink) of alcohol increases breast cancer risk by 9% (95% CI: 4% to 13%) and that a monotonic linear relation exists between increasing alcohol intake and increased breast cancer risk [Smith-Warner et al., 1998]. A more recent publication from the Malmo Diet and Cancer Cohort corroborated these results, reporting that alcohol intake, particularly the consumption of wine, increased breast cancer risk [Mattisson et

al., 2004]. This analysis included 89,602 person years of follow-up and 342 cases of breast cancer. High wine intake was associated with a two-fold increased risk of breast cancer (RR=2.12, 95% CI: 1.24 to 3.60). Total alcohol also demonstrated an increased risk, however the result was insignificant [Mattisson et al., 2004]. Other prospective cohort studies have produced similar results in postmenopausal women [Stolzenberg-Solomon et al., 2006;Tjonneland et al., 2003;Petri et al., 2004]. This relation is also thought to be mediated through estrogen pathways. Numerous studies have illustrated that women consuming 1-2 drinks per day on average have higher circulating levels of estrone, estradiol and dehydroepiandrosterone sulfate (DHEAS) [Dorgan et al., 2001;Onland-Moret et al., 2005;Reichman et al., 1993].

Other dietary variables have also been studied, with special attention to dietary fat and saturated fat. Fat intake was thought to be an important risk factor for breast cancer because of its potential to increase estrogens [Michels et al., 2007]. However, prospective cohort studies have found inconsistent results in the association between fat intake and breast cancer risk. Two research groups have conducted pooled analyses of the prospective cohort studies [Boyd et al., 1993;Smith-Warner et al., 2001a]. Boyd and colleagues (1993) pooled seven prospective studies and observed a pooled relative risk of 1.03 (95% CI: 0.92 to 1.96) for the highest versus lowest intake of fat. The other study conducted by Smith–Warner and colleagues pooled eight separate cohort studies conducted around the world and observed a relative risk of breast cancer of 1.00 (95% CI: 0.98 to 1.03) for every 5% increase in fat intake [Smith-Warner et al., 2001a]. However, Boyd and colleagues updated their 1993 literature review in 2003 and included

all studies published up to 2003 [Boyd et al., 2003]. They found that regardless of study type (cohort or case-control) the highest quartile of dietary fat consumption compared to the lowest quartile was associated with an increase in breast cancer risk (RR=1.13, 95% CI: 1.03 to 1.25). The relative risk for saturated fat intake was similar (RR=1.19, 95% CI: 1.06 to 1.35). This meta-analysis included 45 studies that contained 25,015 cases of breast cancer and 580,000 controls or comparison participants. As well, the results of a recent population-based cohort study found significant increases in breast cancer risk with the consumption of higher levels of dietary fat and saturated fat [Thiebaut et al., 2007]. Corroborating with these results is the Women's Health Initiative trial that examined the effects of a low-fat dietary intervention on breast cancer risk. After an average eight years of follow-up, fat consumption was significantly reduced and the intervention group demonstrated a reduced risk of developing breast cancer (HR=0.91, 95% CI: 0.83 to 1.01), although the results were not statistically significant. These more recent results suggest that there may be an association between dietary fat and breast cancer risk.

A pooled analysis examining the effect of fruit and vegetable consumption in adulthood on breast cancer risk found null results [Smith-Warner et al., 2001b]. These results corroborate with the European Prospective Investigation into Cancer and Nutrition (EPIC) prospective cohort study that included more than 500,000 participants from 10 different European countries [Slimani et al., 2002]. After an average of 5.4 years of follow-up, they found no evidence of an association between fruit and vegetable consumption and breast cancer risk [van Gils et al., 2005]. The relative risk for the

highest versus lowest quartile of vegetable consumption for breast cancer was 0.98 (95% CI: 0.84 to 1.14) and the respective relative risk for fruit consumption was 1.09 (95% CI: 0.94 to 1.25). The consistent lack of association observed in studies of diet and breast cancer have been attributed to either a true lack of association or to measurement error [Michels et al., 2007]. Dietary assessment methods such as like 24-hour dietary recalls and food frequency questionnaires, have long been criticized for both systematic and random errors [Subar et al., 2003], that can result in an underreporting of total energy intake as well as underreporting of other macronutrients, such as protein intake [Subar et al., 2003]. Since this error results in underreporting of dietary components including dietary fat, carbohydrate and alcohol [Subar et al., 2003], an impact on the modeling of diet-breast cancer associations exists with an attenuation of the associations being the most common occurrence. Given this under-reporting, the associations observed thus far between alcohol intake and breast cancer may be even stronger than has been observed.

2.2.5 Obesity

As previously mentioned, obesity in premenopausal years is associated with decreased breast cancer risk, whereas in postmenopausal years, obesity increases breast cancer risk [Dumitrescu and Cotarla, 2005]. A pooled analysis combined seven prospective cohort studies that included almost 340,000 women and 4,385 incident breast cancer cases [van den Brandt et al., 2000]. These investigators found that postmenopausal women with a BMI over 28 kg/m^2 had a relative risk of developing breast cancer of 1.26 (95% CI: 1.09 to 1.46) compared to women with a BMI of less than 21 kg/m^2. Premenopausal women with a BMI of more than 31 kg/m^2 had a relative risk of 0.54

(95% CI: 0.34 to 0.85) compared to women with a BMI of less than 21 kg/m^2. The EPIC cohort study also examined the association between body size and breast cancer risk [Lahmann et al., 2004]. In the analysis that included 103,344 postmenopausal women, those not taking HRT had a 30% excess risk of developing breast cancer if their BMI was greater than 30 kg/m^2 compared to women with a BMI less than 25 kg/m^2 (RR=1.31, 95% CI: 1.08 to 1.59). Among HRT users, there was an inverse association between BMI and breast cancer risk although the trend across tertiles was not statistically significant [Lahmann et al., 2004]. This modification by HRT use was also observed in the Women's Health Initiative, a prospective cohort study conducted in the United States [Morimoto et al., 2002]. These researchers discovered that only non-HRT users demonstrated an increased risk of breast cancer with obesity (RR=2.52, 95% CI: 1.62 to 3.93, comparing BMI > 31.1 to BMI <22.6) [Morimoto et al., 2002]. The increased risk of breast cancer caused by obesity in post-menopausal women is proposed to be attributable to the increased exposure to endogenous estrogens, as previously discussed [Stephenson and Rose, 2003].

2.2.6 Physical activity

There have been many studies that have examined the association between physical activity and breast cancer [Friedenreich and Cust, 2008;Monninkhof et al., 2007;Friedenreich et al., 2001;Vainio et al., 2002]. Physical activity is known to favorably alter breast cancer risk factors such as SHBG, insulin, central adiposity, and sex hormone levels [International Agency of Cancer Research, 2002]. It is through these mechanisms that physical activity is thought to reduce the risk of breast cancer. In a

recent review of the association between physical activity and breast cancer, the relation was strongest in postmenopausal women [Monninkhof et al., 2007]. Case-control and cohort studies demonstrated risk reductions ranging from 20-80% in women who were physically active with the average risk reduction between 25-30% and clear evidence of a dose-response effect with increasing activity levels associated with even greater risk reductions [Monninkhof et al, 2007].

2.2.7 Family history of breast cancer

Family history of breast cancer is known to increase risk of breast cancer [Dumitrescu and Cotarla, 2005]. A meta-analysis that included 58,209 breast cancer cases and 10,986 controls from 52 epidemiologic studies, observed that breast cancer risk increased with increasing number of first-degree family relatives with breast cancer [2001]. The relative risk for people with one first-degree relative with breast cancer, compared to women with no family history of breast cancer was 1.80 (95% CI: 1.69 to 1.91). The respective relative risk for women with three or more affected first-degree relatives was 3.90 (95% CI: 2.03 to 7.49) [2001]. Mutations in high-penetrance genes, BRCA1 and BRCA2, are responsible for 80-90 percent of hereditary breast cancer [de Jong et al., 2002]. These genes, however, are not often found in cases of sporadic breast cancers [de Jong et al., 2002]. All genetic mutations that are known to be associated with breast cancer only account for 5-10 percent of all breast cancers [Dumitrescu and Cotarla, 2005]. This finding implies that breast cancer is largely mediated by environmental factors, or environmental-gene interactions.

In summary, epidemiologic evidence has elucidated several modifiable and non-modifiable breast cancer risk factors. The role of estrogens in the etiology of breast cancer is well developed and clearly indicates the relations that are observed between breast cancer risk and age at menarche, parity, menopause, HRT use, obesity after the menopause, and possibly alcohol and dietary fat consumption. Physical activity acts through more complex pathways and possibly through genetic predisposition. Familial breast cancer, although a very strong breast cancer risk factor accounts for only approximately 5% of all breast cancer cases, and hence is not a major determinant for breast cancer risk. Having reviewed the overall analytic epidemiology of breast cancer, the following sections will focus on the scientific literature regarding the associations between breast cancer and mammographic density and with dietary and serum cholesterol.

2.3 Mammographic Density and Breast Cancer

Mammographic density refers to the proportion of dense tissue, thought to be composed of stromal and epithelial tissue, in the breast [Boyd et al., 2005]. High mammographic density is an established risk factor for breast cancer [Boyd et al., 2005] . In a review of 78 articles on the relation between mammographic density and breast cancer, the authors conclude that density in more than 50% of the breast has an attributable risk of approximately 30% [Boyd et al., 2005]. Furthermore, they state that a density of 75 percent or more increases risk by four to five fold compared to women with primarily fatty breast tissue [Boyd et al., 2005]. Researchers have been aware of the relation between breast cancer and mammographic density for over 40 years. In 1976,

Wolfe was the first to propose a classification system that categorized breast density patterns on mammograms into four risk categories [Wolfe, 1976]. N1, P1, P2 and DY categories rated breast density patterns, respectively, from least at risk to dangerously at risk for breast cancer. Today, Wolfe's classification system is still widely used yet support is gaining for more accurate, reproducible, continuous measurements using computer-assisted methods [Warner et al., 1992]. This technique provides a quantitative measurement of total breast area and dense tissue area thereby permitting an estimation of the proportion of dense tissue to be made. Brisson and colleagues have shown that it is the proportion of dense tissue, as opposed to the absolute area of dense tissue that is more important in classifying women at high risk for breast cancer [Brisson et al., 2003]. Their study also found that estimating percent density provides a more accurate gradient of risk of breast cancer than Wolfe's classifications. Research using more quantitative methods for breast density measurement has demonstrated that the relation between mammographic density and breast cancer is even stronger than once believed [Boyd et al., 2005].

Although there is a strong relation between breast cancer and mammographic density, little is known about the etiology of dense tissue in the breast. Martin and Boyd's review, conducted in 2008, summarizes the mechanisms that may explain the strong association between mammographic density and breast cancer [Martin and Boyd, 2008]. They begin by relating changes in mammographic density to the mitotic activity of the breast modeled by Pike and colleagues in 1983 [Pike et al., 1983]. Mitotic activity of the breast epithelium is highest following menarche, slows with each birth and is

lowest after the menopause. With age, mammographic density decreases, is less extensive in parous women compared to nulliparous women, and is lower with multiple births [Martin and Boyd, 2008]. There is also a decline in percent density of approximately 8% that is observed with the menopause [Boyd et al., 2002b]. Pike's model shows that the mitotic activity in the breast results in cell proliferation and therefore increases the probability of genetic damage. Martin and Boyd (2008) suggest that mammographic density may represent a cumulative exposure to stimuli (estrogens) which promote cell division, thus predisposing breast tissue cells to genetic damage [Martin and Boyd, 2008].

Mammographic density is also positively associated with combined estrogen-progestin HRT use and negatively with Tamoxifen [Martin and Boyd, 2008]. The direction of these associations is the same as shown with breast cancer risk. The reduction in mammographic density with Tamoxifen use has been 10% or less in intervention studies [Cuzick et al., 2003]. Mammographic density has been shown to increase 3.1-4.7% over the control group in a one year estrogen-progestin intervention [Greendale et al., 1999]. Other studies have shown similar effects [Boyd et al., 2005;McTiernan et al., 2005;Persson et al., 1997;Rutter et al., 2001].

Parity, menopause, and exogenous hormones are all associated with breast cancer and mammographic density, and display the same directional effect on mammographic density as with breast cancer risk. Body weight is also associated with mammographic density and breast cancer, although in the opposite direction in postmenopausal women.

Increased postmenopausal weight is associated with an increase in breast cancer risk, and with a decrease in mammographic density [Boyd et al., 2006]. With age, fat tissue in the breast increases and dense stromal and epithelial tissue decreases [Martin and Boyd, 2008]. Since excess fat in postmenopausal years is thought to increase breast cancer risk, it is counterintuitive to observe a decrease in percent density with obesity as one would expect mammographic density and obesity to act in the same direction on breast cancer risk. This inverse relation implies that obesity is a negative confounder in the relation between mammographic density and breast cancer, and studies have shown that controlling for body composition variables, does in fact increase the estimated breast cancer risk [Brisson et al., 1984;Lam et al., 2000]. These observations imply that obesity and mammographic density may act independently on breast cancer risk and represent different etiologic pathways.

Mammographic density is associated with many breast cancer risk factors, and is a predictor of breast cancer risk. From the epidemiologic evidence, it seems that estrogen may have an important role in the development of mammographic density, however more research is needed to fully elucidate the biological mechanisms.

2.4 Serum Cholesterol and Breast Cancer

Cholesterol is a fatty molecule that is both manufactured by the liver and absorbed through food consumption. Cholesterol and other fats are transported in the blood stream in the form of particles called lipoproteins. The most common and significant particles are called low-density lipoproteins (LDL-C) and high-density

lipoproteins (HDL-C) [1994]. Cholesterol has many functions in the body, but the one of greatest importance to breast cancer is its function as a precursor to sex hormone synthesis (Figure 2). While these hormones play many important roles in female physiology, of relevance to this study is their strong association with increased breast cancer risk. Estrone and estradiol are two sex hormones that have been especially linked to this process. As previously stated, for postmenopausal women, the primary source of estrogen is from the aromatization of androstenedione, an adrenal androgen found in fat tissue [Bernstein, 2002]. In an earlier review on breast cancer risk factors, women with higher levels of circulating estrogen had up to 3.6 times the risk of breast cancer compared to women with low concentrations of endogenous estrogens [Hulka and Moorman, 2001]. Since cholesterol is a precursor to this process, it is hypothesized that high cholesterol levels will increase the amount of bioavailable estrogen, increasing the risk of breast cancer [Vatten and Foss, 1990]. Complicating the relation is the established observation that estrogen decreases circulating levels of cholesterol [Barrett-Connor et al., 1997;Greendale et al., 1999]. In postmenopausal women, estrogen replacement therapy is commonly used to improve lipid profiles for primary prevention of coronary artery diseases [Herrington et al., 2000;Grodstein et al., 2001]. Estrogen and cholesterol levels are clearly interrelated. The observed affect of estrogen on serum cholesterol levels suggest that low serum cholesterol levels may be indicative of a high estrogenic environment. Hence cholesterol may be negatively associated with breast cancer risk.

Epidemiologic studies that have attempted to illustrate the association between serum cholesterol and breast cancer have produced inconsistent results. In 2005, a small

case-control study examined the association between serum lipids and breast cancer and found that breast cancer patients (n=56) had higher triglycerides (TG) and lower HDL-C levels compared to controls (n=44) [Michalaki et al., 2005]. In 2001, Manjer and colleagues examined the relation between markers of hyperinsulinaemia and breast cancer in a cohort study consisting of 9,738 women [Manjer et al., 2001]. After three years of follow up, 112 pre-menopausal and 157 postmenopausal cases of incident breast cancer were identified. The relative risk for breast cancer in postmenopausal women increased with rising quartiles of serum cholesterol levels, with the highest cholesterol levels corresponding to a relative risk of 1.6 times the risk in the lowest quartile (p-value for trend = 0.05). These results remained statistically significant even after adjusting for other important breast cancer risk factors, such as age, current hormone replacement therapy use, smoking, alcohol consumption, height, weight and oral contraceptive use. The relative risks for pre-menopausal women were non-statistically significant. A case-control study consisting of 54 pre- and postmenopausal incident breast cancer cases and 42 age- and sex-matched controls found similar results [Ray and Husain, 2001]. Ray and Husain examined the relation between lipids, lipoproteins, vitamins and breast cancer and found that cases had significantly higher levels of total serum cholesterol ($p<0.05$), triglycerides and LDL-C ($p<.01$) than controls [Ray and Husain, 2001]. HDL-C was found to be lower in breast cancer patients compared with controls ($p<.001$). A prospective study that focused on Norwegian overweight and obese postmenopausal women found comparable results [Furberg et al., 2004]. With a follow-up of 17.2 years, the researchers found that the risk of postmenopausal breast cancer was inversely related to quartile of HDL-C ($p_{trend} = .02$) [Furberg et al., 2004]. Women with HDL-C levels

above 1.64mmol/L compared to those with levels below 1.20mmol/L had a relative risk of developing breast cancer of 0.75 (95% CI = 0.58 to 0.97). Interestingly, when the analysis was stratified by BMI category (<25kg/m^2 and ≥25kg/m^2), only the heaviest subgroup retained the HDL-C association. In this group the relative risk of postmenopausal breast cancer for the highest versus lowest HDL-C levels was 0.43 (95% CI = 0.28 to 0.67).

While these studies demonstrate a positive relation between cholesterol and breast cancer other studies have shown opposite or null relationships. Eliassen and colleagues (2005) examined statin use and serum lipids and the risk of breast cancer with data from the Nurses Health Study [Eliassen et al., 2005]. This analysis only examined total cholesterol, and not components of cholesterol. In this study, 79,994 women were followed prospectively for 12 years and self-reported their cholesterol levels. The relative risk for breast cancer in postmenopausal women with cholesterol levels greater than 240 mg/dL compared to 180 mg/dL was 1.04 (95% CI: 0.91 to 1.17) [Eliassen et al., 2005]. A limitation of this study was the self-reported nature of the cholesterol values and the narrow range of cholesterol levels. These two factors could have contributed to the null result. A very similar prospective study with 31,209 Norwegian women, observed no evidence of a relation between breast cancer risk and blood lipid levels after 7-13 years of follow-up [Gaard et al., 1994]. After adjustment for known risk factors of breast cancer, the relative risk of breast cancer of women in the highest quartile of total cholesterol compared with women in the lowest quartile was 0.87 (95% CI: 0.61 to1.23). The corresponding relative risks and confidence intervals were 0.82 (95% CI: 0.58 to

1.16) for triglycerides, 1.02 (95% CI: 0.73 to1.42) for HDL-C, and 0.93 (95% CI: 0.67 to 1.29) for LDL-C. Previously, Hiatt and colleagues also found no relation between breast cancer and serum cholesterol levels in a cohort of 95,179 women [Hiatt et al., 1982].

Other studies have shown that HDL-C levels are positively associated with breast cancer or breast cancer risk factors [Punnonen et al., 1987;van Stiphout et al., 1987;Heiss et al., 1980;Sacks et al., 1975;Snook et al., 1985;Castelli et al., 1977;Haskell et al., 1984;Matthews et al., 1989;Glueck et al., 1980]. HDL-C and breast cancer have been shown to be higher in northern European countries compared to Asian countries [Punnonen et al., 1987], in women who are nulliparous compared to parous [van Stiphout et al., 1987], and in women of higher socioeconomic status compared to women of lower SES [Heiss et al., 1980]. HDL-C has also been shown to be positively associated with dietary fat intake [Sacks et al., 1975;Snook et al., 1985], alcohol consumption [Castelli et al., 1977;Haskell et al., 1984], endogenous hormones [Matthews et al., 1989], and leanness in premenopausal women [Glueck et al., 1980]. All of these factors associated with HDL-C are known or postulated to also increase breast cancer risk.

The research literature proposes that cholesterol may be a biologic marker of increased risk for breast cancer, yet the results are inconsistent. The inconsistency could be attributed to the small sample sizes used and the complex nature of cholesterol, estrogen and breast cancer [Eliassen et al., 2005]. In addition, in some studies, modifying effects of statin use and other cholesterol-lowering drugs were not considered in these

analyses. Omission of medication data could contribute to the inconsistent results observed. It appears that abnormal levels of HDL-C, whether too high or too low, are associated with breast cancer risk.

2.5 Dietary Cholesterol and Serum Cholesterol

Although most cholesterol in the body is produced by the liver, food sources can exacerbate serum cholesterol, LDL-C, HDL-C and triglyceride levels over time. In food, cholesterol is found in eggs, dairy products, meat, and poultry with particularly high levels contained in egg yolks and organ meats. Fish generally contains less cholesterol than other meats, but some shellfish is high in cholesterol [Roehl E, 1996]. Fatty foods also contribute to high cholesterol levels.

If dietary cholesterol has a significant effect on serum cholesterol levels, dietary interventions focusing on low cholesterol diets could modify breast cancer risk. Researchers have, however, had difficulty producing consistent results in dietary interventions. It is thought that there are responders and non-responders to dietary cholesterol intake [Glueck et al., 1980;Katan et al., 1986;Katan and Beynen, 1987;Katan et al., 1988]. A review on serum responses to dietary fat and cholesterol concluded that apolipoprotein gene polymorphisms are associated with an individual's plasma lipid response to dietary fat and cholesterol [Abbey, 1992] and this response may account for the differences between individuals, suggesting that genetics may play role in responsiveness to dietary changes. It has been counter argued, however, that it is rare for serum cholesterol levels not to respond to dietary changes in cholesterol, and that it is

simply the magnitude of response that varies [Katan et al., 1986;Katan et al., 1988]. In 1987, Katan and Beynen used a repeated measures design to examine the effects of a high-cholesterol diet on serum cholesterol levels in people whose serum cholesterol level was unusually susceptible to consumption of cholesterol [Katan and Beynen, 1987]. They fed 32 participants a low-cholesterol diet for the first half of the trial and then followed with a high-cholesterol diet for the second half of the study and measured the change in serum cholesterol level. These researchers found a positive correlation between dietary cholesterol and serum total cholesterol ($r = 0.41$, $p<0.05$) as well as with HDL-C, yet the findings were not statistically significant ($r = 0.31$, $p=0.09$). It appears as though dietary cholesterol may influence serum cholesterol, although this effect may depend on the genetic predisposition of the individual and their responsiveness to dietary cholesterol.

Some studies have examined dietary interventions in women at risk for breast cancer. Henderson and colleagues randomly assigned 45-65 year old women to either an intensive low-fat diet intervention or to a control group [Henderson et al., 1990]. This pilot project's aim was to test the feasibility of a long term dietary intervention in women at risk for breast cancer. After two years of follow-up, women on the low-fat diet had significantly lower serum cholesterol levels than the control group with a between group difference of 15.5 mg/dL, corresponding to 125% of the expected difference. However, the Women's Health Initiative, for which the previous study was the pilot study, did not find significant changes in any of the cholesterol levels after three years of follow-up [Prentice et al., 2006]. Adding to these two studies is a regression analysis that combined

the results of 141 different groups of subjects from studies that examined the effects of dietary cholesterol on serum cholesterol [Hegsted et al., 1993]. The authors concluded that dietary cholesterol and fatty acids do increase serum cholesterol and must be considered in studies that examine the effects of these factors. This study illustrates that diet can have an effect on serum cholesterol levels. It is not clear however, whether or not dietary cholesterol affects breast cancer risk.

2.6 Dietary Cholesterol and Breast Cancer

The varying incidence rates of breast cancer observed across the world have led researchers to believe that lifestyle factors must be associated with this disease [Parkin, 2004]. Diet is one lifestyle factor that has long been proposed to contribute to these differing breast cancer rates [Armstrong and Doll, 1975]. Ecological [Prentice and Sheppard, 1990] and case-control studies [Boyd et al., 2003] have all shown a positive association between dietary fat and breast cancer risk. It has also been shown that cholesterol in the diet is directly associated with levels of saturated fat in foods, therefore cholesterol cannot be ruled out as being a contributing factor to this observed relationship [Hu et al., 2001]. Cohort studies examining the association between dietary cholesterol and breast cancer have not found evidence of an association [Michels et al., 2007]. A meta-analysis of seven major cohort studies found no association between dietary consumption of cholesterol and breast cancer [Hunter et al., 1996]. Out of the seven cohort studies, only the Nurses' Health Study found a weak positive relation between cholesterol intake and breast cancer risk in 89,494 women who were enrolled in the study (RR = 1.12, 95%CI = 1.03-1.23) [Willett et al., 1992]. However, a more recent analysis

of data from the Nurses' Health Study did not find evidence of an association between dietary cholesterol and breast cancer [Eliassen et al., 2005]. Postmenopausal women consuming greater than 240 mg/dL of cholesterol compared to less than 180 mg/dL of cholesterol had a relative risk of breast cancer of 1.04 (95% CI: 0.91 to 1.17). In 2006, they repeated the analysis by categorizing breast cancer by estrogen/progesterone receptor status and still found no evidence of an association [Kim et al., 2006].

Although dietary cholesterol used to be of great interest to cancer scientists [McMichael et al., 1984], more recent studies examining diet and breast cancer focus on fat consumption and do not report dietary cholesterol in the results [Prentice et al., 2006;Thiebaut et al., 2007]. As reviewed above, there does appear to be an increased risk of breast cancer associated with higher fat and saturated fat intakes. Studies examining the effects of cholesterol on breast cancer incidence are limited. Although dietary cholesterol is highly associated with saturated fat in the diet [Hu et al., 2001], studies examining the relation between dietary cholesterol and breast cancer have found no evidence of an association [Michels et al., 2007].

2.7 Cholesterol and Mammographic Density

Cholesterol and mammographic density have both been linked to breast cancer, yet little is known about their association with each other. A few studies have examined the relation between serum cholesterol levels and mammographic density [Aiello et al., 2005;Boyd et al., 1989;Boyd et al., 1995b;Maskarinec et al., 2001]. The study design, sample, methods and main results were reviewed in detail (Table 1). Maskarinec and

colleagues were the first group to assess the relation between serum cholesterol and mammographic density in postmenopausal women [Maskarinec et al., 2001]. They recruited 39 multi-ethnic women (16 Caucasian, 10 Japanese, 3 Native Hawaiian, 9 Chinese, and 1 Korean) from a medical centre in Honolulu, HI, an ethnically diverse state. Using both dense breast area and percent density as outcomes, they observed statistically significant inverse associations with HDL-C after controlling for important covariates (B=-0.013 and B=-0.44, respectively for dense breast area and percent density) [Maskarinec et al., 2001]. This study also noted that Asian women had a statistically significant smaller amount of dense tissue in the breast compared to Caucasian and Native Hawaiian women, however Asian women also had greater percent densities than these other ethnicities. This finding was attributed to the smaller breast size of the Asian women [Maskarinec et al., 2001].

Aiello and colleagues measured baseline levels of circulating sex hormones (free estradiol), insulin-like growth factor I, serum lipids and mammographic density in 88 sedentary, postmenopausal women enrolled to participate in a 12 month exercise intervention [Aiello et al., 2005]. Mammographic density was estimated using computer-assisted technology. The women were currently not using hormone replacement therapy (HRT) and had a body mass index greater than 25 kg/m^2. When mammographic density was assessed as a continuous variable, total cholesterol and LDL-C levels were positively associated with mammographic density in former users of HRT (p=0.053 and p=0.047, respectively). In never users of HRT, the observed associations were in the opposite direction. Both total cholesterol and LDL-C were inversely associated with quartiles of

percent density (p=0.044 and p=0.071, respectively). When percent density was considered as a categorical variable, positive associations were observed with total cholesterol and LDL-C in former users of HRT, corroborating with the categorical analysis. There was no evidence of an association between cholesterol levels and mammographic density in never users of HRT in the categorical analysis. The latter study did not find any evidence of a relation between HDL-C and mammographic density, like the former study. Since these populations were quite different in terms of ethnicity, it would be important to repeat these measures in larger samples to test the repeatability of the results.

An earlier study examined serum cholesterol and plasma lipids in 46 pre-menopausal women [Boyd et al., 1989]. This study differed in its measurement of mammographic density, using Wolfe's patterns instead of computer-assisted methods. In the univariate analysis, HDL-C was positively associated with high risk mammographic density patterns and LDL-C and TG were inversely associated with high risk mammographic density patterns. However, in a multivariate analysis that controlled for the effects of percentage body fat, parity, consumption of alcohol and dietary fat, only HDL-C retained statistical significance. Boyd and colleagues later conducted a similar study in pre-menopausal women using continuous measures of mammographic density [Boyd et al., 1995b]. They measured plasma lipids, and lipoproteins in women without breast cancer, but with varying degrees of mammographic density. After controlling for the effects of age and BMI, mammographic density was significantly and positively associated with plasma HDL-C and urinary excretion of mutagen malondialdehyde

(MDA- a marker of lipid peroxidation), and negatively with LDL-C, triglycerides, and apoprotein B (a protein constituent of LDL-C). These results corroborate with their previous findings. In the multivariate analysis, however, only apoprotein B and urinary excretion of MDA remained significantly associated with mammographic density.

Whether or not these results can be compared to the studies conducted with postmenopausal women with regards to risk of breast cancer is questionable, as the disease experience differs by menopausal status [Friedenreich, 2004]. Regardless, these studies do imply that cholesterol could be related to mammographic density, although the cholesterol constituent of most importance to mammographic density is not clear. Low-density lipoprotein and HDL-C were found to be both positively and inversely associated with mammographic density, hence more research may be needed to clarify this association. However, the results of the studies between never users of HRT [Aiello et al., 2005] and premenopausal women [Boyd et al., 1989;Boyd et al., 1995b] are consistent since LDL-C was inversely associated with mammographic density. In users and past-users of HRT, the trend was in the opposite direction with an inverse association found with HDL-C [Maskarinec et al., 2001], and a positive association found with LDL-C [Aiello et al., 2005]. These results suggest that exogenous hormones may modify the relation between mammographic density and serum cholesterol.

Other studies have examined the role of dietary interventions, that included changes in dietary cholesterol, on mammographic density [Knight et al., 1999;Vachon et al., 2000;Boyd et al., 1989;Brisson et al., 1989]. The design, sample, methods and main

results were examined in detail (Table 2). The Minnesota Breast Cancer Family Cohort examined the diets of 1,508 women who had a family member diagnosed with breast cancer in 1944 [Vachon et al., 2000]. The follow-up began in 1990, when women were asked to fill in a food frequency questionnaire and have a mammogram. Mammographic density was assessed using computer-assisted technology and presented as percent density. No statistically significant associations were found between total caloric intake, total fat, saturated fat or cholesterol and mammographic density in postmenopausal women [Vachon et al., 2000].

In another study, Knight and colleagues examined the effect of a 2 year low-fat, high carbohydrate diet on mammographic density [Knight et al., 1999]. They found significant changes in percent density in the experimental group at the end of the study period, even after controlling for weight loss [Knight et al., 1999]. Examination of specific dietary variables revealed that change in cholesterol intake was the variable most strongly associated with change in dense area and percent density. Specifically, cholesterol intake was reduced from a median of 229 mg/day to 150 mg/day which resulted in an average reduction of 3.27 cm^2 and 3.52 in dense area and percent density, respectively. A breast cancer case-control study also examined this relation in the 645 controls that were included in the study [Brisson et al., 1989]. In this study, total dietary cholesterol was positively associated with percent density, after controlling for age, body weight, parity and education. Lastly, Boyd and colleagues examined the association between dietary cholesterol and mammographic density in a cross-sectional, univariate analysis and did not find evidence of an association between these variables [Boyd et al.,

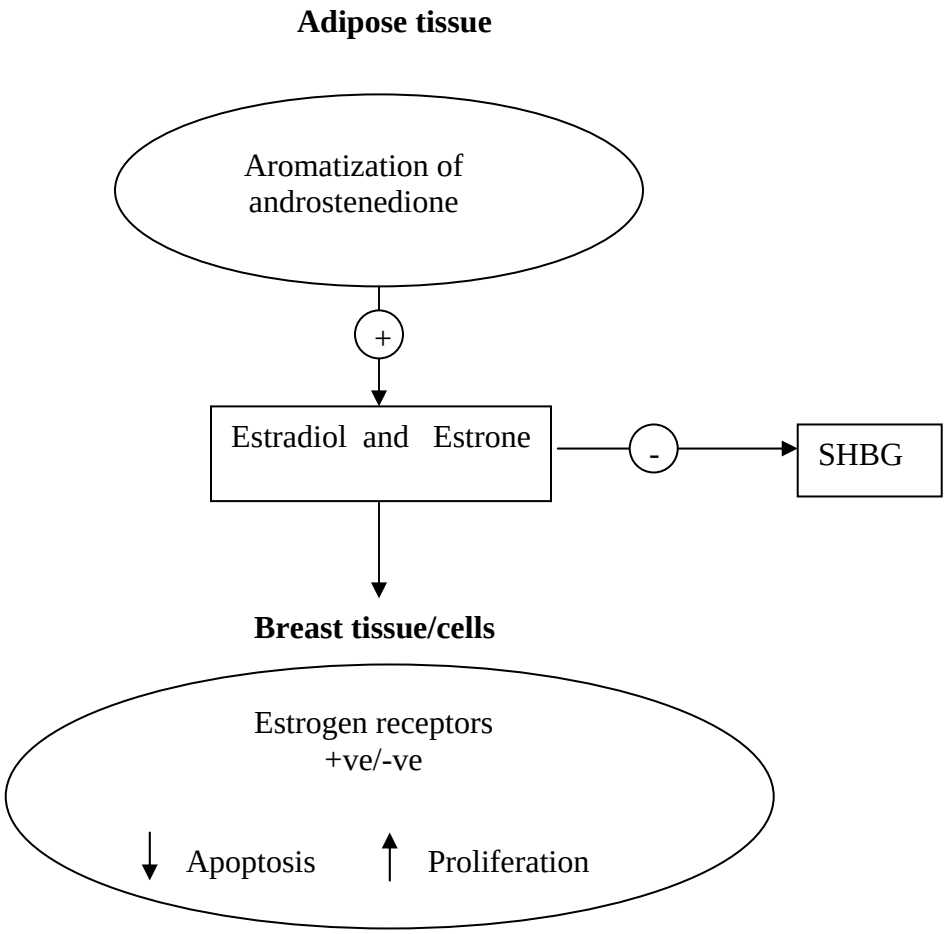
1989]. All of these results combined, suggest that an association between dietary cholesterol and mammographic density may exist, however more research is needed to fully evaluate this association. More recent studies exploring diet and mammographic density have not reported results on dietary cholesterol, and instead have focused on dietary fat or dietary patterns [Nagata et al., 2005;Nordevang et al., 1993;Sala et al., 2000;Takata et al., 2007;Thomson et al., 2007;Masala et al., 2006]. High fat and meat dietary patterns [Takata et al., 2007], a low polyunsaturated fat to saturated fat ratio [Thomson et al., 2007], and high total fat and saturated fat [Nagata et al., 2005] were found to be associated with greater mammographic densities. However, other studies [Masala et al., 2006;Nordevang et al., 1993;Sala et al., 2000] found no significant associations between dietary fat and dense tissue in the breast. The studies examining the association between dietary cholesterol and mammographic density are inconclusive. However, knowing that dietary cholesterol interventions could impact serum cholesterol levels, this relation is worth exploring further.

2.8 Summary

Literature on the relation between dietary and serum cholesterol and mammographic density is limited. By studying the separate components, researchers have provided convincing evidence for a need to concentrate efforts on examining the relation between dietary and serum cholesterol and mammographic density as they relate to the risk of breast cancer. Although the relation is complex, the role of cholesterol in estrogen synthesis implies that cholesterol levels, particularly HDL-C levels, could be a biologic marker for breast cancer risk. Lowering dietary cholesterol can also lower

serum cholesterol thus making cholesterol a likely modifiable risk factor for breast cancer. Preliminary data on the relation between cholesterol and mammographic density suggest that cholesterol may be associated with this known risk factor for breast cancer. More research is needed to confirm these findings in similar and diverse populations. Furthermore, research examining mammographic density should use computer-assisted methods to measure percent density in order to obtain a more accurate estimate of risk. Lastly, studies need to include measures of dietary cholesterol when examining the relation between cholesterol and mammographic density to determine the effect that diet has on this relation.

Figure 1: Role of estrogen in breast cancer etiology in postmenopausal women



SHBG – sex hormone binding globulin

Figure 2: Major pathways of steroid metabolism (adapted from [Miller, 1990])

cholesterol				
↓				
pregnenolone	↔	progesterone		
↓		↓		
DHA	↔	Δ4 androstenedione	↔	testosterone
				↓
		↓		
estrone sulphate	↔	**estrone**	↔	**estradiol**

DHA – dehydroepiandrosterone.

Table 1: Summary of studies examining the association between mammographic density and serum cholesterol

Author, Country, Year	Study design and population*	No. of Participants	Associations with mammographic density	Adjusted variables	Subgroup analysis
Boyd et al., Canada, 1989	Cross-sectional Pre-menopausal Aged 30-50 years	30 with MD≥75% 16 with MD≤25%	**Univariate:** **Total cholesterol:** NS **HDL-C:** +ve (p=0.0001) **LDL-C:** -ve (p=0.001) **VLDL-C:** NS **TG:** -ve (p=0.007) **Final model:** **HDL-C:** +ve (F-value=10.3, p=0.003)	Percent body fat, family history of breast cancer, parity, saturated fat intake (% calories), alcohol	N/A
Boyd et al., Canada, 1995	Cross-sectional Pre-menopausal Aged 29-51 years	273	**Age and BMI adjusted:** **Total cholesterol:** NS **HDL-C:** +ve (p=0.03) **LDL-C:** -ve (p=0.02) **VLDL-C:** NS **TG:** -ve (p=0.02) **Final model:** **All lipids**: NS	Parity, BMI, Apoprotein B, mutagen malondialdyhyde (MDA) excretion, alcohol, sum of skinfold thickness	N/A
Maskarinec et al., USA, 2001	Cross-sectional Post-menopausal Multi-ethnic Mean age range: 58-66 years	39	**Percent density as outcome:** **HDL-C:** -ve (B=-0.44, p=0.001) **Dense area as outcome:** **HDL-C**: -ve (B=-0.013, p=0.03)	Percent density model: BMI, estrogen use, age at menarche, intake of soy protein Dense area model: Estrogen use, family history of breast cancer	N/A

Table 1: Summary of studies examining the association between mammographic density and serum cholesterol

Author, Country, Year	Study design and population*	No. of Participants	Associations with mammographic density	Adjusted variables	Subgroup analysis
Aiello et al., USA, 2005	Cross-sectional Postmenopausal Aged 50-75 years	88	**Categorical MD** **HRT never users (n=43):** **All lipids:** NS **HRT former users (n=40):** **Total cholesterol:** +ve (p=0.03) **HDL-C:** NS **LDL-C:** +ve (p=0.03) **Continuous MD** **HRT never users (n=43):** **Total cholesterol:** -ve (p=0.053) **HDL-C:** NS **LDL-C: -ve** (p=0.047) **HRT former users (n=40):** **Total cholesterol:** +ve (p=0.044) **HDL-C**: NS **LDL-C:** +ve (p=0.071)	Age, ethnicity, years since menopause, percent body fat	Prior hormone therapy use (never vs. former), and latency (< 5yrs vs. ≥5yrs)

MD- mammographic density; HRT-hormone replacement therapy; HDL-C – high-density lipoprotein; LDL-C – low-density lipoprotein; TG- triglycerides; N/A – not applicable; NS – not statistically significant

Table 2: Summary of studies examining the association between mammographic density and dietary cholesterol

Author, country, year	Study design and population*	No. of Participants	Associations with mammographic density	Adjusted variables	Subgroup analysis
Knight et al., Canada, 1999	Randomized controlled trial Premenopausal at baseline, post menopausal at end of study Mean age: 49 years	78	**Percent density:** Dietary cholesterol: +ve (B=5.77, p=0.001) Total fat: +ve (B=9.21, p=0.004) Saturated fat: +ve (B=8.57, p=0.002) **Dense area:** Dietary cholesterol: +ve (B=6.21, p=0.001) Total fat: NS Saturated fat: +ve (B=6.07, p=0.05)	Change in total calorie intake, weight change, family history of breast cancer	N/A
Vachon et al., USA, 2000	Cross-sectional 80% postmenopausal Aged 40-90 years	1508	**Percent density:** Dietary cholesterol: NS Total fat: NS Saturated fat: Pre-menopausal women only -ve (p=0.03)	Caloric intake, age, age^2, BMI, WHR, physical activity, age at menarche, age at first birth, number of births, alcohol, smoking, family history of breast cancer, HRT use, oral contraceptive use	Menopausal status

Table 2: Summary of studies examining the association between mammographic density and dietary cholesterol

Author, country, year	Study design and population*	No. of Participants	Associations with mammographic density	Adjusted variables	Subgroup analysis
Brisson et al., Canada, 1989	Population-based case-control Aged 40-62 years	645 controls	**Total Percent density:** Dietary cholesterol: +ve (p=0.01) Total fat: NS Saturated fat: NS	Age, body weight, parity, education	N/A
Boyd et al., Canada, 1989	Cross-sectional Pre-menopausal women Aged 30-50 years	30 with MD≥75% 16 with MD≤25%	**Univariate:** Dietary cholesterol: NS Total fat: NS Saturated fat: NS	N/A	N/A

N/A – not applicable; NS – not statistically significant; MD – mammographic density

Chapter Three: METHODS

3.1 Study design – ALPHA Trial

The data used in this secondary analysis originate from the Alberta Physical Activity and Breast Cancer Prevention Trial (ALPHA Trial), a two-armed randomized controlled intervention trial conducted in Edmonton and Calgary between 2003 and 2006 that examined the impact of a 12-month moderate-to-high intensity aerobic exercise intervention on biologic mechanisms possibly related to breast cancer risk. There are no papers published yet using these data, hence, a brief outline of the research design, sampling strategies and data collection procedures used in the ALPHA Trial will be discussed with specific attention to the measurements being used in this study.

3.2 Sampling

The eligible population for the ALPHA Trial consisted of women in Calgary and Edmonton who were postmenopausal, between the ages of 50-74, and who were sedentary at baseline. A detailed outline of all of the eligibility criteria is provided in Appendix 1. This population was chosen for reasons important to the aim of the parent study, but that are relevant to the present analysis as well. First, postmenopausal women experience fewer estrogen hormone fluctuations than premenopausal women, making the collection of blood for analysis of estrogen less complicated [Hankinson et al., 1995]. Since estrogen is related to both mammographic density and cholesterol [Clemons and Goss, 2001] it is important for the validity of the serum cholesterol measures, and thus the overall study results, that estrogen levels are constant. Second, women in this age

group who are sedentary can still reduce their breast cancer risk through physical activity [Monninkhof et al., 2007;Friedenreich and Cust, 2008]. Enrolling sedentary women at baseline was important since exercise alters cholesterol levels, thus having a homogeneous study sample provides control for the impact of exercise on the serum measures. Third, the upper age range of 74 was chosen since recruitment was done initially from the Alberta Breast Screening Program (ABSP) for which women up to age 69 years can be screened. Since letters of invitation were sent to all women who had been in the program up to five years previously, women up to age 74 could be recruited for the ALPHA Trial. Lastly, overweight, postmenopausal women are at high risk for breast cancer because of increased endogenous estrogen levels [Lahmann et al., 2004;Cauley et al., 1989;Gram et al., 1997;Nelson et al., 1988]. The primary rationale for the ALPHA Trial was to increase understanding of the biological mechanisms underlying the association between exercise and its influence on breast cancer risk in women who have an increased risk for breast cancer given their age and lifestyle. The ultimate objective of this research was to elucidate how physical activity can be used to reduce risk in these postmenopausal, sedentary women.

The primary objectives for the ALPHA Trial, for which the sample size estimations were based, were to detect differences between the intervention and control groups for serum estrogens and body composition. Based on previous literature, it was expected that women in the exercise versus the control groups would differ in fat mass by 5-10% [Ross and Janssen, 2001] and in estrogen levels by 1-47% [Cauley et al., 1989;Nelson et al., 1988;Verkasalo et al., 2001] by the end of the year-long intervention.

The estimations were based on a normal distribution formula for comparing means based on two independent samples with a two-sided alpha of 0.05 and a power of 80% [Friedenreich, 2002]. Initial group sizes of 167 women were sought in Edmonton and Calgary to allow for 10% loss to follow up. The study enrolled 320 women with exactly half randomized to each of the intervention and control arms of the trial.

The present project is a cross-sectional, descriptive analysis of the baseline data of the women enrolled in the ALPHA Trial. The focus of this analysis is the association between cholesterol and mammographic density in sedentary, postmenopausal women.

3.3 Recruitment and eligibility

Potentially eligible women were identified using letters of invitation from the ABSP and via media advertisements. The databases at the Calgary and Edmonton ABSP were used for the mass mailings. The databases provided information about the eligibility criteria and, therefore, could be used to identify women suitable for the study. The criteria identified at the ABSP included: 1) living in Edmonton or Calgary, 2) 50-74 years of age, 3) English-speaking, 4) radiologist rating of breast density of ≥ 0% and 5) a normal mammographic report as of February 1, 2003. Letters of invitation were sent to 4,543 women by Dr. Tim Terry, the chief radiologist at ABSP, of which 1,284 women agreed to be considered for this Trial based on the initial telephone screening. 2,170 women responded to three separate media campaigns held in Calgary and Edmonton and to pamphlets and posters that were distributed in Calgary and Edmonton, primarily in physicians' offices. These physicians were part of the Alberta Family Physicians

Research Practice Network (AFPRN), a network that is dedicated to improving the care of patients through involvement in research. An agreement was signed between AFPRN and the co-investigators of the ALPHA Trial, in which the AFPRN agreed to mail out recruitment information to 920 family physicians in Alberta inviting them to help recruit women for the ALPHA Trial. Out of these mailings, 67 physicians responded favourably and agreed to display posters and pamphlets in their offices, as well as to discuss the trial with eligible patients.

After an initial telephone screen of all interested women (n=3,454), 2,536 women remained eligible and were further assessed using the Participant Eligibility Questionnaire (PEQ). The PEQ excluded 1,960 more women leaving 576 interested and eligible to participate. A detailed participant flow chart is included in Figure 3 and includes specific reasons for exclusion. Of these women, 542 attended an information session; 308 originating from media campaigns and AFPRN recruiting efforts and 234 originating from the ABSP. After the information session, at which time informed consent was obtained (Appendix 2: Informed Consent Form) and several baseline health questionnaires were completed, a series of tests were conducted to further assess participant eligibility. These tests included in this order: 1) the PARmed-X (signed by their family physician that deemed them healthy to undertake this program), a fasting blood test (to screen for underlying metabolic conditions that would be a contraindication for an exercise intervention of this kind), a sub-maximal fitness test on a treadmill (to ensure that they were neither too fit for the study or unfit for this program (indicated by a maximum oxygen consumption greater than 34.5 ml/kg/min), a mammogram (for those

women who were not recruited through the ABSP to ensure that all had some level of breast tissue density above the fatty tissue level and to get the baseline mammogram). After eligibility was established with these baseline screening tests, a computed tomography (CT) scan and a dual x-ray absorptiometry (DXA) scan were also taken to measure body composition. After these tests, 320 women remained and were randomized into the trial. In total, 155 women from Calgary and 165 women from Edmonton were randomized into the trial. All study participants provided informed consent at the outset of the study and all data have been and will be kept confidential in locked cabinets at the Alberta Cancer Board.

3.4 Data Collection

3.4.1 Serum lipids

Blood samples were collected from all of the participants prior to randomization as part of the screening process. Measures of total cholesterol, high and low density lipoprotein (HDL-C and LDL-C, respectively), and triglycerides (TG) were estimated at baseline in units of mmol/L. LDL-C was calculated using the formula:

LDL-C = Total cholesterol – Triglycerides/2.2 – HDL-C.

A fasting blood draw was taken after a minimum ten hour fast. Participants also recorded the date and time of last food or drink ingested (except water), and the date and time of last exercise performed. They also provided information on all medications, vitamins or herbal supplements that were taken in the 24 hours prior to blood draw (Appendix 3:

Participant Blood Questionnaire). Blood was collected and analyzed at the Calgary Laboratory Services at the Foothills Medical Centre in Calgary using a Hitachi 747 Analyzer (Tokyo, Japan) following a standardized protocol [Calgary Laboratory Services, 2002]. In Edmonton, blood was collected at the Cross Cancer Institute in Edmonton and then sent to the University of Alberta Hospital for analysis of lipids using Synchron LX® Systems Lipid Calibrator (Fullerton, California). The respective labs provided the tubes for the blood taken for screening purposes. The phlebotomist at the laboratory recorded the date and time of blood collection on the bottom of the requisition form as well as the date and time the participant last ate or drank anything (except water) therefore giving two measures of these important variables. These two factors are of interest since recent food uptake produces transient increases in plasma triglycerides and decreases of LDL-C and HDL-C [Calgary Laboratory Services, 2002]. All blood samples were labeled with ALPHA as the person's last name, the study code number as the person's first name and the collection time and date. A unique identifier label was also used for each blood draw. This identifier was used to link the blood sample to the participant ID and information about the blood draw. Those participants that had to return to have further blood drawn were assigned another unique identifier label on the second blood draw. Table 3 outlines the laboratory services that were performed for screening purposes.

Table 3: Blood screening criteria for participants in the ALPHA Trial

Blood Screening Test	**Cut-off values used for exclusion in the ALPHA Trial**
Complete Blood Count	
Hemoglobin	120-160 g/L
Hematocrit	0.36-0.48 L/L
Red Blood Cell (RBC)	4.0-5.6
Mean corpuscular volume (MCV)	82-100 fL
Mean corpuscular hemoglobin concentration (MCHC)	320-360 g/L
Red cell distribution width (RDW)	11.0-16.0%
Platelet count	150-400
White blood cell (WBC)	4.0-11.0
Neutrophils	2.0-9.0
Lymphocytes	0.5-3.3
Monocytes	0.0-0.1
Eosinophils	0.0-0.7
Basophils	0.0-0.2
Lipids and Endocrine	
Total Cholesterol	> 7.44 mmol/L
Low Density Lipoprotein (LDL-C)	> 4.92 mmol/L (if over 5.0 treat and then can return to study if stable after 3 months)
High Density Lipoprotein (HDL-C)	< 0.72 mmol/L
Triglycerides (TG)	> 2.56 mmol/L
Thyroid Stimulating Hormone (TSH)	0.16-7.2 mU/L
General Chemistry	
Fasting Glucose	> 7.0 mmol/L
Creatinine	36-120 umol/L
Alanine Aminotransferase (ALT)	> 48 U/L

All blood was collected, processed and stored within 12 hours of collection at the respective laboratories. The fasting time was only collected for 226 participants. In Edmonton, the participants were not accompanied to the blood draw with a research staff member and therefore the Participant Blood Questionnaire was not checked over for completeness before blood draw. Even though the laboratory staff had instructions to record the fasting time, this record was not done for all participants. There is no reason to believe these women had not fasted though as it is standard procedure for lab personnel to ask the participants if they had fasted for 12 hours. If they had not fasted, the laboratory staff asked them to return another day. The mean time spent fasting was 12.9 hours with a range of 10 to 20 hours. Only two participants exceeded 16 hours of fasting, one of which was 17.8 hours and the other was 20 hours. These participants had their blood collected at 9:30 a.m. and 2:57 p.m., respectively. Time from collection to analysis was on average three hours and 11 minutes with a range of one hour eight minutes to seven hours and 23 minutes. These values were well within acceptable ranges for blood processing.

3.4.2 Dietary cholesterol

Dietary cholesterol was assessed using the National Cancer Institute's 12-month Diet History Questionnaire, modified for Canadian dietary and nutrient content differences [Csizmadi et al., 2007;Subar et al., 2001;Thompson et al., 2002]. The diet questionnaire consists of 130 food items and includes both portion size and dietary supplement questions (Appendix 4: Diet History Questionnaire). Completion of the questionnaire takes, on average between 1-1.5 hours. This self-administered

questionnaire was completed at baseline prior to randomization into the exercise and control groups. Dietary cholesterol (mg), a main exposure, and total fat (g), saturated fat (g), total calories (kcal) per day and alcohol consumption (servings/day), covariates of interest for this study, were estimated from these data using the *Diet*Calc* software program (version 1.4.2). The Canadian version of the questionnaire had been previously developed by researchers at the Alberta Cancer Board [Csizmadi et al., 2007]. This study set out to modify the Canadian version of the Diet History Questionnaire (DHQ) to better reflect food availability and fortification practices in Canada. In total, 2411 foods were identified that were most likely to differ between Canada and the US. Twenty-five percent of these foods were modified for folate, 11% for vitamin D, 10% for calcium and between 7 and 10 percent for remaining nutrients. These modifications were made in the Canadian nutrient database after being tested in a population of 13,181 men and women aged 35-69 years [Csizmadi et al., 2007]. The modified version of the DHQ is now being routinely used in several observational epidemiologic studies conducted by researchers at the Alberta Cancer Board including in the Alberta Cohort Study (Tomorrow Project®) [Bryant et al., 2006].

3.4.3 Mammographic density

Mammographic density was measured at baseline and at the end of study as an ALPHA Trial secondary outcome. All mammograms were taken at the ABSP sites using the same type of mammography unit at both sites. The median time between the information session and the baseline mammogram was 13 days. Information about the use of mammograms was explained to the participants at the initial information session

and informed consent was obtained for access to the participant's mammograms in order to determine mammographic density. The radiologists at the respective ABSP centres read all mammograms. The radiologists routinely assigned a subjective rating of density in five categories (fatty, <25%, 25-49%, 50-74%, ≥75%). This information, along with the date of the mammogram, date of birth of the participant, the ALPHA ID and the Screen Test ID were sent to the ALPHA Trial Study Coordinators and recorded in the Access® tracking database. ABSP staff followed up any abnormal mammograms.

Mammograms were then sent to Princess Margaret Hospital in Toronto where they were digitized using a Lumisys® 85 laser film scanner. Subject identifiers were permanently cropped from the saved images. Mammograms were read by Dr. Norman Boyd, a co-investigator on the ALPHA Trial and an international expert on mammographic density. Dr. Boyd was blind to any information about the subject at the time of digitization and reading of the mammograms. This precaution ensured that reader bias would not be an issue in the results of the density estimations made from the mammograms.

Mammograms were read in five batches of 140-142 films by Dr. Boyd using Cumulus 1.08® and Cumulus 3®, an interactive computer imaging software program developed by Dr. Martin Yaffe, a medical physicist at Sunnybrook Hospital in Toronto and co-investigator on the ALPHA Trial who has pioneered these methods for estimating the area of mammographic density. Only one mammogram for each time point was sent for each subject. In most cases, the right craniocaudal view was sent unless there was

scar tissue or another mark on the right breast. In those cases the left craniocaudal view was used. Baseline and end-of-study mammograms of the same participant were sent in the same batch to reduce the potential of introducing measurement error into the final analysis. If a participant did not return for the end-of-study mammogram, the baseline mammogram was not analyzed. This stipulation affected 17 participants, nine who were lost to follow-up during the trial and eight who did not return to have their end-of-study mammogram. Thus, the sample size was reduced to 303 women for this analysis.

Dr. Boyd set the density thresholds to distinguish between dense and non-dense areas of the breast and estimated total breast area and total dense tissue area for each participant. The outlines around the breast distinguish between the breast and the background and density thresholds distinguish between fatty tissue and dense tissue. Both thresholds are sensitive and can alter the appearance of the breast composition therefore it is important that only one investigator read the mammograms for each aspect for the reason of reliability. A subset of 100 mammograms was read by an inexperienced reader, Dr. Christy Woolcott, so that measures of inter-rater reliability could be calculated. Dr. Woolcott followed the same procedures as outlined above for setting the thresholds and reading the mammograms as done by Dr. Norman Boyd. Dr. Woolcott also attended a workshop training so that she was familiar with the techniques. To assess for intra-rater reliability Dr. Norman Boyd re-read 30 pairs of films between batches and 20 pairs of films within the same batch so that inter- and intra-batch reliability could be calculated, respectively. The measurements used in this project are those of Dr. Norman Boyd and the intra-class correlations were calculated by Dr. Christy Woolcott.

Using total breast area and total dense tissue area, we were able to estimate percent dense area as well as total non-dense area. Both measures of absolute dense tissue area and percent dense area have been shown to provide accurate risk assessments and are superior to previously used broad categorical measures of mammographic density [Brisson et al., 2003].

After the mammograms were read, they were returned to the ABSP and the density data were sent to the ALPHA Trial staff for cleaning and analysis. One step in this process was to transfer the measures from pixels to centimetres squared. To accomplish this data conversion, we multiplied the total breast area and total dense area by a factor of 0.000676. This conversion factor was used because one pixel in the digitized images was equivalent to 6.76×10^{-4} cm^2.

The time between baseline mammogram and blood draw was assessed to check the temporal nature of the outcome and main predictor variables. On average, blood draw was taken 46 days (SD=68.3) after the mammogram with half of the participants having their blood drawn 26 days afterwards. However, the days between these two measurements ranged between having the blood drawn 247 days before the mammogram to 362 days after the mammogram. All of these data were checked for data entry errors as well as reasons for the time discrepancies. In total, there were 58 participants where the difference between these two measures was greater than 90 days and 22 participants where the difference was greater than 180 days. Crude regression models that included

and excluded these participants were run to determine if these extreme cases affected the relations of interest. No significant differences were found between the estimated coefficients. From a biological viewpoint, we would not expect lipid levels or mammographic density levels to change considerably over a one year period [Keleman et al., 2008]. The commencement of lipid lowering drugs or estrogen replacement therapy would be of concern as these medications/hormones would affect lipid levels and mammographic density, respectively and would thus alter the cross-sectional nature of the data. Since the women were already enrolled in the study and they knew that beginning estrogen therapy would make them ineligible for the study, there is little risk of women commencing estrogen therapy between these two measurement time-points. The women could have started lipid-lowering drug therapy, but this information would have been recorded on the participant blood questionnaire for those that had their blood drawn following the mammogram, which was the majority of the women who had a difference of 90 or 180 days. These medication data were taken into consideration in the exploration of effect modification and confounding and neither statin use nor other cholesterol lowering drugs had an effect. Since we did not notice a difference in the regression analyses and there is little evidence to suggest that this temporal issue would affect the validity of our results, we decided that these participants would remain in the analysis.

3.4.4 Covariates

All covariates were measured at baseline and some of the factors were measured using a variety of different methods. Demographic variables (age, education, marital status), reproductive variables (age at menarche, age at menopause, number of live births,

and age at first pregnancy), hormone history use (hormone replacement therapy (HRT) use, duration of HRT use, years since HRT use), and disease history variables (history of hypercholesterolemia, history of thyroid disease, family history of breast cancer) were all obtained using the Baseline Health Questionnaire (Appendix 5). This questionnaire was administered at the first information session and was also used as part of the eligibility screening for the ALPHA Trial. Anthropometric covariates were measured using three different methods. First, weight, height, waist and hip circumferences were measured at the baseline fitness tests by the exercise trainers. Second, subcutaneous and intra-abdominal fat were measured using a CT scan. Third, total fat tissue and total lean tissue were measured using DXA scans that permitted the estimation of percent body fat. Dietary variables (total calories, fat, saturated fat, carbohydrates, protein, mono-unsaturated fat, poly-unsaturated fat, and alcohol consumption) were measured using the DHQ previously discussed.

3.5 Data Analysis

A cross-sectional analysis to examine the association between cholesterol and mammographic density was performed. This analysis used baseline data from 303 sedentary, postmenopausal women between the ages of 50-74 years who voluntarily enrolled in this study for whom complete data were available for the two main factors under investigation, namely, mammographic density and serum and dietary cholesterol. The distribution patterns of the outcome variables, percent density, absolute dense area and all explanatory variables, total cholesterol, HDL-C, LDL-C, TG and dietary cholesterol, were examined using descriptive statistics (mean, median, standard

deviations, box-plots and histograms). With the use of graphs, outliers were detected and assessed to determine if they were true outliers or as a result of incorrect data entry. This review resulted in the deletion of one observation from the total cholesterol and TG analysis. In these instances, the lipid levels were deemed to be measurement errors as the values were beyond the normal range of lipid levels. A Cook's D test [Katz, 1999] was performed after deletion of these observations and for both total cholesterol and TG, the predictions of the linear equation improved significantly. This graphing process was completed with each covariate included in the analysis.

The main explanatory variables were then examined against the outcome variables to check for assumptions of linearity (plotting the conditional mean against lipid levels), normality (plot of the residuals against the quantiles of the standard normal [Katz, 1999]) and constant variance (plot of residuals against fitted values of the outcome). Based on these diagnostic tests, it was observed that percent density and total dense area did not meet the assumptions required for linear regression. Multiple transformations of these two outcome variables were used and included square-root, log, squared, and reciprocal transformations. Of these, square-root transformation modified the fit of the model best to meet the assumptions of linear regression, particularly the assumptions of linearity and normality. Thus, square-root percent dense area and square-root dense area were used as the two main outcome variables.

Multiple linear regression was the most appropriate statistical method to use in this analysis because of the continuous nature of mammographic density, and for the

assessment of confounding and effect modification. Models were developed for each lipid and dietary cholesterol separately, one with square-root percent density as the outcome and the other with square-root dense tissue as the outcome. Therefore, ten models in total were constructed. Covariates were added to the models using a forward inclusion multiple regression process. As a supplementary analysis, the relation between dietary and serum cholesterol was tested using simple linear regression.

All covariates were originally selected based on possible relations with the main effect variables previously established in the literature. To gain a better understanding of the data collected in the ALPHA Trial and the relation between covariates and main effect variables, tables were created that listed mean values of the mammographic density and lipids by quartiles of covariates (data not shown). Analysis of variance tests were performed to determine if differences among covariates was significant. From this output, we observed that square-root percent mammographic density changed significantly according to age, weight, body mass index, hip circumference, waist circumference, waist-hip ratio, subcutaneous fat, intra-abdominal fat, percent body fat, ever having benign breast disease, ever having hypercholesterolemia, percent calories from saturated fat, and breast thickness. Square-root dense area behaved differently since it was not significantly altered according to levels of body mass index, waist-hip ratio, ever having hypercholesterolemia and percent calories from saturated fat. These differences could be attributable to the biological relation between percent density and adiposity, since breast adipose tissue is included in the denominator of the calculation. Since dense area does not include adipose tissue, relations with adipose tissue and other

variables highly associated with adipose tissue may not exist. Therefore, even though these two outcomes (square-root percent density and square-root dense area) were highly correlated (r=0.91), they seemed to be associated differently with lipids and covariates and it was decided that both would remain as main outcome measures.

Next, univariate analyses were performed with both lipid and mammographic density as outcomes to determine which covariates were linearly associated with the continuous variables and the strength of the associations.

3.6 Effect modification

Variables that were thought to modify the relation between the outcome and predictor variables were examined for effect modification using a multistage method. This analysis was exploratory because little is known of the relation between cholesterol and mammographic density and the factors that modify this relation. First, regression models were run for different levels of the potential effect modifier and the regression lines were graphed to determine if the slopes appeared to differ quantitatively between the levels of the covariate. Second, when the relation between the outcome and the main predictor appeared to change by the effect modifier, regression models including interaction terms were constructed to determine if the coefficient associated with the interaction term was large in magnitude and statistically significant ($p<0.10$). This process yielded only one statistically significant interaction term and modest coefficients for the interaction terms even though some relations seemed to differ quantitatively in the graphing process. Because of this apparent contradiction, a third process was undertaken:

regression lines were re-graphed by levels of the interaction variable using lowess (locally weighted least squares) plots [Katz, 1999]. Lowess plots use a locally weighted regression method to fit a smooth curve through a scatterplot, helping to reveal trends in the data. This technique provided a more accurate depiction of the regression in the subgroup analysis and allowed an examination of the effect of outlying points on the scatterplot. The lowess plots revealed that in most cases, a few outlying points were influencing the regression line in one direction or the other, making the differences between lines on the regression graphs appear greater than they actually were.

In the relation between mammographic density and serum lipids, the following variables were assessed for effect modification: total caloric intake, saturated fat intake, alcohol consumption, waist circumference, family history of breast cancer, HRT use and statin use. In the relation between mammographic density and dietary cholesterol, we assessed alcohol consumption, waist circumference, HRT use and statin use.

3.7 Confounding

For the analysis of confounding, regression graphs were also created. A crude regression line was plotted alongside a covariate-adjusted regression line and the graphs were examined for separation between the lines. The regression coefficients were also examined to determine if the crude and adjusted estimate for the rate of change of mammographic density for unit increase in lipid level was substantially different. A significant change in effect estimate was determined if the adjusted estimate differed from the crude by a magnitude greater than one standard deviation of the crude estimate.

This criterion was determined to be meaningful as a one standard deviation change in the estimate relates to the distribution of the estimate in the population and would represent a substantial change. Only variables that had known or suspected relations with the outcome and predictor variables were included in the analysis of confounding. Age was forced into the model because of the known associations between age and mammographic density and cholesterol.

Variables that met the above criteria were then placed in a correlation matrix to determine the extent of multicollinearity between the variables. Covariates that were highly correlated ($r>0.70$) and that represented similar concepts (eg. intra-abdominal fat and waist circumference), were then examined for the extent to which they confounded the primary relation. The covariate that had the greatest effect on the estimated coefficient of interest, and therefore confounded the relation the greatest, was added into the model first. All covariates that were highly related to the added confounder from a biological viewpoint were eliminated from further analysis. Continuing the modeling process, the remaining covariates were added one at a time. The covariate that significantly altered the coefficient of interest the greatest was added next into the model. The R^2 value was also examined to see if it increased with the addition of a new variable. This examination was undertaken with caution though as R^2 value naturally increases with the addition of any new variable to the model and it does not necessarily mean that the model is a better fit [Kleinbaum et al., 2007]. Covariates were added to each model separately. This process stopped when the coefficient associated with the primary relation no longer changed significantly.

In the relation between mammographic density and serum lipids the following variables were tested in the models to see if they significantly confounded the relation: age, waist circumference, saturated fat intake, past year physical activity, alcohol consumption, as well as variables that affected lifetime hormone exposure; for instance time since menopause, HRT use, time since HRT use, age at first birth and parity. These variables have all been found to be associated with mammographic density [Boyd et al., 2005] and have either been found to be associated with serum lipids, or are related indirectly to serum lipids. When examining the relation between mammographic density and dietary cholesterol, we examined the confounding effects of alcohol consumption, weight, total calories consumed, saturated fat consumed, age, and exercise in the models.

Missing data were handled using a pair-wise deletion method where an observation was only eliminated from the analysis when no data were available for the variable of interest. All statistical tests were two-sided. A p-value of less than 0.05 was considered statistically significant. All analyses were performed using SAS 9.

3.8 Ethical Considerations

All study participants provided their informed consent at the outset of the ALPHA Trial and all data have been kept in locked cabinets at the Alberta Cancer Board and access to the electronic data is restricted to a few study personnel with password protection. All personal identifying information has been removed from all data files being used in this study thus the researcher was not able to identify study participants.

The risks and benefits were also outlined to the participants prior to obtaining informed consent and this study does not alter these in any way. In order to ensure that this study was ethically sound, Dr Christine Friedenreich, Principal Investigator of the ALPHA Trial, requested ethics approval for this thesis project as a modification to the ALPHA Trial. The approval of this modification by the Office of Medical Bioethics preceded the commencement of this project (Appendix 6).

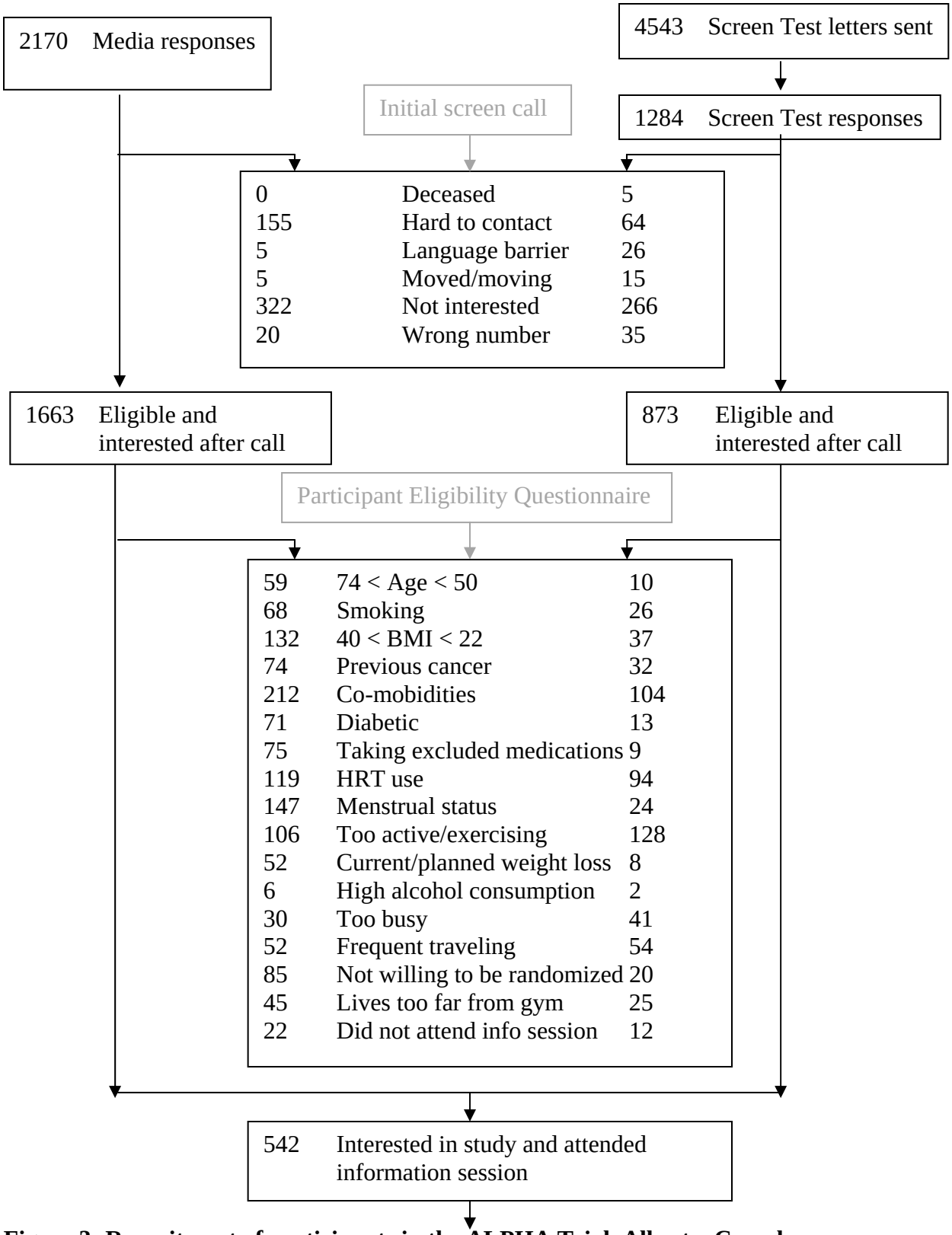
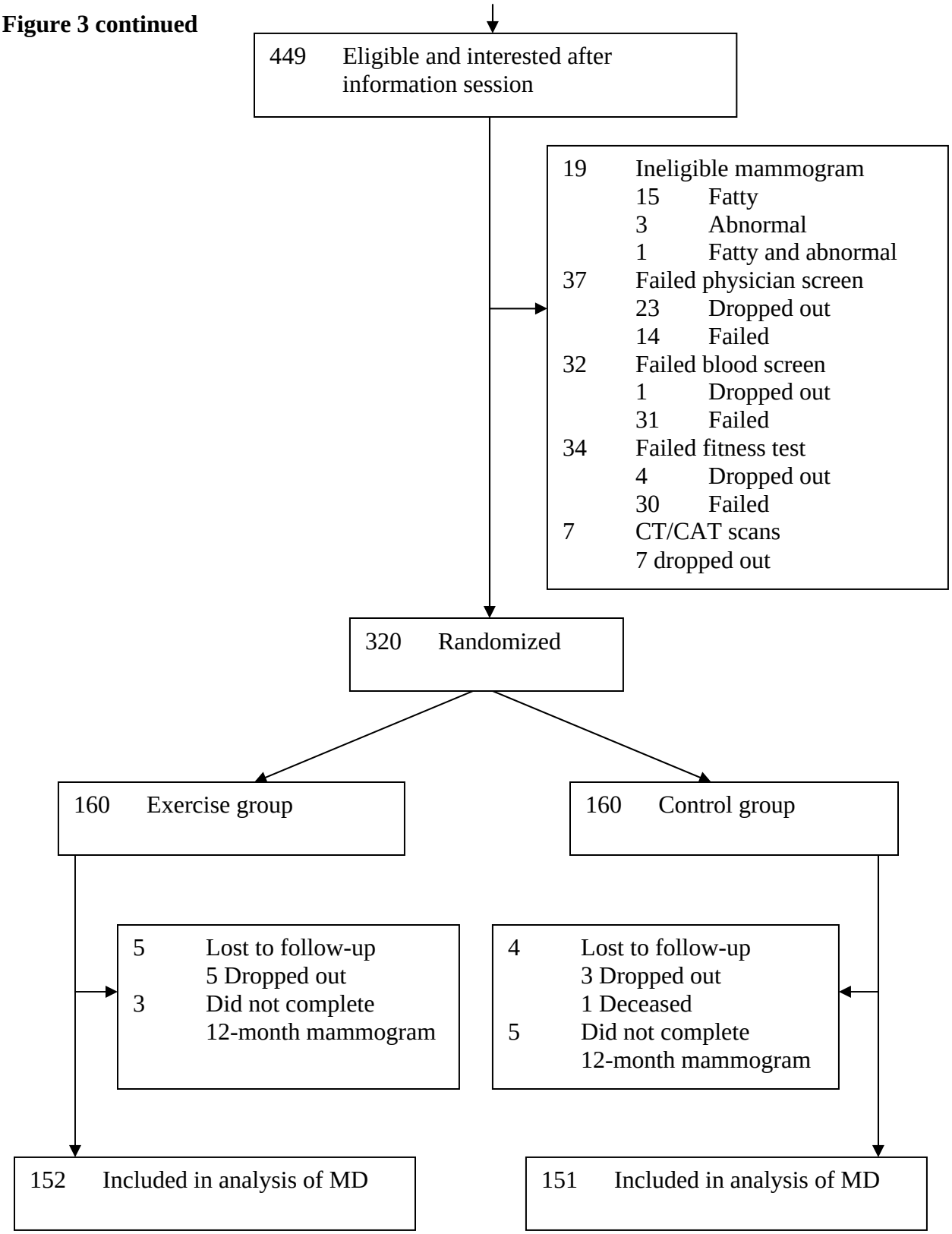


Figure 3: Recruitment of participants in the ALPHA Trial, Alberta, Canada

Figure 3 continued



Chapter Four: RESULTS

4.1 Participant characteristics

The socio-demographic and lifestyle characteristics of the study population were examined (Tables 4 and 5). In total, there were 303 postmenopausal women from Calgary and Edmonton, most of whom were Caucasian (90.4%). The mean age of the study participants was 60.2 years (SD=5.6yrs), ranging from 50 to 74 years by study design. The vast majority of the sample population (75.2%) was married or in common-law relationships. The women were well educated with 67.0% having completed college, trade school, or university degree. Women who enrolled into the study had been postmenopausal for an average of 9.7 years (SD=6.3). The majority of women were parous (90.4%), with an average of two children each. The average age at first birth was 24.6 years (SD=4.9). Under half of the women in this sample had previously used hormone replacement therapy (HRT) (47%), and ceased HRT use approximately 3.8 years (SD=5.9) prior to enrolment into the study. The average duration of HRT use was 6.3 years (SD=5.3).

The average total energy intake among the 299 participants who completed the Diet History Questionnaire was 1524 kilocalories per day (SD=555). These women consumed an average of 55 grams of fat per day (SD=28.7), that equalled 32 percent of the total calories consumed. The average cholesterol in their diets was 166 milligrams per day (SD=82.4). The women consumed 0.36 servings of alcohol per day on average

with the majority of women consuming between 0.1 and 1 drink per day (73.2%). Only 32 women reported drinking an average of more than one alcoholic serving per day.

The majority of this sample of women was overweight with a mean BMI of 28.9 kg/m^2 (SD=4.3) and a mean percent body fat of 42.0 percent (SD=5.2). In total, 79.2 percent of the participants had a BMI of 25 kg/m^2 or greater, with a maximum value of 43.4 kg/m^2. On average these women had gained 16.8 kilograms (SD=9.4) since the age of 30 years. The mean waist circumference was 88.3 centimetres (SD=10.3), which is the criterion cut-point for women with metabolic syndrome [2002].

Twenty percent of the women in this study had a first degree family history of breast cancer and 24.9 percent had reported having been diagnosed with benign breast disease. Approximately one-third of the sample had also been diagnosed with hypercholesterolemia in the past, however, only 14.2 percent had been on cholesterol lowering statin drugs in the year prior to the study and only 10.6 percent were taking statin drugs on the day of blood draw.

4.2 Serum lipid characteristics

The descriptive characteristics of the serum lipids of the study sample are shown in Table 6. Histograms depicting the distribution of these variables are in Figure 4. One participant was excluded from analyses of all serum lipids because of an extreme value of 16.2 mmol/L for serum cholesterol that was deemed to be either a laboratory

measurement error or a recording error. After exclusion of this participant, the mean serum cholesterol in this sample was 5.7 mmol/L (SD=0.9). The eligibility cut-point for entry into the study was a serum cholesterol value greater than 7.4 mmol/L used to eliminate any unhealthy individuals for whom an exercise intervention would be contraindicated. In total, thirteen participants had values above this limit. These cut-point criteria were established for the purpose of the exercise intervention of the ALPHA Trial. If participants who had serum cholesterol levels that exceeded our eligibility cut-point were cleared by their physicians through the PARmed-X form (a physical activity-specific checklist used by physicians to clear patients for a physical activity program, [Canadian Society for Exercise Physiologists, 2008]), then they were considered eligible for the study. Hence, this study population did include some women that had lipid levels outside the normal range but who were otherwise healthy and consequently eligible for the present analysis.

The mean triglyceride level was 1.5 mmol/L (SD=0.7), with a median value of 1.4. Although the distribution of this variable was slightly skewed, transformations did not significantly affect the assumptions of the linear model and did not improve the fit of the model; therefore triglycerides were retained in their original format. There was one extreme value of 8.3 mmol/L, but it belonged to the same participant that was excluded above. This value further justified our rationale for excluding this participant from all serum lipid analyses. Twenty-six women had triglyceride levels above the study cut-point of 2.56 mmol/L.

Mean HDL-C levels were 1.59 mmol/L (SD=0.34) and mean LDL-C levels were 3.40 mmol/L (SD=0.67). Five women could not be included in the LDL-C analysis because their triglyceride levels were too high making the calculation of LDL-C inaccurate [Calgary Laboratory Services, 2002]. Ten women had LDL-C levels above the cut-point of 4.92 mmol/L and no women had HDL-C levels below the cut-point of 0.72 mmol/L.

4.3 Correlation between serum lipid measures

The association between the different serum lipid measures was assessed using Pearson correlation coefficients (r) and the precision around these correlations examined with the 95% confidence intervals (Table 7). There was strong correlation between measures of LDL-C and total cholesterol (r=0.93, 95% CI: 0.91 to 0.94). This high correlation was expected because total cholesterol is comprised of mostly LDL-C [Calgary Laboratory Services, 2002]. HDL-C and cholesterol were not correlated, with a correlation coefficient of 0.07 (95% CI: -0.04 to 0.18). LDL-C and HDL-C were inversely associated with one another (r=-0.16, 95% CI: -0.27 to -0.05). Triglycerides were positively associated with total cholesterol (r=0.35, 95% CI: 0.25 to 0.44), and LDL-C (r=0.23, 95% CI: 0.12 to 0.33) and inversely associated with HDL-C (r=-0.48, 95% CI: -0.56 to -0.39).

4.4 Correlation between serum lipids and covariates

The associations between serum lipid measures and important covariates are reported in Tables 8 and 9. Age at menarche, number of live births, total fat intake, HRT use, and a history of benign breast disease were not significantly related to any of the lipid measures ($p>0.05$). Of all the body size measures, waist circumference and intra-abdominal fat were the most strongly associated with all lipid measures, especially HDL-C ($r=-0.37$ and $r=-0.39$, respectively) and TG ($r=0.35$ and $r=0.44$, respectively). Higher alcohol intake was associated with higher HDL-C and lower TG levels, possibly illustrating the cardio-protective effects of alcohol [Friedman and Kimball, 1986]. Taking statins, either throughout the year or on the day of blood draw was significantly associated with lower LDL-C and total cholesterol levels. Since the primary action of most statins is to lower LDL-C [Wilt et al., 2004] and since LDL-C and total cholesterol are strongly and positively associated with one another, these results are expected. A history of hypercholesterolemia was associated with a poor lipid profile. This association could be reflective of the relation between body weight and lipid levels as women with this history profile tended to be heavier (results not shown).

4.5 Associations between serum lipids and dietary cholesterol

The association between dietary cholesterol and serum cholesterol was assessed by scatterplots (Figure 5). From these graphs it appears as though there is no association between serum lipids and dietary cholesterol as all regression lines are horizontal and there is large variability of data points about the regression line.

To further explore the relation between these two variables, regression models were created with serum lipids modeled as the outcome and dietary cholesterol modeled as the predictor variable (Table 10). Since the magnitude of the beta coefficients is not significantly different from zero, there is no evidence that dietary cholesterol is associated with serum lipid levels.

An exploratory analysis of effect measure modification by alcohol consumption, weight, and statin use was undertaken. There was no evidence that these variables significantly modified any of the relations between dietary cholesterol and serum lipids. Total calories consumed, saturated fat consumed, alcohol intake, age, and exercise were all examined as potential confounders yet none of these variables were found to significantly alter the beta-coefficient based on previous criteria. Therefore, in this study sample, there is no evidence of an association between dietary cholesterol and serum lipid levels.

4.6 Mammographic density characteristics

Characteristics of mammographic density in this study sample are described in Table 6. Histograms of mammographic density variables and their transformations are presented in Figure 6. The range of percent density in this sample was between zero and 56 percent. One of the exclusion criteria for entry into the ALPHA Trial was having a subjective radiologist rating of zero percent. This stipulation was incorporated so that the participants were in a risk category at baseline that was amenable to change by the

exercise intervention. For this analysis, a wider range of percent density values aids in the assessment of the relation with serum lipids and does not affect the validity of the results. Fifteen participants entered into the trial with a percent density of zero. In the exercise trial, the relation between non-dense tissue in the breast and biomarkers was of great interest, therefore even though these women had little to no dense tissue they were still considered to be in a risk category that was amenable to change. The mean percent density was 17.6% (SD=13.4). On average, the women had a total breast area of 152.2 cm^2 (SD=53.0), total dense tissue area of 25.0 cm^2 (SD=21.2) and fatty tissue levels of 127.2 cm^2 (SD=53.8). All median values were less than the mean, illustrating the positively skewed distribution of these data.

4.7 Inter- and intra-rater reliability

To determine the reliability of mammographic density measures, Dr. Boyd read 30 films between batches (inter-batch) and 20 films within the same batch (intra-batch). Recall, that the mammograms were read in five different batches, with 140-142 films in each batch. The intra-class correlation coefficients were very close to one [Woolcott, 2006] for all measures of mammographic density. Specifically, the inter-batch correlations for dense area and percent density were 0.95 (95% CI: 0.90 to 0.98) and 0.94 (95% CI: 0.89 to 0.97), respectively. The intra-batch correlations were similar with 0.95 (95% CI: 0.87 to 0.98) reported for dense area, and 0.95 (95% CI: 0.88 to 0.98) for percent density. These high correlation coefficients suggest that there is high agreement between the repeated measures. In terms of inter-rater reliability between Dr. Boyd's and

Dr. Woolcott's measures, the ICC inter-batch reading was 0.96 (95% CI: 0.92 to 0.98) for dense area and 0.97 (95% CI: 0.92 to 0.99) for percent density. The respective ICCs for intra-batch were 0.91 (95% CI: 0.78 to 0.96) and 0.86 (95% CI: 0.69 to 0.94). Although this project only used the measures from Dr. Boyd, the established inter-rater reliability is sufficiently high that this computerized method of measuring mammographic density using Cumulus® is appropriate when there are multiple readers.

4.8 Correlation between mammographic density measures

Associations between the different mammographic density measures were subsequently examined (Table 11). Square-root percent density and square-root dense area were highly and positively correlated (r=0.91, 95% CI: 0.89 to 0.93) as were total breast area and non-dense area (r=0.92, 95% CI: 0.90 to 0.94). Square-root percent density and non-dense area were negatively correlated (r=-0.60, 95% CI: -0.67 to -0.53) suggesting that the greater the level of fat tissue in the breast the lower the proportion of dense tissue to total breast tissue. This finding coincides with the equation for calculating percent density where non-dense tissue makes up the majority of the denominator. This relation is widely observed in the literature [Boyd et al., 2005]. Square-root dense area did not correlate well with total breast area (r=0.09, 95% CI: -0.02 to 0.20) and was negatively associated with non-dense area (r=-0.29, 95% CI: -0.39 to -0.18).

4.9 Correlation between mammographic density and covariates

Next, the associations between mammographic density and important covariates were examined (Table 12 and 13). Site, age at menarche, number of live births, time since menopause, HRT use, duration of HRT use, time since last HRT use, family history of breast cancer, fat intake, percent fat intake, alcohol intake and statin use on the day of blood draw and throughout the past year were not significantly associated with either square-root percent density or square-root dense area, although the correlation between alcohol and percent density approached statistical significance ($r=0.10$, $p=0.07$). All of the body composition measurements were significantly associated with the outcome variables. Subcutaneous fat and waist circumference held the strongest relations with both square-root percent density and square-root dense area. This relation differs slightly from the lipid measures where lipids were most highly associated with waist circumference and intra-abdominal fat. Age at first birth was positively related to both the density measures, indicating that the older a woman was when she had her first baby, the greater her amount of absolute and relative dense tissue. This relation is not established in the literature, but age at first birth is often controlled for in studies examining the relation between mammographic density and breast cancer risk [Boyd et al., 2005] suggesting that there is a relation with mammographic density. Having a history of benign breast disease was also strongly related to square-root percent density and square-root dense area, with higher relative and absolute dense tissue in those with a positive history.

4.10 Associations between mammographic density and serum lipids

4.10.1 Square-root percent density

The strength and direction of the linear relations between square-root percent density and all of the serum lipids is illustrated using scatterplots (Figure 7). From these graphs it can be observed that there is a slight negative slope between cholesterol, LDL-C and TG and square-root percent density. HDL-C is positively associated with square-root percent density. These graphs also illustrate the high variability of the predicted outcome for each level of the independent variable. This analysis provides an indication that serum lipids may not be a good predictor of mammographic density.

An exploratory analysis of effect-measure modification by total caloric intake, saturated fat intake, alcohol consumption, waist circumference, family history of breast cancer, HRT use and statin use was undertaken. Alcohol consumption was found to modify the relation between triglycerides and percent density significantly. This effect-measure modification was observed on an additive scale, was statistically significant ($p=0.008$) and was illustrated in both a graph of the regression lines for each level of alcohol consumption as well as in a regression model that included an interaction term for those women who drank greater than one serving of alcohol per day (Figure 8). Figure 8 illustrates a dose-response relation with effect of daily alcohol consumption on the relation between triglycerides and percent density. As alcohol consumption increases, the relation becomes strongly inverse suggesting that those who drink more have a lower

percent density for each millimole increase in triglyceride level compared to those who drink moderate amounts of alcohol or none at all.

To explore this analysis further, women were categorized according to their alcohol consumption and various characteristics were compared across the groups (Table 14). From this analysis it was observed that women who drank more than one serving of alcohol per day on average were different from women with other consumption patterns in terms of average total calories, fat and cholesterol consumed per day. Compared to those who consumed less than one drink per day or who abstained from drinking, more women who drank greater than one drink per day had previously used HRT (53.1% vs. 50.5 and 29.8%) and previously smoked (46.9% vs. 32.0 and 13.0%). Furthermore, more of them were married (98% vs. 74.7% and 68.1%) and more tended to have had completed greater than high-school education (84.4% vs. 65.8 and 61.8%).

Based on this analysis, the results for the relation between percent density and TG were presented stratified by alcohol consumption (Table 15). The remaining lipids were left un-stratified and are presented in Table 18. In the stratified analysis of TG, a significant association was found between TG and percent density in the group drinking greater than one drink of alcohol per day on average. This relation remained after adjustment for age and waist circumference. In the adjusted model, there is a negative association between TG and percent density. Interpretation of the coefficient suggests that there is a decrease of 0.94 square-root percent density for each millimole increase in

TG. Since this estimate is difficult to interpret, back transformations were performed to provide mean percent density values for given values of waist circumference and age category (Table 17). A strong negative relation between percent density and TG was found in those who consume more that one drink per day on average while the other two drinking categories demonstrated a weak positive relation. Hence, this study found evidence of a negative relation between TG and percent density in women who drank more than one drink per day on average.

In order to examine the relation between square-root percent density and cholesterol, HDL-C and LDL-C, a multiple regression analysis was performed (Table 18). From these analyses we were able to determine if these serum lipids were good predictors of mammographic density in this population. In the crude analysis, significant relations were observed between all lipid measures and square-root percent density. Confirming the previous graphs (Figure 7), cholesterol and LDL-C were both inversely associated with percent density, while HDL-C was positively associated with percent density. Age and waist circumference were found to significantly confound the relation between percent density and all serum lipids. In the age-adjusted analysis, all of the estimated beta-coefficients for lipids remained statistically significant, despite wide confidence intervals for HDL-C. When further adjustment for waist circumference was performed, all estimates were attenuated to non-significant results. Thus, after adjusting for biologically important confounders of age and waist circumference, there was no evidence of a relation between cholesterol, HDL-C and LDL-C and percent density.

4.10.2 Square-root dense area

The linear relations between square-root dense area and all the serum lipids are also illustrated with scatterplots (Figure 9). From these graphs it can be observed that there is a slightly negative slope depicting the relation between cholesterol, LDL-C and square-root dense area. HDL-C is slightly positively associated with square-root dense tissue while there does not appear to be a relation between triglycerides and dense area. Overall, the associations seem weaker than those relations with the relative measure of percent density, illustrated by the near horizontal slope of the regression lines.

An exploratory analysis of effect modification was repeated for the relation between lipids and dense area and alcohol consumption was once again found to modify the relation between TG and square-root dense area (Table 16). In the crude analysis, a significant association between TG and dense area exists in those women who consumed greater than one alcoholic drink per day with a beta coefficient of -1.55 (95%CI=-2.83 to -0.27), but this result was attenuated after controlling for age and waist circumference (β=-0.81, 95%CI=-1.97 to 0.36). In the other two levels of alcohol consumption there were no statistically significant associations between TG and dense area before or after adjustment for biologically significant confounders. Therefore, regardless of alcohol consumption patterns, there is no evidence of an association between TG and dense breast area in these data.

Multiple regression analyses were performed to determine the strength of the linear relation between the remaining serum lipids and square-root dense tissue (Table 19). In the crude analysis, only LDL-C was weakly and negatively associated with dense area (B=-0.30, 95% CI: -0.60 to -0.00). None of the other cholesterol measures were statistically significantly related to dense area. These results confirm the linear regression graphs in Figure 9 which show horizontal slopes for the regression lines. Adjusting for age and waist circumference further attenuated the results and increased the width of the confidence intervals. Thus, this study found no evidence of a relation between cholesterol, HDL-C and LDL-C with dense breast area.

4.11 Associations between mammographic density and dietary cholesterol

4.11.1 Univariate associations with dietary cholesterol

To explore the relation between cholesterol and mammographic density further, we examined the association between cholesterol in the diet and mammographic density. First, we investigated the association between dietary cholesterol and important continuous and categorical covariates using Pearson correlation coefficients (r) and ANOVA, respectively (Table 20 and 21). Dietary cholesterol was not statistically significantly correlated with waist circumference, BMI, intra-abdominal fat, sub-cutaneous fat, total body fat or past year physical activity level. Dietary cholesterol was statistically significantly and positively associated with total caloric intake, dietary fat intake, saturated fat intake and percent calories from fat. It was also weakly and positively associated with alcohol consumption and weakly and inversely associated with

age. In the categorical analysis, mean dietary cholesterol did not significantly differ between site, HRT use, family history of breast cancer, or statin use, although the past year statin users did appear to have lower dietary cholesterol intakes (161.6 mg/day for statin users vs. 194.1 mg/day for non statin users). Those who consumed no alcohol had significantly lower dietary cholesterol than those who consumed some alcohol per day. This result could be indicative of differing dietary patterns associated with drinkers or could be a spurious result due to the small sample size in the non-drinker group (n=48).

4.11.2 Square-root percent density and square-root dense area

To gain information about the strength and direction of the association between dietary cholesterol and the mammographic density variables we created scatterplots of these variables plotted against each other (Figure 10). From the slope of the regression lines and the wide dispersion of points around the regression line, there appears to be a weak negative association between percent density and dietary cholesterol and no relation between dense area and dietary cholesterol.

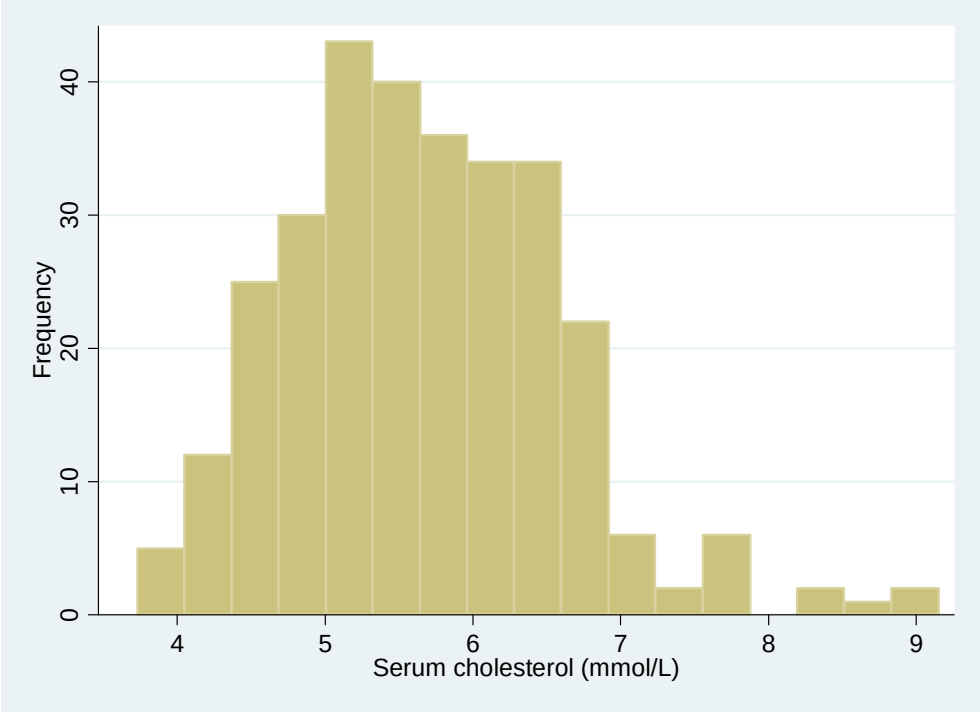
To examine the relation between dietary cholesterol and the mammographic density variables, linear regression models were developed (Table 22 and 23). The beta-coefficients representing the relation with percent density is very small indicating that for a one milligram per day increase in dietary cholesterol there is a decrease of 0.001 square-root percent density that was not statistically significant. The same estimate of effect is observed for dense area, but the confidence intervals are wider indicating that

this estimate is less precise. The low R^2 values indicate that dietary cholesterol does not explain any variance in serum cholesterol and is not a good predictor of serum cholesterol.

An exploratory analysis of effect modification by alcohol consumption, weight, HRT use and statin use was undertaken but no evidence for modification by these variables was observed. Confounding by alcohol consumption, saturated fat consumed, total calories consumed, age, weight and exercise was also assessed. There was no evidence that any of these factors confounded the relation between mammographic density and dietary cholesterol. Therefore, there is no evidence for a relation between mammographic density measures and dietary cholesterol in this study sample.

Figure 4: Distribution patterns of serum cholesterol

a) Serum cholesterol



b) High-density lipoprotein

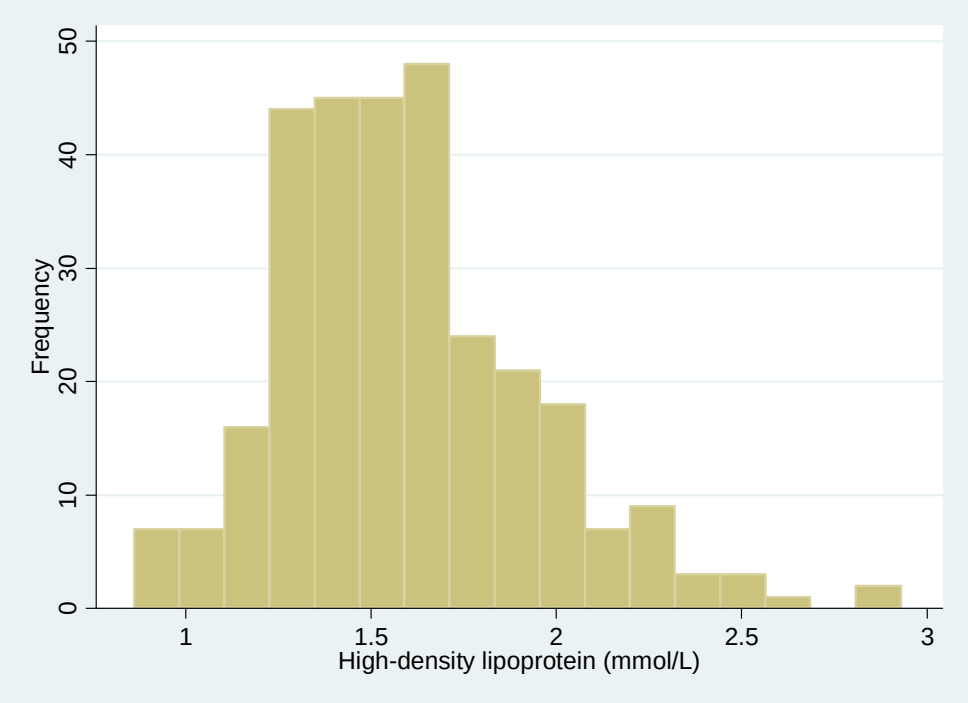
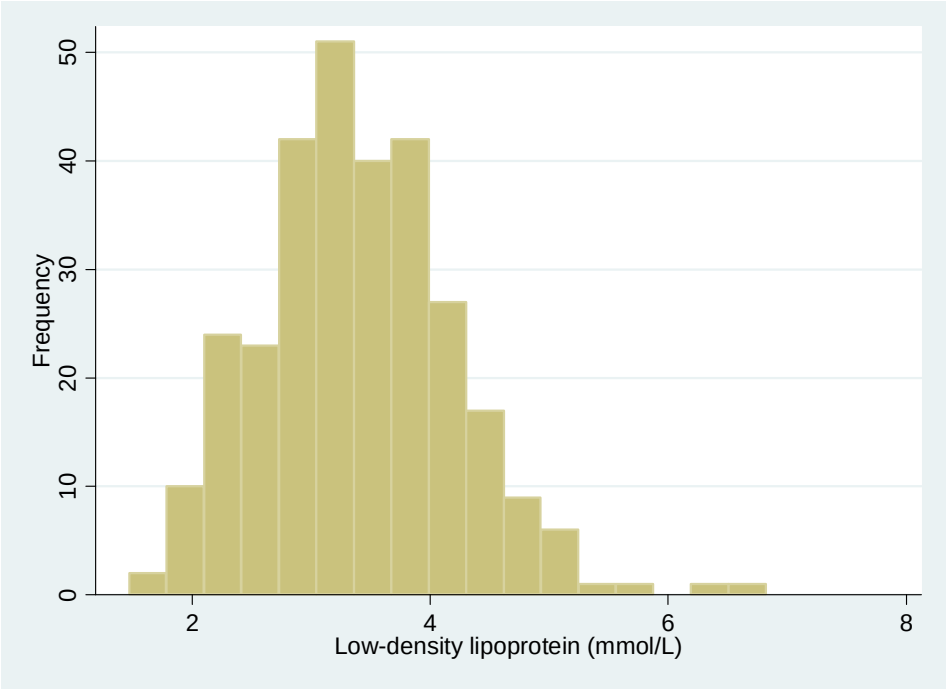


Figure 4 continued

c) Low-density lipoprotein



d) Triglycerides

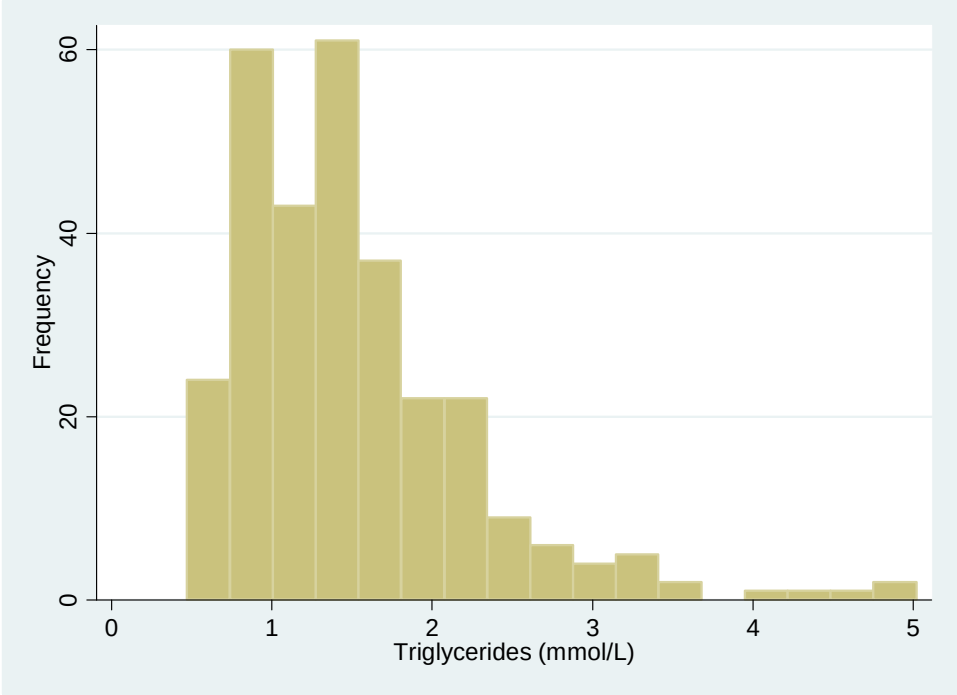
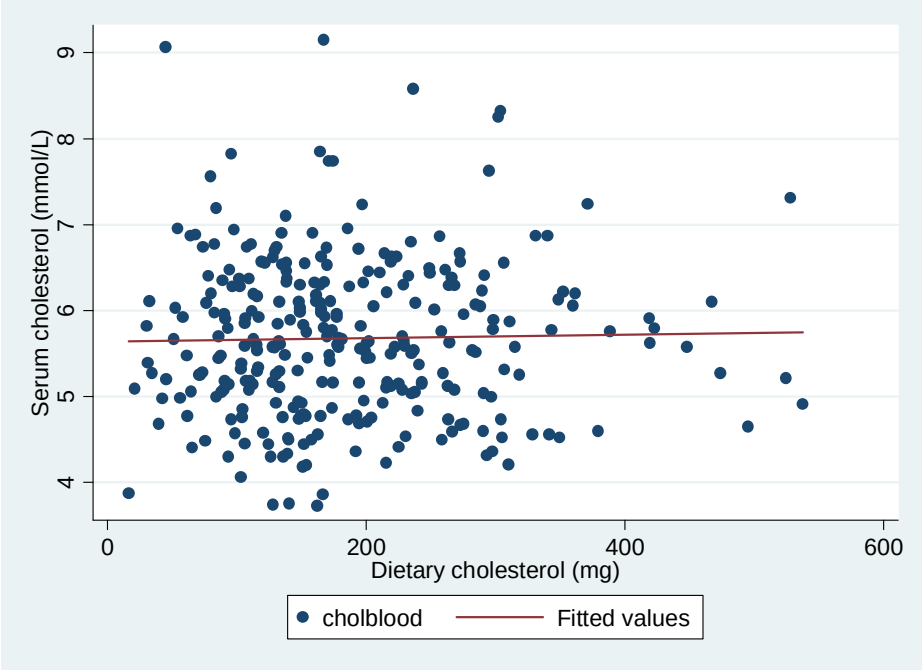


Figure 5: Linear regression model depicting the relation between dietary cholesterol and serum lipids

a) Serum cholesterol



b) Serum high-density lipoprotein

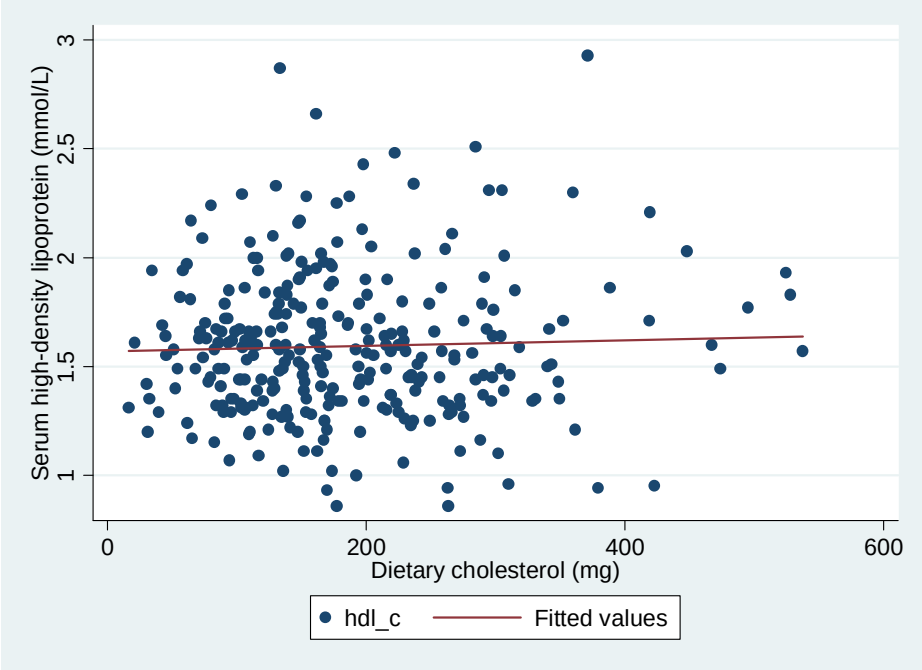
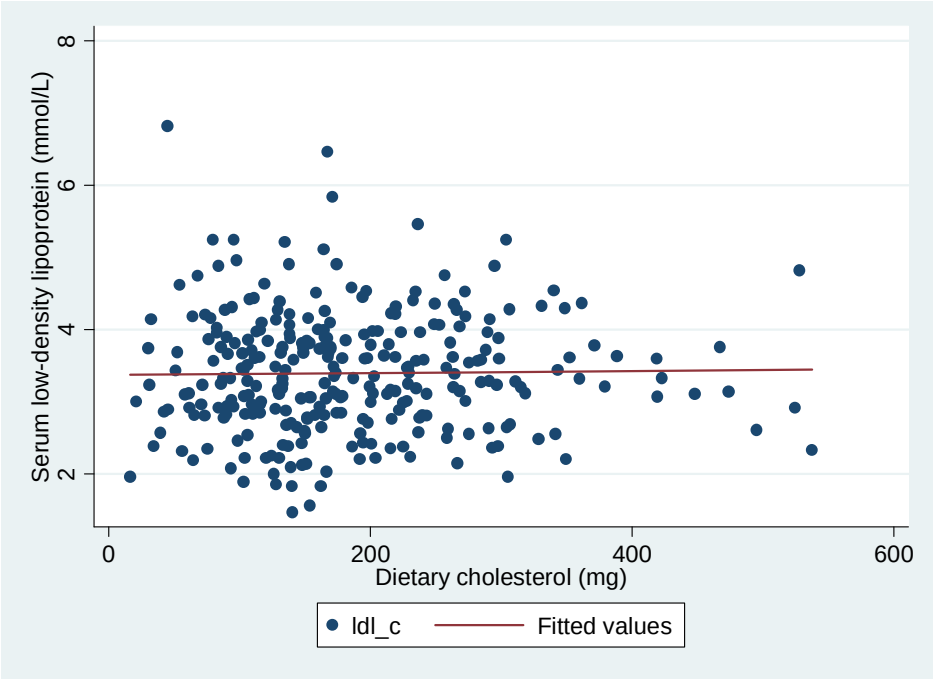


Figure 5 continued

c) Serum low-density lipoprotein



d) Serum triglycerides

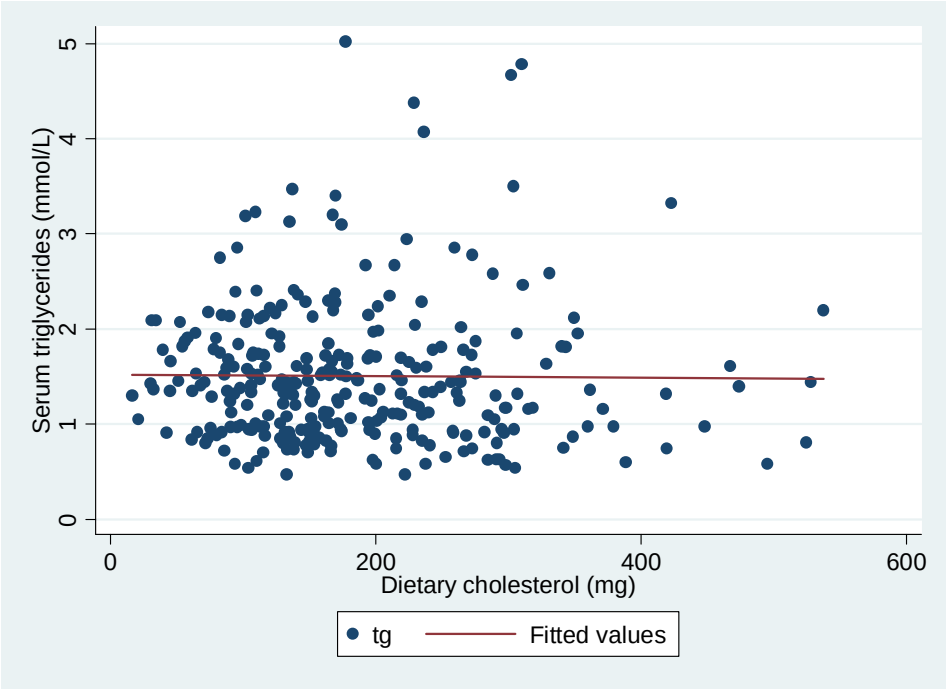
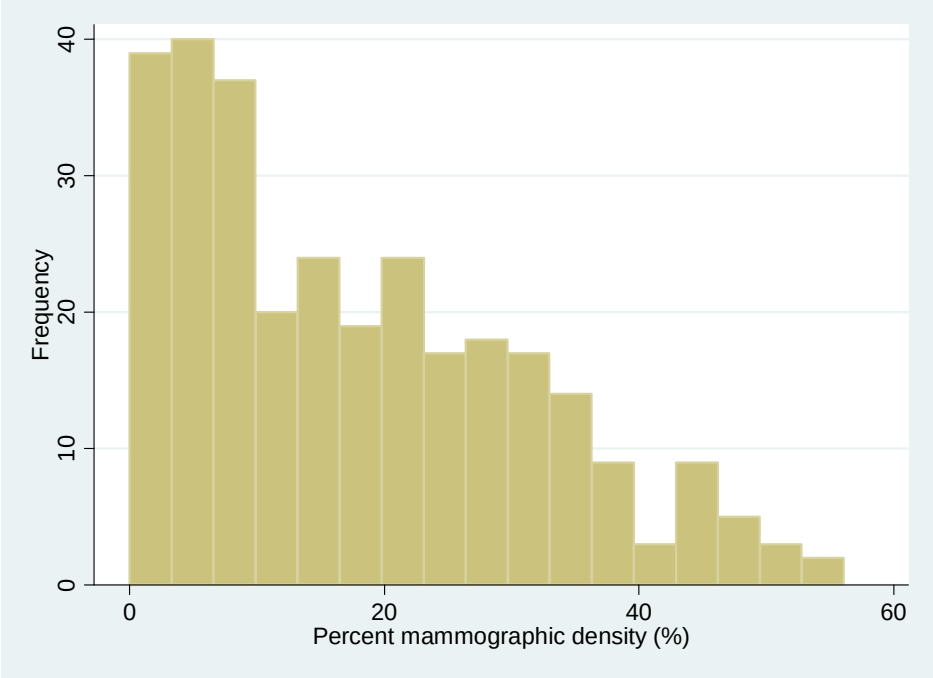


Figure 6: Distribution patterns of mammographic density

a) Percent mammographic density



b) Square-root percent mammographic density

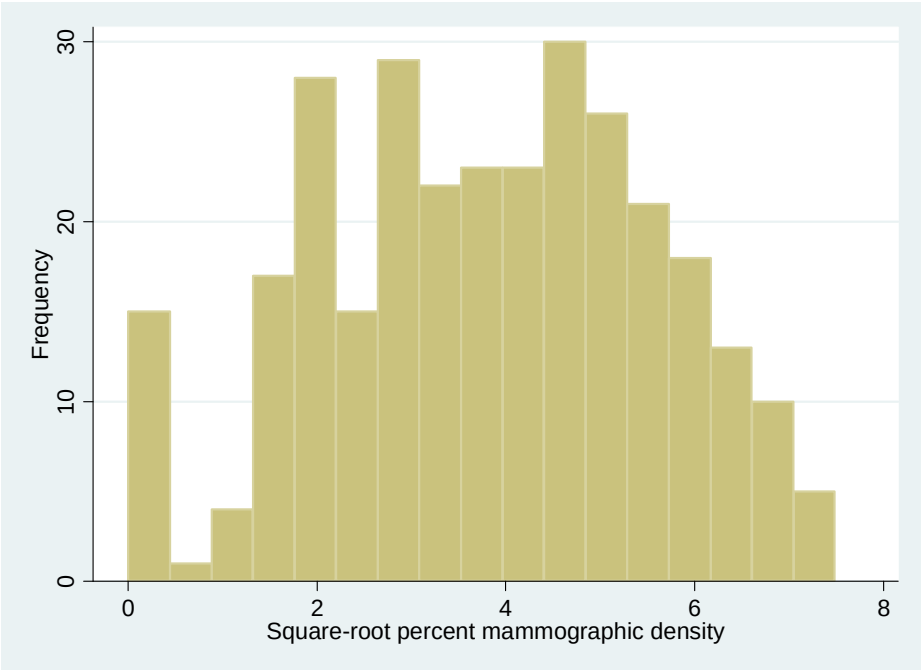
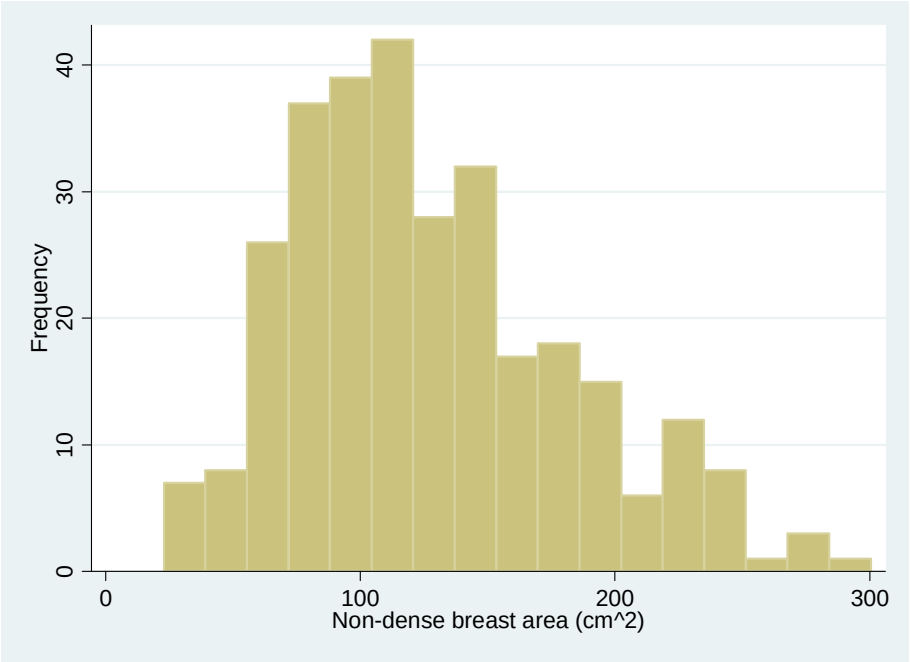


Figure 6 continued

c) Non-dense breast area



d) Total breast area

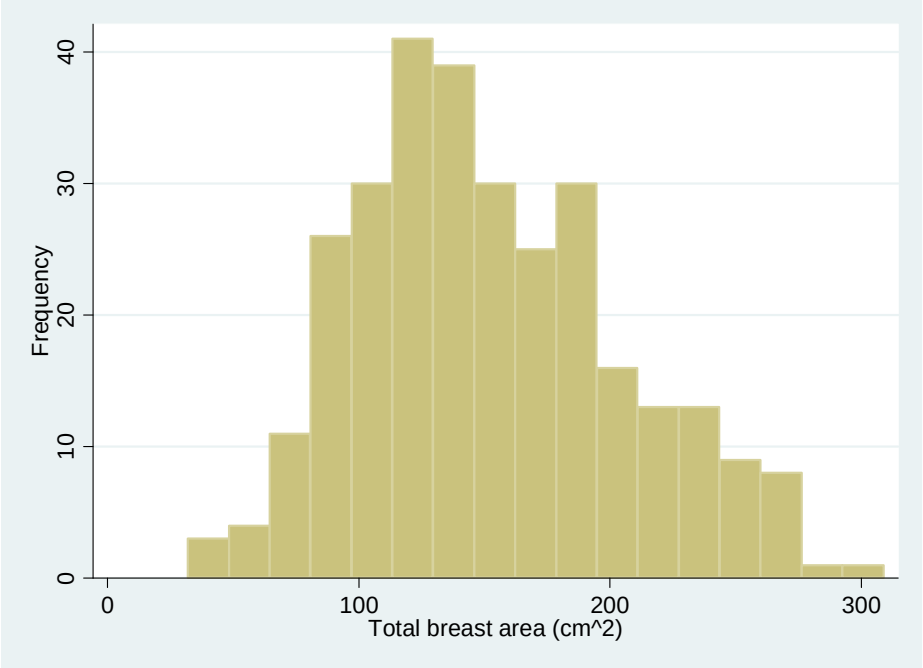
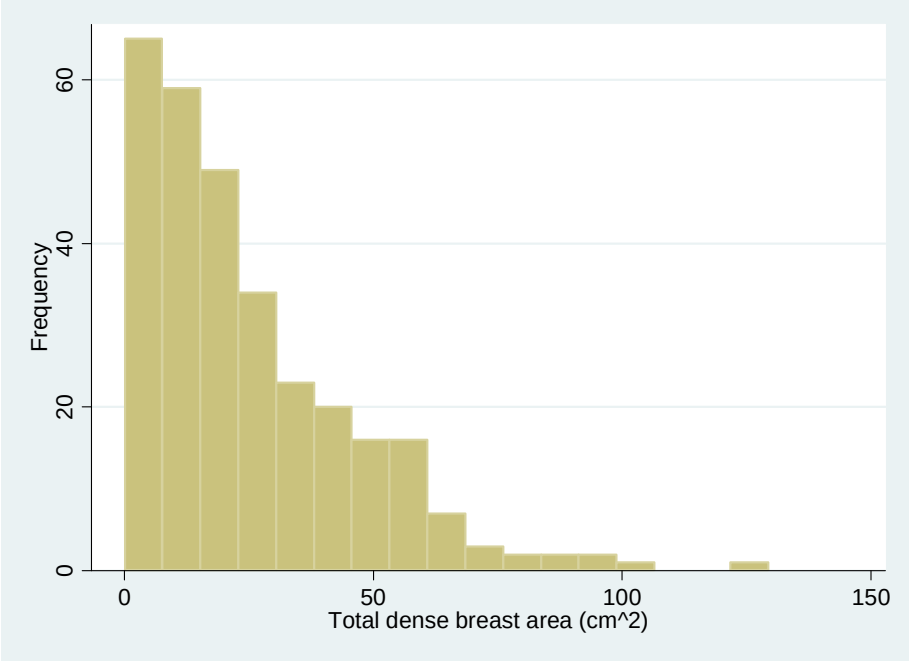


Figure 6 continued

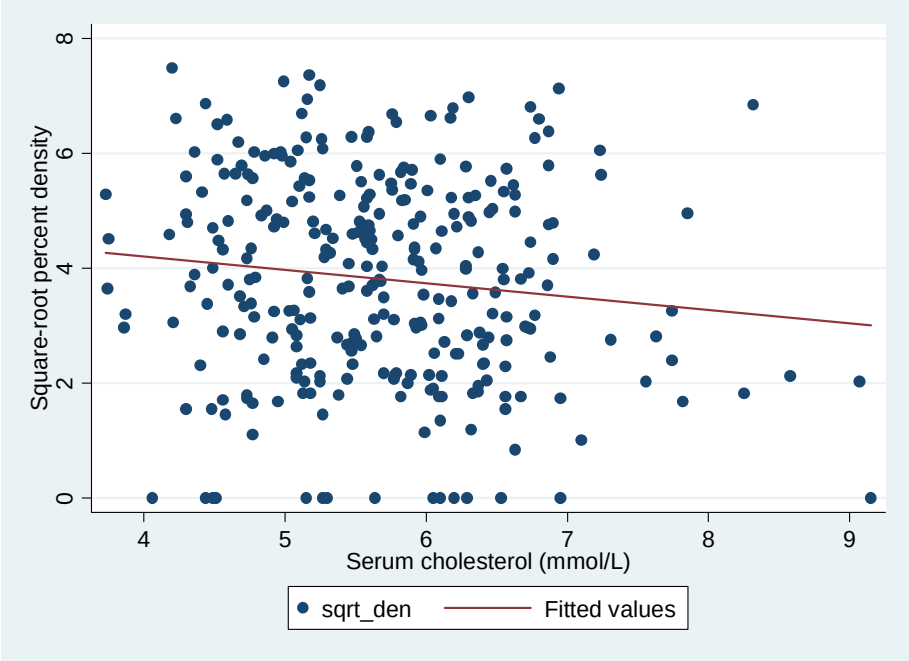
e) Dense breast area



f) Square-root dense breast area

Figure 7: Linear regression model depicting the relation between square-root percent density and serum cholesterol

a) Serum cholesterol



b) Serum high-density lipoproteins

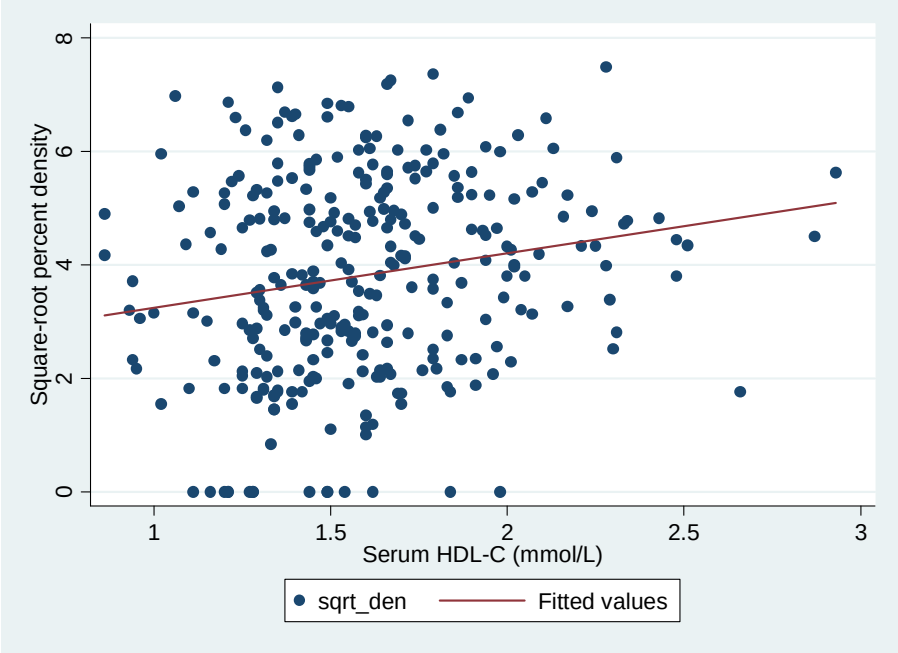
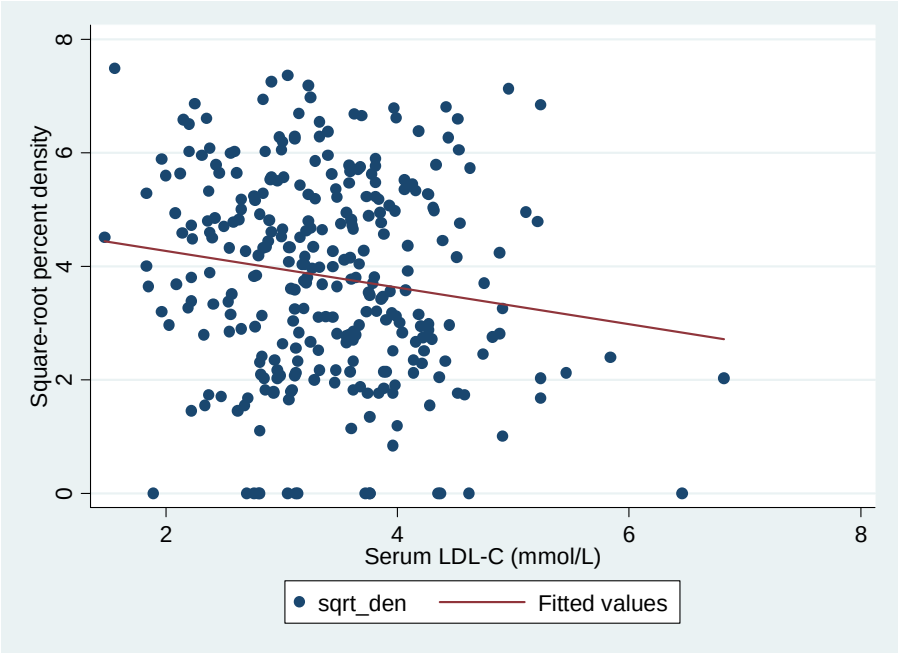


Figure 7 continued

c) Serum low-density lipoproteins



d) Serum triglycerides

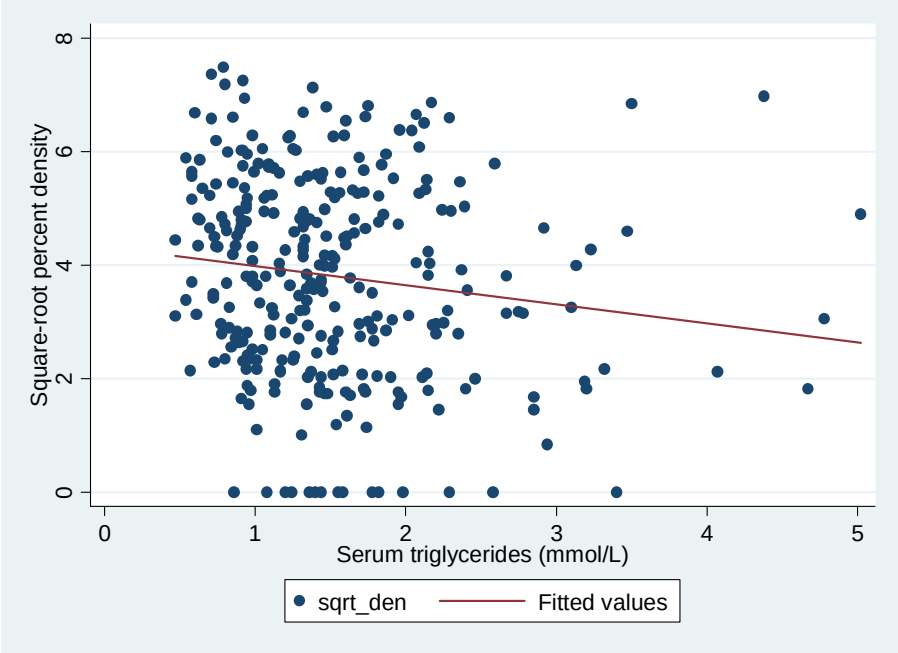
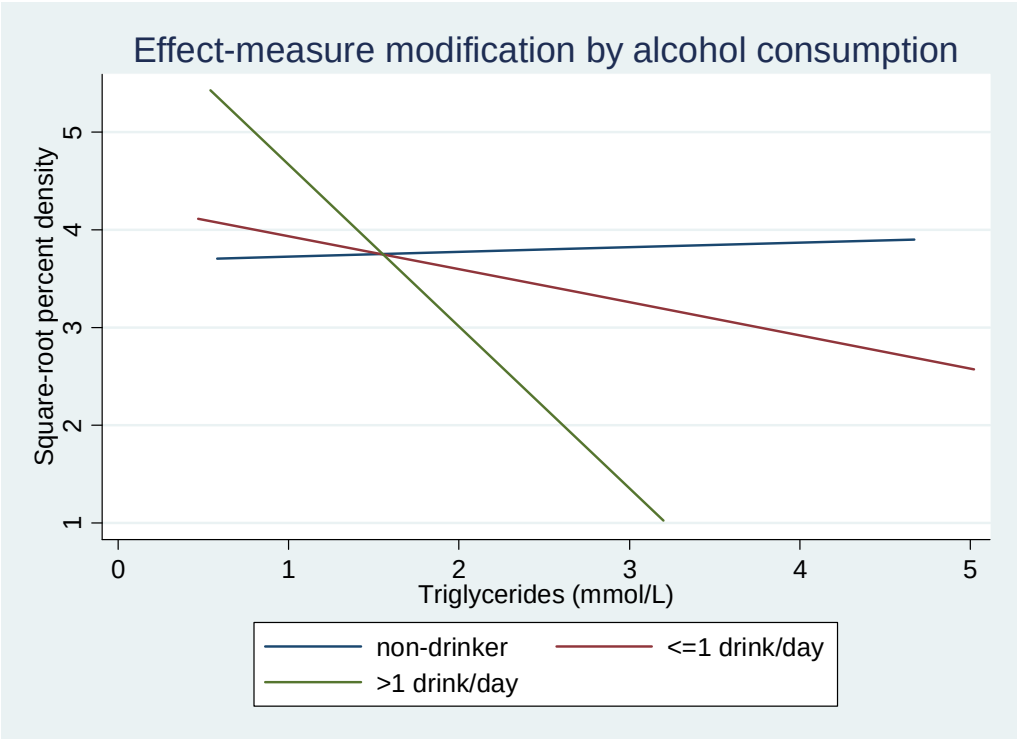


Figure 8: Effect modification of the relation between square-root percent density and triglycerides by levels of alcohol consumption



Regression model:

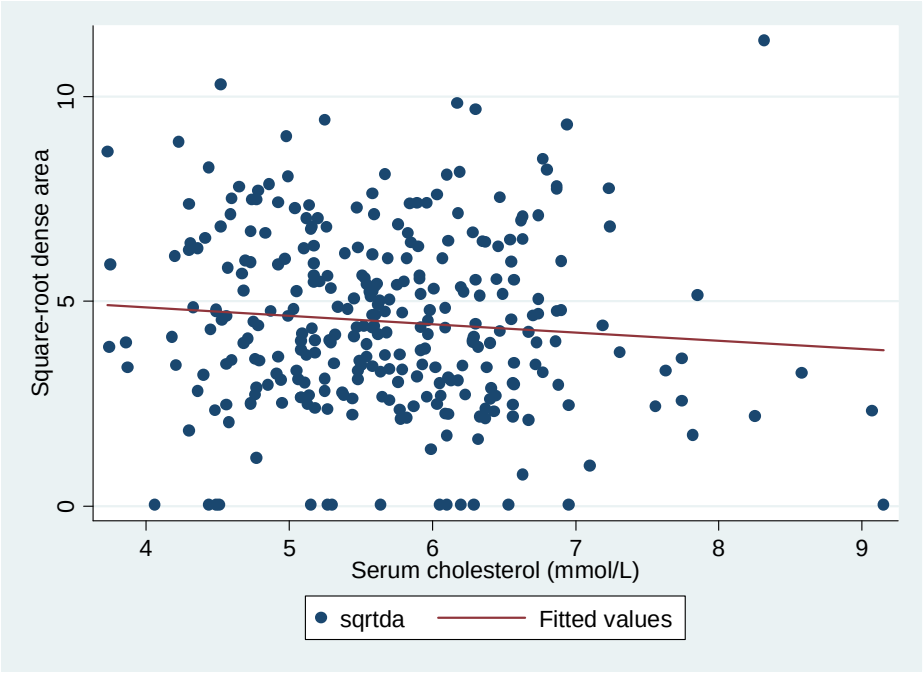
Square-root percent density = $B_o + B_1(TG) + B_2(<=1\ drink/day) + B_3(>1\ drink/day) + B_4(TG * <=1drink/day) + B_5(TG * >1drink/day)$

```
      Source |       SS       df       MS              Number of obs =     300
-------------+------------------------------           F(  5,   294) =    2.93
       Model |  44.6477583     5  8.92955166           Prob > F      =  0.0134
    Residual |  895.116102   294  3.04461259           R-squared     =  0.0475
-------------+------------------------------           Adj R-squared =  0.0313
       Total |  939.763861   299  3.14302294           Root MSE      =  1.7449

------------------------------------------------------------------------------
Sqrt % Density      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          TG |   .0869324   .2911401     0.30   0.765    -.4860506    .6599153
<=1drink/day |   .6656336   .5982665     1.11   0.267    -.5117941    1.843061
 >1drink/day |   2.711892   .9568508     2.83   0.005      .828747    4.595037
 TG*<=1drink |  -.4262341   .3336232    -1.28   0.202    -1.082827    .2303583
  TG*>1drink |   -1.74171   .6524023    -2.67   0.008    -3.025681   -.4577393
       _cons |   3.611816   .5319884     6.79   0.000     2.564828    4.658804
------------------------------------------------------------------------------
```

Figure 9: Linear regression model depicting the relation between square-root dense tissue area and serum cholesterol

a) Serum cholesterol



b) Serum high-density lipoprotein

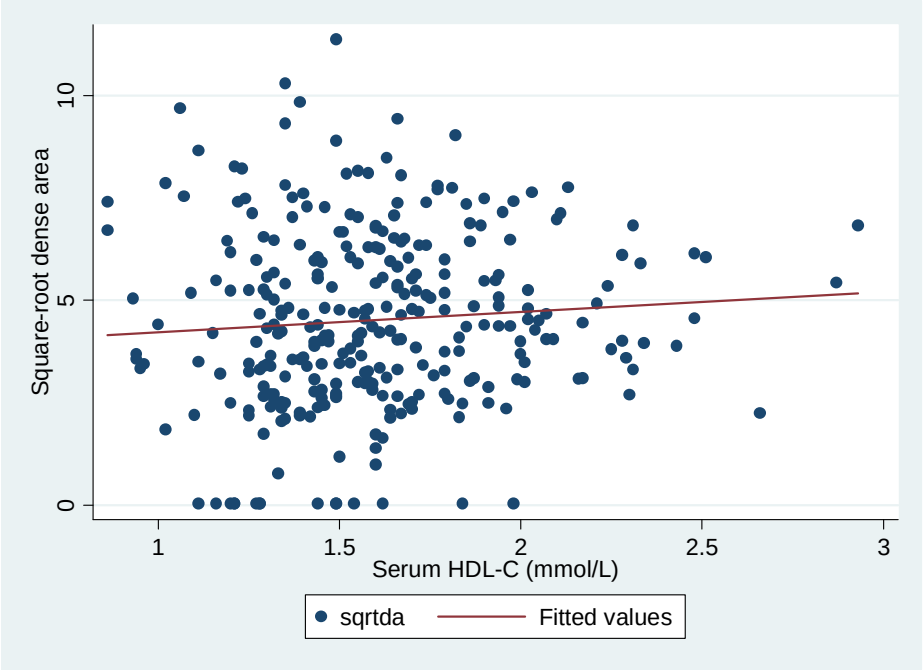
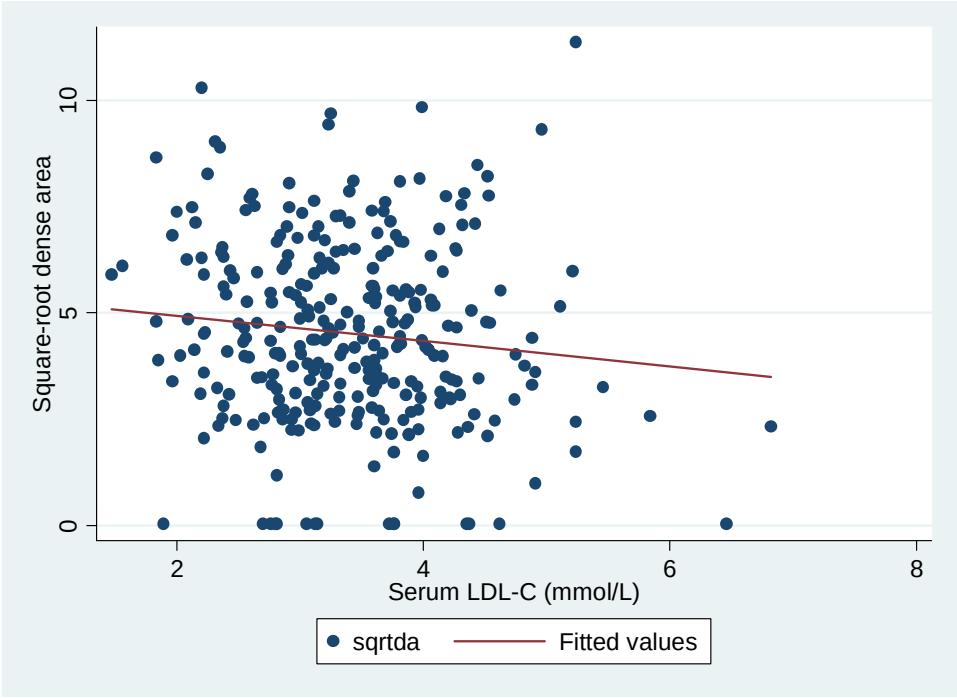


Figure 9 continued

c) Serum low-density lipoprotein



d) Serum triglycerides

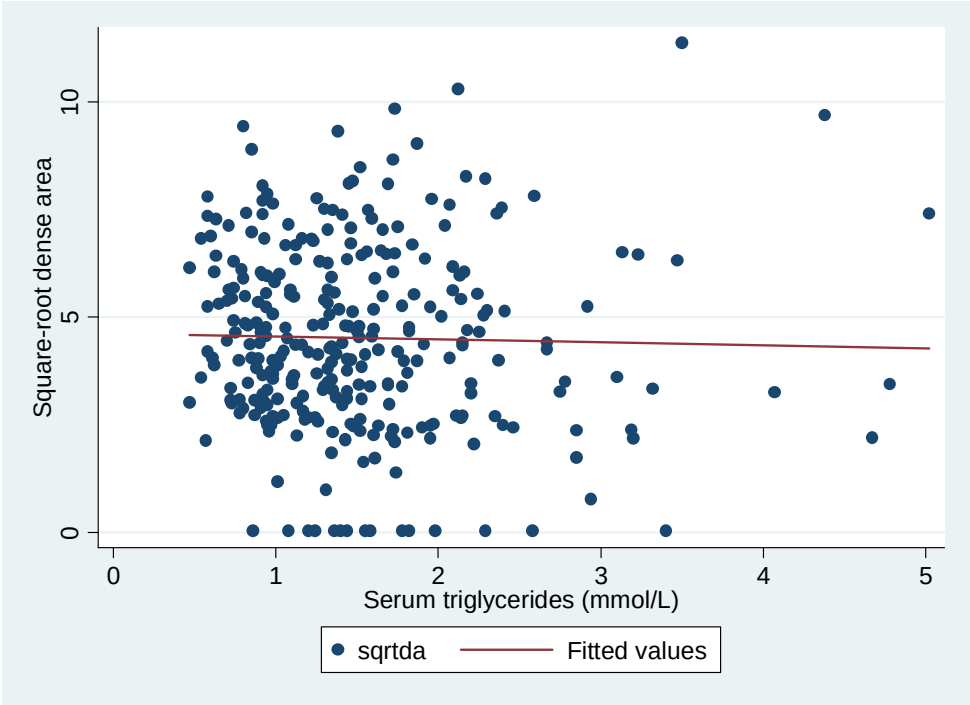
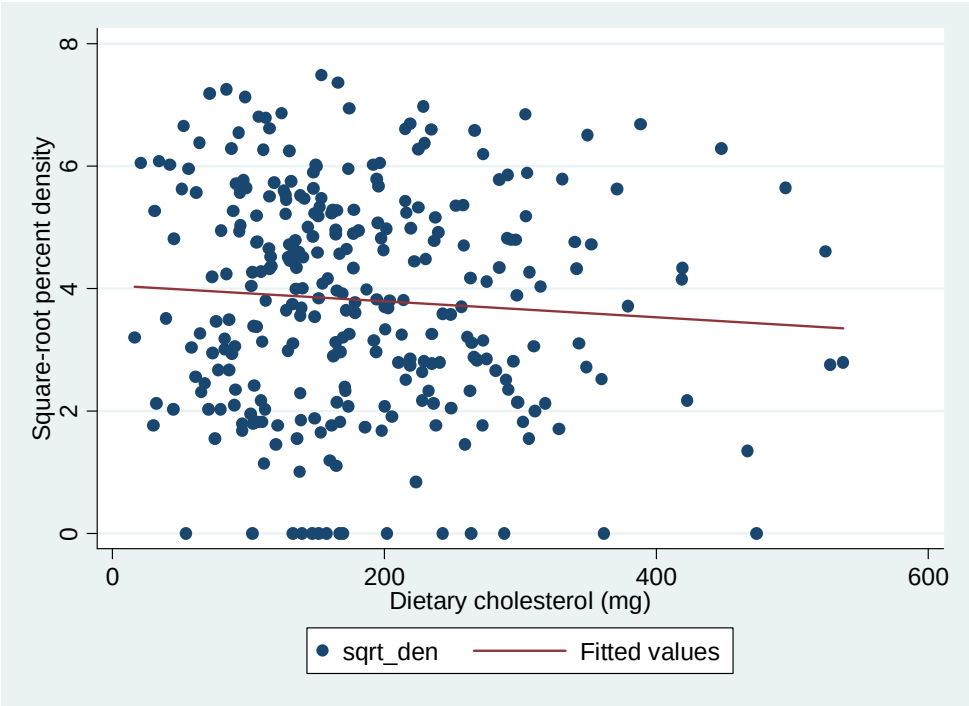


Figure 10: Linear regression model depicting the relation between mammographic density and dietary cholesterol

a) Square-root percent density



b) Square-root dense area

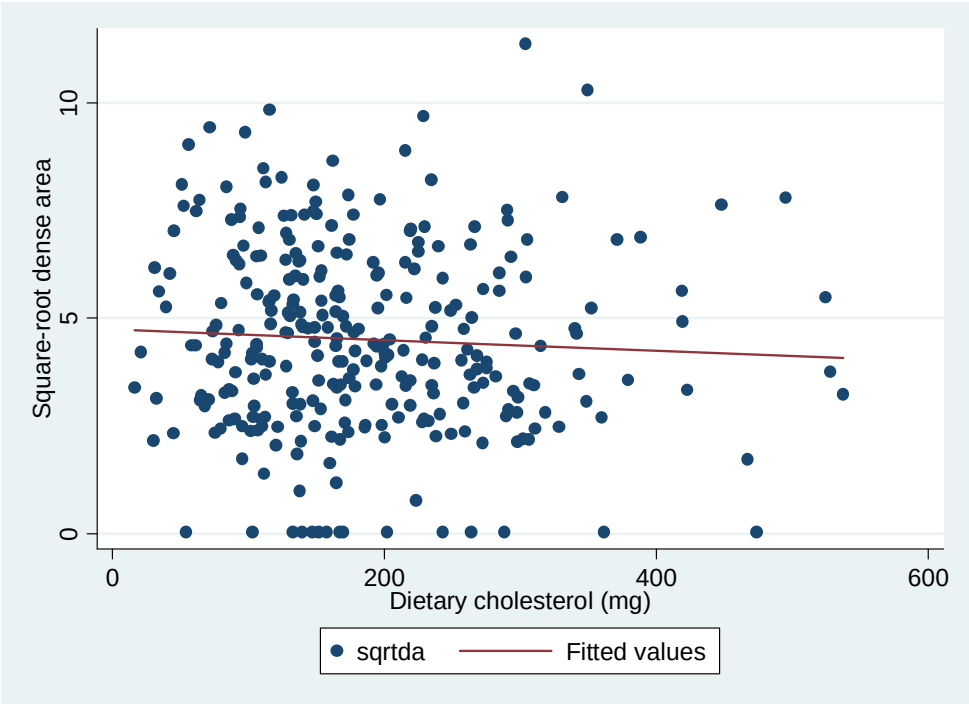


Table 4: Mean, median and range of descriptive variables of ALPHA Trial participants*, Alberta, Canada 2003-2006

Variable	N	Mean	(SD)	Median	Range
Age (years)	303	60.2	(5.6)	60.0	50-74
Age at first birth (years)	274	24.6	(4.9)	23.0	16-43
Live births (number)	302	2.4	(1.3)	2.0	0-8
Age at menarche (years)	299	12.8	(1.5)	13.0	9-19
Years since menopause (years)	250	9.7	(6.3)	8.0	1-32
Body mass index (kg/m^2)	303	28.9	(4.3)	28.4	20.3-43.4
Waist circumference (cm)	303	88.3	(10.3)	87.3	65.3-126.0
Intra-abdominal fat (cm^2)	303	99.9	(55.0)	93.5	14.9-363.9
Sub-cutaneous fat (cm^2)	303	324.1	(114.1)	314.3	75.3-729.0

Table 4 continued

Variable	N	Mean	(SD)	Median	Range
Total calories (kcal/day)	299	1524.9	(555.3)	1448.6	411.4-4783.9
Total fat (g/day)	299	55.5	(28.7)	50.1	12.3-278.1
Fat (% of total calories)	299	32.2	(7.6)	31.9	12.0-61.0
Saturated fat (g/day)	299	17.1	(8.9)	15.6	3.9-63.4
Cholesterol (mg/day)	299	165.7	(82.4)	149.8	16.5-633.5
Alcohol consumption (serv/day)	299	0.36	(0.5)	0.14	0-3.3
HRT duration (years)[+]	134	6.3	(5.3)	5.0	0.08-25.0
Time since HRT (years)[+]	138	3.8	(5.9)	2.0	0-39
Past year physical activity (MET-hrs/wk/yr)	303	122.0	(68.2)	108.3	13.5-537.7

[+]Among the women who had ever used HRT
*Among those with baseline measurements of mammographic density

Table 5: Distribution of categorical descriptive variables of ALPHA Trial participants, Alberta, Canada 2003-2006

Variable	Category	Frequency	Percent
Site	Calgary	152	50.3
	Edmonton	150	49.7
Education	University degree	74	31.0
	College or trade school	109	36.0
	High school or less	98	32.4
Marital status	Married/common-law	228	75.2
	Divorced/separated	56	13.9
	Widowed/never married	32	10.6
HRT use ever	Yes	142	47.0
	No	160	53.0
History of hypercholesterolemia	Yes	96	31.8
	No	206	68.2
Benign breast disease	Yes	75	24.9
	No	226	75.1
Family history of breast cancer	Yes	63	20.8
	No	239	79.2
Statin use – past year	Yes	43	14.2
	No	260	85.8
Statin use - day of blood draw	Yes	32	10.6
	No	271	89.4
Body mass index (kg/m2)	< 25	63	20.8
	25-<30	125	41.3
	≥ 30	115	37.9
Alcohol consumption (serv/day)	0	48	16.1
	0.1-<1	219	73.2
	≥ 1	32	10.7
HRT duration (years)	Never user	160	55.8
	0-<5	60	19.8
	≥ 5	74	24.4

Table 6: Mean, median and range of mammographic density and serum lipid measures

Variable	N	Mean (SD)	Median	Range
Mammographic density variables				
Percent density (%)	303	17.6 (13.4)	14.6	0-56.1
Total area (cm^2)	303	152.2 (53.0)	145.0	32.0-308.8
Total dense area (cm^2)	303	25.0 (21.2)	18.9	0-129.3
Total non-dense area (cm^2)	303	127.2 (53.8)	116.7	22.8-300.4
Serum lipid variables				
Total cholesterol (mmol/L)	302	5.68 (0.91)	5.62	3.73-9.12
Triglycerides (mmol/L)	302	1.51 (0.74)	1.36	0.47-5.02
HDL-C (mmol/L)	302	1.59 (0.34)	1.56	0.86-2.93
LDL-C (mmol/L)	297	3.40 (0.67)	3.33	1.24-5.84

Table 7: Pearson correlation coefficients (r) measuring association between plasma lipid measures

	Cholesterol	**HDL-C**	**LDL-C**	**Triglycerides**
	r 95% CI[a] (p-value)	r 95% CI (p-value)	r 95% CI (p-value)	r 95% CI (p-value)
Cholesterol	----------	0.07 -0.04 to 0.18 (0.21)	0.93 0.91 to 0.94 (<0.001)	0.35 0.25 to 0.44 (<0.001)
HDL-C	0.07 -0.04 to 0.18 (0.21)	----------	-0.16 -0.27 to -0.05 (0.006)	-0.48 -0.56 to -0.39 (<0.001)
LDL-C	0.93 0.91 to 0.94 (<0.001)	-0.16 -0.27 to -0.05 (0.006)	----------	0.23 0.12 to 0.33 (<0.001)
Triglycerides	0.35 0.25 to 0.44 (<0.001)	-0.48 -0.56 to -0.39 (<0.001)	0.23 0.12 to 0.33 (<0.001)	----------

[a]confidence interval

Table 8: Pearson correlation coefficients (r) measuring associations between serum lipids and continuous covariates

Variable	Cholesterol	High-density lipoprotein	Low-density lipoprotein	Triglycerides
	r 95% CI[a] (p-value)	r 95% CI (p-value)	r 95% CI (p-value)	r 95% CI (p-value)
Age	0.13 0.02 to 0.24 (0.03)	0.03 -0.08 to 0.14 (0.60)	0.07 -0.04 to 0.18 (0.22)	0.12 0.01 to 0.23 (0.05)
Age at menarche	0.10 -0.01 to 0.21 (0.07)	-0.05 -0.16 to 0.06 (0.41)	0.09 -0.02 to 0.20 (0.13)	0.10 -0.01 to 0.21 (0.08)
Number of live births	0.08 -0.03 to 0.19 (0.15)	-0.02 -0.13 to 0.09 (0.74)	0.09 -0.02 to 0.20 (0.11)	0.02 -0.09 to 0.13 (0.79)
Age at first birth	-0.14 -0.25 to 0.03 (0.02)	0.06 -0.05 to 0.17 (0.31)	-0.13 -0.24 to -0.02 (0.03)	-0.09 -0.20 to 0.02 (0.15)
Time since menopause	0.09 -0.02 to 0.20 (0.14)	-0.01 -0.12 to 0.10 (0.89)	0.04 -0.07 to 0.15 (0.54)	0.13 0.02 to 0.24 (0.05)
Duration of HRT use	0.21 0.04 to 0.37 (0.02)	0.12 -0.05 to 0.28 (0.18)	0.14 -0.03 to 0.30 (0.11)	0.08 -0.09 to 0.25 (0.36)
Time since last use of HRT	0.12 -0.05 to 0.28 (0.18)	-0.24 -0.39 to -0.08 (0.005)	0.18 0.01 to 0.34 (0.04)	0.13 -0.04 to 0.29 (0.14)
Waist circumference	0.13 0.01 to 0.24 (0.03)	-0.37 -0.46 to -0.27 (<0.001)	0.16 0.05 to 0.27 (0.007)	0.35 0.25 to 0.45 (<0.001)
Body mass index	0.09 -0.02 to 0.20 (0.11)	-0.26 -0.36 to -0.15 (<0.001)	0.10 -0.01 to 0.21 (0.09)	0.26 0.15 to 0.36 (<0.001)

Table 8 continued

Variable	Cholesterol	High-density lipoprotein	Low-density lipoprotein	Triglycerides
	r 95% CI[a] (p-value)	r 95% CI (p-value)	r 95% CI (p-value)	r 95% CI (p-value)
Intra-abdominal fat area	0.17 0.06 to 0.28 0.004	-0.39 -0.48 to -0.29 <0.001	0.18 0.07 to 0.29 0.002	0.43 0.33 to 0.52 <0.001
Sub-cutaneous fat area	0.07 -0.04 to 0.18 (0.26)	-0.24 -0.34 to -0.13 (<0.001)	0.09 -0.02 to 0.20 (0.11)	0.17 0.06 to 0.28 (0.002)
Fat intake	-0.04 -0.15 to 0.07 (0.52)	0.02 -0.09 to 0.13 (0.71)	-0.03 -0.14 to 0.08 (0.66)	0.00 -0.11 to 0.11 (0.99)
Percent fat intake	-0.03 -0.14 to 0.08 (0.64)	0.11 -0.00 to 0.22 (0.05)	-0.06 -0.17 to 0.05 (0.32)	-0.04 -0.15 to 0.07 (0.50)
Saturated fat intake	-0.02 -0.13 to 0.09 (0.78)	0.00 -0.11 to 0.11 (0.94)	-0.01 -0.12 to 0.10 (0.84)	0.01 -0.10 to 0.12 (0.89)
Alcohol intake	0.05 -0.06 to 0.16 (0.40)	0.25 0.14 to 0.35 (<0.001)	0.02 -0.09 to 0.13 (0.78)	-0.12 -0.23 to -0.01 (0.03)
Past year physical activity	0.02 -0.09 to 0.13 (0.64)	-0.06 -0.17 to 0.05 (0.28)	0.02 -0.09 to 0.13 (0.76)	0.06 -0.05 to 0.17 (0.31)

[a] 95% confidence interval

Table 9: Mean serum lipids by levels of categorical covariates with test of significance from Analysis of Variance (ANOVA)

Variable	Categories	Cholesterol	High-density lipoprotein	Low-density lipoprotein	Triglycerides
		mean (p-value)[a]	mean (p-value)	mean (p-value)	mean (p-value)
Site	Edmonton	5.71	1.53	3.49	1.55
	Calgary	5.76	1.66	3.30	1.54
		(0.71)	(<0.001)	(0.06)	(0.92)
Parous	Yes	5.75	1.59	3.41	1.54
	No	5.58	1.65	3.21	1.57
		(0.44)	(0.37)	(0.20)	(0.83)
HRT ever	Yes	5.66	1.61	3.40	1.49
	No	5.76	1.59	3.41	1.56
		(0.43)	(0.61)	(0.86)	(0.45)
Benign breast disease	Yes	5.72	1.63	3.30	1.47
	No	5.71	1.58	3.44	1.54
		(0.94)	(0.26)	(0.20)	(0.53)
Family history of breast cancer	Yes	5.60	1.57	3.43	1.32
	No	5.75	1.60	3.40	1.58
		(0.35)	(0.48)	(0.80)	(0.03)
History of hypercholesterolemia	Yes	6.09	1.54	3.61	1.92
	No	5.54	1.62	3.31	1.34
		(<0.001)	(0.05)	(0.003)	(<0.001)
Alcohol consumption (servings)	0	5.58	1.55	3.25	1.62
	0.1-1	5.68	1.57	3.44	1.51
	>1	5.77	1.81	3.38	1.26
		(0.66)	(<0.001)	(0.39)	(0.09)
Statin use – day of blood draw	Yes	5.21	1.55	2.89	1.80
	No	5.79	1.60	3.45	1.51
		(0.006)	(0.47)	(<0.001)	(0.07)
Statin use – past year	Yes	5.38	1.54	3.05	1.85
	No	5.79	1.60	3.45	1.49
		(0.03)	(0.27)	(0.004)	(0.01)

[a] p-value is from ANOVA

Table 10: Beta coefficients from simple linear regression modeling dietary cholesterol and serum lipids

Lipid	Crude		
	β (r)	**95% CI[a]**	**R^2**
Cholesterol	0.0002 (0.03)	-0.0007 to 0.001	0.0007
HDL-C	0.0001 (0.04)	-0.0002 to 0.0005	0.0001
LDL-C	0.0002 (0.03)	-0.0007 to 0.001	0.0007
Triglycerides	-0.0001 (-0.02)	-0.0009 to 0.0006	0.0004

β=beta-coefficient; r=Pearson correlation coefficient CI=confidence interval; R^2 = explained variance
Note: serum lipids are modeled as the dependent variable

Table 11: Pearson correlation coefficients (r) measuring association between mammographic density measures

	Square-root percent density	**Total area**	**Square root dense area**	**Non-dense area**
	r 95% CI[a] (p-value)	r 95% CI[a] (p-value)	r 95% CI[a] (p-value)	r 95% CI[a] (p-value)
Square-root percent density	----------	-0.278 -0.38 to -0.17 (<0.001)	0.911 0.89 to 0.93 (<0.001)	-0.600 -0.67 to -0.53 (<0.001)
Total area	-0.278 -0.38 to -0.17 (<0.001)	----------	0.091 -0.02 to 0.20 (0.11)	0.921 0.90 to 0.94 (<0.001)
Square-root dense area	0.911 0.89 to 0.93 (<0.001)	0.091 -0.02 to 0.20 (0.11)	----------	-0.286 -0.39 to -0.18 (<0.001)
Non-dense area	-0.600 -0.67 to -0.53 (<0.001)	0.921 0.90 to 0.94 (<0.001)	-0.286 -0.39 to -0.18 (<0.001)	----------

[a]confidence interval

Table 12: Pearson correlation coefficients (r) measuring associations between mammographic density and continuous covariates

Variable	Square-root percent density	Square-root dense area
	r 95% CI[a] (p-value)	r 95% CI (p-value)
Age	-0.12 -0.22 to -0.01 (0.04)	-0.09 -0.20 to 0.02 (0.10)
Age at menarche	-0.04 -0.15 to 0.07 (0.52)	-0.01 -0.12 to 0.10 (0.84)
Number of live births	-0.07 -0.18 to 0.04 (0.21)	-0.05 -0.16 to 0.06 (0.38)
Age at first birth	0.15 0.03 to 0.26 (0.01)	0.12 0.00 to 0.24 (0.05)
Time since menopause	-0.09 -0.20 to 0.02 (0.18)	-0.05 -0.16 to 0.06 (0.41)
Duration of HRT use	-0.03 -0.20 to 0.14 (0.69)	0.02 -0.15 to 0.19 (0.82)
Time since last HRT use	-0.11 -0.27 to 0.06 (0.20)	-0.12 -0.28 to 0.05 (0.18)
Waist circumference	-0.49 -0.57 to -0.40 (<0.001)	-0.27 -0.37 to -0.16 (<0.001)
Body mass index	-0.45 -0.54 to -0.36 (<0.001)	-0.24 -0.34 to -0.13 (<0.001)

Table 12 continued

Variable	Square-root percent density	Square-root dense area
	r 95% CI[a] (p-value)	r 95% CI (p-value)
Intra-abdominal fat	-0.36 -0.45 to -0.26 (<0.001)	-0.18 -0.29 to -0.07 (0.002)
Sub-cutaneous fat	-0.49 -0.57 to -0.40 (<0.001)	-0.28 -0.38 to -0.17 (<0.001)
Fat intake	0.04 -0.07 to 0.15 (0.48)	0.04 -0.07 to 0.15 (0.51)
Percent fat intake	-0.04 -0.15 to 0.07 (0.47)	-0.06 -0.17 to 0.05 (0.29)
Saturated fat intake	0.03 -0.08 to 0.14 (0.60)	0.05 -0.06 to 0.16 (0.43)
Alcohol intake	0.10 -0.01 to 0.21 (0.07)	0.04 -0.07 to 0.15 (0.47)
Past year physical activity	-0.06 -0.17 to 0.05 (0.31)	-0.06 -0.17 to 0.05 (0.30)

[a]CI = confidence interval

Table 13: Mean mammographic density by levels of categorical covariates with test of significance from Analysis of Variance (ANOVA)

Variable	Categories	Square-root percent density	Square-root dense area
		mean (p-value)[a]	mean (p-value)
Site	Edmonton	3.76	4.58
	Calgary	3.82	4.40
		(0.79)	(0.47)
Parous	Yes	3.78	4.50
	No	3.91	4.32
		(0.72)	(0.66)
HRT ever	Yes	3.82	4.54
	No	3.77	4.44
		(0.81)	(0.71)
Benign breast disease	Yes	4.37	5.24
	No	3.59	4.22
		(0.001)	(<0.001)
Family history of breast cancer	Yes	3.97	4.64
	No	3.75	4.45
		(0.37)	(0.54)
History of hypercholesterolemia	Yes	3.45	4.23
	No	3.96	4.61
		(0.02)	(0.15)
Alcohol consumption (servings)	0	3.76	4.53
	0.1-1	3.74	4.45
	>1	4.25	4.77
		(0.32)	(0.73)
Statin use – day of blood draw	Yes	3.37	4.27
	No	3.85	4.53
		(0.15)	(0.53)
Statin use – past year	Yes	3.36	4.20
	No	3.88	4.56
		(0.08)	(0.32)

[a] p-value is from test of ANOVA

Table 14: Comparison of selected continuous and categorical characteristics across levels of past year average alcohol consumption

Variable	Non-drinker (n=48)	< 1 drink/day (n=218)	≥ 1 drink/day (n=32)	p-value*
Age (years)	59.9	60.3	61.3	0.52
Triglycerides (mmol/L)	1.6	1.5	1.3	0.09
Calories	1578.2	1483.8	1838.7	0.003
Fat (grams)	56.7	54.6	68.5	0.05
Percent calories from fat (%)	32.0	32.6	32.1	0.86
Dietary cholesterol (mg)	150.7	192.1	229.6	0.005
Sub-cutaneous fat (cm^2)	324.3	328.8	285.0	0.13
Body mass index (kg/m^2)	28.9	29.1	27.5	0.16
Exercise (METhrs/wk/yr)	133.2	121.5	109.1	0.30
First birth age (years)	25.1	24.6	24.2	0.74
Live births (number)	2.1	2.4	2.5	0.39
HRT duration (years)	6.9	6.2	6.5	0.91
HRT ever (%)	29.8	50.5	53.1	0.03
Smoking ever (%)	13.0	32.0	46.9	0.001
Married/common-law (%)	68.1	74.7	93.7	0.03
Greater than high-school (%)	61.8	65.8	84.4	0.08

Table 15: Association between triglycerides and square-root percent density, by levels of average past year alcohol consumption

Lipid	Non-drinker (n=47)		≤1 drink/day (n=214)		> 1 drink/day (n=32)	
	Crude	Adjusted[a]	Crude	Adjusted[a]	Crude	Adjusted[a]
	β 95% CI[b] (r)	β 95% CI[b] (partial r)	β 95% CI[b] (r)	β 95% CI[b] (partial r)	β 95% CI[b] (r)	β 95% CI[b] (partial r)
Triglycerides	0.05 -0.61 to 0.71 (0.02)	0.15 -0.44 to 0.73 (0.08)	-0.34 -0.66 to -0.02 (-0.14)	0.19 -0.12 to 0.50 (0.08)	-1.65 -2.69 to -0.62 (-0.51)	-0.94 -1.79 to -0.10 (-0.40)

[a] Adjusted for age (categories) and waist circumference (continuous)
[b] CI: confidence intervals around the parameter estimates

Table 16: Association between triglycerides and square-root dense area, by levels of average past year alcohol consumption

Lipid	Non-drinker (n=47)		≤1 drink/day (n=214)		> 1 drink/day (n=32)	
	Crude	Adjusted[a]	Crude	Adjusted[a]	Crude	Adjusted[a]
	β 95% CI[b] (r)	β 95% CI[b] (partial r)	β 95% CI[b] (r)	β 95% CI[b] (partial r)	β 95% CI[b] (r)	β 95% CI[b] (partial r)
Triglycerides	0.31 -0.53 to 1.14 (0.11)	0.29 -0.52 to 0.10 (0.11)	-0.06 -0.45 to 0.33 (-0.02)	0.34 -0.08 to 0.75 (0.11)	-1.55 -2.83 to -0.27 (-0.41)	-0.81 -1.97 to 0.36 (-0.26)

[a] Adjusted for age (categories) and waist circumference (continuous)
[b] CI: confidence intervals around the parameter estimates

Table 17: Mean percent mammographic density for 55-59 year old women with a waist circumference of 88 cm, by levels of alcohol consumption

	Mean percent mammographic density (%)		
	Triglyceride= 0.60 mmol/L	Triglyceride= 1.5 mmol/L	Triglyceride= 2.56 mmol/L
Non-drinker	11.6	13.1	15.1
≤ 1 drink/day	13.0	14.3	15.9
> 1 drink/day	21.9	13.8	6.7

Table 18: Beta-coefficients from multiple linear regression model assessing the associations between serum lipid measures and square-root percent density

Lipid	Crude		Age-adjusted[a]		Age & waist circumference adjusted	
	β (r)	95% CI[b]	β (partial r)	95% CI	β (partial r)	95% CI
Cholesterol	-0.23 (-0.12)	-0.45 to -0.01	-0.19 (-0.10)	-0.41 to -0.03	-0.06 (-0.04)	-0.26 to 0.13
HDL-C	0.96 (0.18)	0.34 to 1.54	0.99 (0.19)	0.42 to 1.57	0.06 (0.01)	-0.48 to 0.61
LDL-C	-0.32 (-0.15)	-0.57 to -0.08	-0.28 (-0.13)	-0.53 to -0.04	-0.11 (-0.06)	-0.33 to 0.10

[a] Age categories = 51-54, 55-59, 60-64, 65-74; [b] 95% CI= 95% confidence interval; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein

Table 19: Beta-coefficients from multiple linear regression model assessing the associations between serum lipid measures and square-root dense area

Lipid	Crude		Age-adjusted[a]		Age & waist circumference adjusted	
	β (r)	95% CI[b]	β (partial r)	95% CI	β (partial r)	95% CI
Cholesterol	-0.20 (-0.09)	-0.47 to 0.06	-0.14 (-0.06)	-0.41 to 0.13	-0.06 (-0.02)	-0.32 to 0.20
HDL-C	0.49 (0.08)	-0.22 to 1.21	0.55 (0.09)	-0.15 to 1.26	-0.11 (-0.02)	-0.84 to 0.62
LDL-C	-0.30 (-0.11)	-0.60 to -0.00	-0.24 (-0.09)	-0.54 to 0.06	-0.12 (-0.05)	-0.41 to 0.17

[a] Age categories = 51-54, 55-59, 60-64, 65-74 [b] 95% CI= 95% confidence interval; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein

Table 20: Pearson correlation coefficients (r) measuring associations between dietary cholesterol and continuous covariates

Variable	Dietary cholesterol
	Correlation coefficient 95% CI[a] p-value
Age	-0.14 -0.25 to -0.03 (0.02)
Waist circumference	0.05 -0.06 to 0.16 (0.36)
Body mass index	0.06 -0.05 to 0.17 (0.29)
Intra-abdominal fat	-0.07 -0.18 to 0.04 (0.22)
Sub-cutaneous fat	0.07 -0.04 to 0.18 (0.23)
Total body fat	0.06 -0.05 to 0.17 (0.34)
Alcohol consumption	0.12 0.01 to 0.23 (0.05)
Total caloric consumption	0.56 0.48 to 0.63 (<0.001)
Dietary fat intake	0.67 0.60 to 0.73 (<0.001)

Table 20 continued

Variable	Dietary cholesterol
	Correlation coefficient 95% CI[a] (p-value)
Dietary saturated fat intake	0.72 0.66 to 0.77 (<0.001)
Percent calories from fat	0.51 0.42 to 0.59 (<0.001)
Past year physical activity	0.05 -0.06 to 0.16 (0.37)

[a]confidence interval

Table 21: Mean dietary cholesterol levels (mg/day) by levels of categorical covariates with test of significance from Analysis of Variance (ANOVA)

Variable	Category	Dietary cholesterol
		mean (p-value)
Site	Calgary	187.9
	Edmonton	190.6
		(0.78)
HRT ever	Yes	190.3
	No	187.6
		(0.83)
Family history of breast cancer	Yes	184.7
	No	190.0
		(0.74)
Statin use – past year	Yes	161.6
	No	194.1
		(0.07)
Statin use – day of blood draw	Yes	185.0
	No	190.0
		(0.81)
Alcohol consumption	0	92.3
	0.1-1	186.0
	>1	210.9
		(0.005)

Table 22: Beta-coefficients from linear regression model assessing the association between dietary cholesterol and square-root percent density

Variable	Crude		
	β (r)	**95% CI**	**R^2**
Dietary cholesterol	-0.001 (-0.06)	-0.003 to 0.001	0.004

β=beta-coefficient; r=Pearson correlation coefficient, R^2=explained variance; CI=confidence interval

Table 23: Beta-coefficients from linear regression model assessing the association between dietary cholesterol and square-root dense area

Variable	Crude		
	β (r)	**95% CI**	**R^2**
Dietary cholesterol	-0.001 (-0.05)	-0.004 to 0.002	0.002

β=beta-coefficient; r=Pearson correlation coefficient, R^2=explained variance; CI=confidence interval

Chapter Five: DISCUSSION

5.1 Overview of main results

In this sample of sedentary, postmenopausal women we found that total cholesterol and LDL-C were negatively associated with percent density, while HDL-C was positively associated with percent density in age-adjusted analyses. Serum cholesterol measures were not statistically significantly related to dense tissue area. After controlling for the confounding effects of waist circumference in addition to age, the associations between total cholesterol, HDL-C and LDL-C and percent density were attenuated. Waist circumference explained most of the variance in percent density. We did observe that alcohol consumption modified the relation between serum TG and mammographic density. In those women who consumed more than one drink of alcohol per day on average, percent density decreased with increasing serum triglyceride levels, while in those women who drank less than this amount or no alcohol at all, no relation was evident. Even though body weight and age are known to influence both mammographic density and TG levels, controlling for their effects did not attenuate the results, however the confidence intervals were quite wide. Although the same type of effect modification by alcohol consumption was observed between TG and dense area, the relations were not statistically significant. Dietary cholesterol was highly correlated with dietary factors such as total calories, fat, and saturated fat. There was no evidence of a relation between dietary cholesterol and either mammographic density measure, nor

was there any association observed between dietary cholesterol and serum cholesterol measures.

5.2 Previous Research

Two previous studies have examined the relation between mammographic density and serum lipids in postmenopausal women [Aiello et al., 2005;Maskarinec et al., 2001]. The results of these investigations were conflicting. Aeillo and colleagues found that in women who had used HRT in the past, mammographic density was positively associated with total cholesterol and LDL-C, regardless of whether percent density was analyzed as a categorical or continuous variable [Aiello et al., 2005]. In never-users of HRT the opposite trend was observed; total cholesterol and LDL-C were inversely associated with a continuous measure of percent density but no evidence of a relation was observed in the categorical analysis. These results indicate that HRT use may modify the association between serum lipids and mammographic density. Maskarinec and colleagues conducted a smaller study with a multi-ethnic population living in Hawaii [Maskarinec et al., 2001]. They observed a negative association between HDL-C and percent density and dense tissue area after controlling for confounding variables. It should be noted that some of these women were using HRT at the time of the study, although the exact proportion was not reported [Maskarinec et al., 2001]. Maskarinec's results corroborate with the results of the past HRT users in the previous study. The researchers found that HDL-C was negatively associated with percent density which would coincide with a positive association with LDL-C. Aiello and colleagues suggested that the difference they found between HRT users and never-users was likely attributable to past use of progesterone

therapy [Aeillo et al., 2005], as progesterone, in combination with estrogen has been shown to have greater effects on mammographic density than estrogen-only therapies [Greendale et al., 1999;Lundstrom et al., 1999;Sendag et al., 2001]. They did not, however, collect data on the type of HRT and therefore could not assess this assumption. Maskarinec and colleagues also did not report that they collected this information [Maskarinec et al., 2001]. In our study we collected detailed information regarding the composition of past HRT use and subsequently examined the modifying effects of progesterone-only, estrogen-only, and combined therapy, but found no effect by any HRT formulation. Since we found no evidence of effect modification by HRT use, our sample may be more representative of never-users of HRT. The duration of time between stopping HRT use and entry into the trial was not stated in the Aeillo paper [Aiello et al., 2005]. In our study, one of the inclusion criteria was having stopped HRT use at least 6 months prior to entry into the trial and the average time between HRT use and study enrolment was 3.8 years. It could be that the period between discontinuing HRT use and entry into the trial was shorter in the Aeillo study, and the effects of HRT on serum cholesterol or mammographic density were still present.

The results of our study are contradictory to the results of past-HRT users in these previous studies [Aiello et al., 2005;Maskarinec et al., 2001]. However, our age-adjusted regression results corroborate with the results of non-HRT users in Aeillo's study and are consistent with a previous study by Boyd and colleagues [1989] among premenopausal women who observed that mammographic density was inversely associated with LDL-C and TG and positively associated with HDL-C [Boyd et al.,

1989]. Boyd and colleagues confirmed these results in a later study using a continuous measure of mammographic density [Boyd et al., 1995b]. In contrast to our study, the results of Boyd's study remained significant even after controlling for age and BMI [Boyd et al., 1995b]. The two studies conducted by Boyd's group [Boyd et al., 1989;Boyd et al., 1995b], were conducted with premenopausal women while our study analyzed the data from postmenopausal women. This difference in study populations would mainly effect the distribution of mammographic density in the sample. In their first study they compared women with breast densities of less than 25 percent to women with densities greater than 75 percent [Boyd et al., 1989]. In their second study, the mean percent density in the lowest quintile was 7.3 percent, and in the highest it was 84.3 percent [Boyd et al., 1995b]. In our study, percent density ranged from zero to 56 percent. The smaller range of percent density could explain why our study results did not remain statistically significant after controlling for confounding variables. The other difference between our study and that of Boyd's is that they controlled for BMI and our study controlled for waist circumference. Presumably, waist circumference is more highly associated with serum lipids, and in our study, was more strongly associated with mammographic density than BMI. It could be that there is residual confounding in the studies by Boyd, thus allowing their results to remain significant. However, controlling for BMI in our study did not alter the significance of our results.

As mentioned previously, our study observed a modifying effect of alcohol consumption on the relation between mammographic density and TG. In the four previous studies that examined the relation between serum cholesterol and measures of

mammographic density [Aiello et al., 2005;Boyd et al., 1989;Boyd et al., 1995b;Maskarinec et al., 2001], three included measures of TG [Boyd et al., 1989;Boyd et al., 1995b;Maskarinec et al., 2001] and none of them observed effect modification by alcohol consumption. Examination of our data revealed that the range of TG values, in the greater than one drink per day subgroup, was smaller than the range of TG values in the other alcohol subgroups; whereas the range of percent density values remained constant between groups. The smaller range of TG values caused a steeper slope in the regression analysis and hence, the significant interaction term, indicating that this slope was different from the regression slope depicting the relation in non-drinkers. Since our study is the first to observe this modification by alcohol consumption it is important to question whether the range of TG values was modified by alcohol consumption or was the result of chance.

The relation between alcohol and TG is also inconclusive. Alcohol has independently been shown to increase TG levels one, three and five hours post-consumption [van der Gaag et al., 2000] although long term effects (eight-weeks) of alcohol consumption have not been observed [Baer et al., 2002]. The American Heart Association, however, warns that excessive alcohol consumption could lead to a condition of high-triglyceride levels, known as hypertriglyceridemia [Lichtenstein et al., 2006]. Alcohol may, therefore, increase TG levels, decreasing the frequency of the lower TG values and therefore narrowing the range of values. However, our study found that TG levels were lower in the high alcohol intake subgroup, although not significantly different from the other subgroups (TG=1.3, vs. 1.6 for non-drinkers and 1.5 for less than

or equal to one drink per day, p=0.08). These results are contrary to what may be expected, therefore alcohol may not be the modifying factor in this relation.

The women in the high alcohol consumption sub-group differed on some important variables compared to the other subgroups. They consumed more calories, ate greater amounts of dietary cholesterol and saturated fat, and exercised less. However, their average BMI and area of subcutaneous fat were the lowest (although not statistically significant) and HDL-C levels were the highest of the three groups. More of them were married, had previously smoked and had completed greater than high-school education. These women appear to be representative of the "French paradox" [Renaud and de Lorgeril M., 1992] since they eat greater amounts of saturated fat, yet they appear to be at low risk for cardiovascular disease (high HDL-C levels, moderate LDL-C levels, lower BMI). It is perhaps a combination of these factors that contributes to the smaller range of TG values although the biological mechanisms guiding this hypothesis are difficult to assess.

The sample size of this subgroup was small (n=32) and may provide the most logical explanation for the small range of TG values observed in this group. As sample size increases, the probability of capturing the full range of plausible values increases. It is possible that the narrow range of TG values in this subgroup is the result of random error. In order to test this hypothesis, a larger study capturing a more diverse population of women with diverse alcohol intake patterns would have to be conducted.

This study did not observe a statistically significant association between HDL-C and mammographic density after adjustment for important confounding variables. HDL-C has been shown to be highly associated with other breast cancer risk factors, such as country of residence, parity, socioeconomic status, dietary fat, alcohol, endogenous hormones and pre-menopausal leanness [Boyd and McGuire, 1990]. In a Norwegian study, HDL-C was also found to be inversely associated with leptin, insulin, and DHEA and positively associated with salivary concentration of progesterone [Furberg et al., 2005]. HDL-C has also been shown to be directly associated with mammographic density [Boyd et al., 1989;Boyd et al., 1995b] and mammographic density has been shown to be positively associated with a “healthy metabolic profile” (low BMI, high HDL-C) [Furberg et al., 2005]. Based on these associations, it was expected that HDL-C would be associated with mammographic density in this study. However, in the four previous studies [Aiello et al., 2005;Boyd et al., 1989;Boyd et al., 1995b;Maskarinec et al., 2001] that have examined the association between serum lipids and mammographic density, two studies that found statistically significant positive associations between HDL-C and percent density were conducted in pre-menopausal women [Boyd et al., 1989;Boyd et al., 1995b]. Maskarinec also observed significant associations between HDL-C and percent density and dense area in postmenopausal women, however in the opposite direction to that of Boyd. The direction of our age-adjusted results corroborate with that of Boyd’s group [Boyd et al., 1989;Boyd et al., 1995b]. Again, the direction of results may differ between our study and that of Maskarinec [Maskarinec et al., 2001] because of a modification by HRT use. Some of the 39 women in their study were taking HRT at the time of study whereas the women in our study had not been using HRT for at

least six months prior to study enrolment. Since HDL-C levels are known to decrease after the menopause [Collins, 2008], and since mammographic density also decreases after the menopause [Martin and Boyd, 2008], perhaps this relation is attenuated in postmenopausal women not taking HRT, which could explain why our results did not remain significant after adjustment for waist circumference. As previously mentioned, the range of mammographic density was also smaller in our study compared to Boyd's studies, hence this could also contribute to the null association. Overall, HDL-C remains to be an important cholesterol constituent in the relation with mammographic density and with breast cancer and therefore these relations should continue to be explored.

Dietary cholesterol was not a good predictor of mammographic density in our study. A number of other studies have examined the association between diet and mammographic density [Nagata et al., 2005;Nordevang et al., 1993;Sala et al., 2000;Takata et al., 2007;Thomson et al., 2007;Masala et al., 2006;Brisson et al., 1989;Knight et al., 1999;Vachon et al., 2000;Boyd et al., 1989]. Only four specifically examined the association between mammographic density and dietary cholesterol [Brisson et al., 1989;Knight et al., 1999;Vachon et al., 2000;Boyd et al., 1989], while others included measures of total and saturated fat. In the four studies that included assessments of dietary cholesterol, only two [Knight et al., 1999; Brisson et al., 1989] found a significant association between change in mammographic density and dietary cholesterol. Knight and colleagues examined the effects of a low-fat dietary intervention on the change in mammographic density during menopause. The researchers found that a decrease in dietary cholesterol explained 18% of the variance in the decrease in

mammographic density that occurred during this two-year time period, after adjusting for confounding factors. This observation was only observed in women who were premenopausal at study start and postmenopausal at end of study. In those women that remained pre-menopausal or postmenopausal throughout the study, no association between dietary cholesterol and change in mammographic density was observed. Brisson and colleagues [1989], observed a positive association between dietary cholesterol and mammographic density patterns in the control group [Brisson et al., 1989]. The two cross-sectional studies that examined this relation failed to find an association between dietary cholesterol and mammographic density [Boyd et al., 1989;Vachon et al., 2000]. Overall, there is limited evidence to support an association between dietary cholesterol and mammographic density.

The lack of association observed between dietary factors and mammographic density in cross-sectional studies could be due to a few factors. As will be discussed later in the Limitations section, questionnaire methods of measuring diet have been criticized for capturing inaccurate data which lead to underreporting of total calories [Subar et al., 2003]. This measurement error results in an underestimation of reported fat and alcohol consumption. In our analysis, this would decrease the proportion of values in the upper range of dietary cholesterol and reduce our ability to detect a linear association between dietary cholesterol and mammographic density.

The temporal nature of a cross-sectional study must also be considered when assessing the results of this study. As stated, mammographic density is affected by many

factors throughout the lifecycle. Our diet history questionnaire assessed average consumption of food over the year prior to enrolment into the study. Cholesterol consumption during this time period may not be predictive of mammographic density in the same year. However, the overall trend of dietary patterns that is captured by this questionnaire may still reflect long term trends in consumption. Therefore, the temporal nature of the study may not be an inhibiting factor for detecting a relation between these variables.

In this study, dietary cholesterol was also not a good predictor of serum cholesterol. As stated in the literature review, the responsiveness of an individual's serum cholesterol levels to dietary cholesterol may depend on his or her genetic predisposition [Abbey, 1992]. In particular, it is thought that apolipoprotein gene polymorphisms are associated with an individual's plasma lipid response, and therefore creating "hyper-responders" and "hypo-responders" to dietary cholesterol and fat [Katan et al., 1986]. Dichotomizing the study sample into non-responders and responders may have allowed for detection of an association between dietary cholesterol and serum cholesterol, however this analysis was exploratory and more complex data were not collected.

In summary, our results suggest that serum cholesterol is not a good predictor of percent density, and especially dense tissue area. The effect modification that was observed between TG and percent density by alcohol intake is not easily explained and has not been observed in other studies; hence this modification may be the result of

chance and necessitates further research. Lastly, the nature of the cross-sectional design, in combination with underreporting that is inherent in food frequency questionnaires, may have limited our analyses of dietary cholesterol. These results are, however, consistent with previous cross-sectional studies that have examined this association.

5.3 Association of serum cholesterol with other breast cancer risk factors

Serum cholesterol has been shown to be associated with breast cancer [Furberg et al., 2004;Manjer et al., 2001;Michalaki et al., 2005;Ray and Husain, 2001] and breast cancer risk factors [Castelli et al., 1977;Glueck et al., 1980;Heiss et al., 1980;Matthews et al., 1989;Punnonen et al., 1987;Sacks et al., 1975;Snook et al., 1985;van Stiphout et al., 1987] in previous studies. This study did not examine breast cancer as an outcome but it did measure many breast cancer risk factors. It is possible that the association between lipids and mammographic density operate through other breast cancer risk factors. In order to elucidate these mechanisms, it is important to examine the interrelationships between cholesterol, mammographic density and breast cancer risk factors.

Obesity is associated with serum lipids, mammographic density and breast cancer. In our study, BMI and waist circumference were highly and positively correlated with LDL-C, TG and total cholesterol and negatively associated with HDL-C. Obesity is also known to increase breast cancer risk in postmenopausal women [Stephenson and Rose, 2003]. This trend indicates that a poor lipid profile may be associated with increased breast cancer risk. This observation has been confirmed in four previous studies examining the relation between serum cholesterol and breast cancer risk [Furberg et al.,

2004;Manjer et al., 2001;Michalaki et al., 2005;Ray and Husain, 2001]. Obesity is also independently associated with mammographic density [Boyd et al., 2005]. In our study, waist circumference and BMI were negatively associated with mammographic density and explained most of the variance in the linear regression models. However, there is little evidence to support a strong relation between mammographic density and serum cholesterol. This suggests that cholesterol and mammographic density may influence breast cancer risk through two different pathways involving adipose tissue. For example, the relation between TG and visceral fat may be important in the association between serum cholesterol and breast cancer risk. Recent studies have examined the association between adiponectin, a hormone secreted from adipose tissue, and breast cancer. In each case-control study, adiponectin levels were lower in cases compared to the controls [Chen et al., 2006;Mantzoros et al., 2004;Miyoshi et al., 2003]. Also, a recent review article noted that adiponectin levels have been found to be lower in patients with breast, endometrial, prostate and colon cancer [Abbey, 2002]. Adiponectin also regulates fatty acid metabolism [Barb et al., 2007], and is inversely associated with visceral fat [Kwon et al., 2005] therefore creating a link between lipids, visceral fat and cancer. Mammographic density, on the other hand, is more closely associated with sub-cutaneous fat than visceral fat, as was indicated in our study. This relation makes sense, as the breast is primarily composed of subcutaneous fat. The difference in type of adipose tissue (visceral vs. subcutaneous) suggests that cholesterol may act through a different obesity pathway than mammographic density to affect breast cancer risk. Examining the relation between different types of adipose tissue and mammographic density may also elucidate the mechanisms causing dense tissue in the breast.

Dietary fat and saturated fat have also been associated with serum cholesterol [Hu et al., 2001], mammographic density [Boyd et al., 2005] and breast cancer [Boyd et al., 2003]. A recent meta-analysis concluded that consuming a high amount of fat is associated with a 13 percent increase in breast cancer risk compared to women who consume smaller amount of fat [Boyd et al., 2003]. Our study did not observe significant associations between dietary fat and saturated fat and either serum cholesterol or mammographic density. These results do not help to elucidate any possible interrelation between dietary fat, serum cholesterol and mammographic density. The lack of association between dietary fat and serum cholesterol could be due to the genetic predisposition [Katan et al., 1986] or to measurement error in the Diet History Questionnaire [Subar et al., 2003] (see Study Limitations). As well, dietary fat and mammographic density have only been weakly and inconsistently associated in previous studies [Boyd et al., 2005]. Based on the complex nature of these relations it is difficult to assess the role of dietary fat in the relation between cholesterol and mammographic density.

High physical activity has been well established in the literature to reduce breast cancer risk [Friedenreich and Cust, 2008;Monninkhof et al., 2007]. The beneficial effects of physical activity on serum lipid profiles is also well established [Lichtenstein et al., 2006]. However, we did not observe significant correlations between physical activity and serum cholesterol measures. This null result could be due to the sedentary nature of the sample that we selected for this study. There is no established relation between

physical activity and mammographic density [Boyd et al., 2005], and this study's results corroborate with this observation. This suggests that the mechanisms by which physical activity affects breast cancer may differ from the mechanisms which associate serum lipids with mammographic density.

Lastly, having a family history of breast cancer was associated with lower TG levels. Total cholesterol, LDL-C and HDL-C did not differ between these two groups. Boyd and colleagues also observed this correlation with TG in pre-menopausal women [Boyd et al., 1989] which then prompted him to examine serum lipids amongst cases of familial breast cancer [Boyd et al., 1995c]. In the multiple regression analysis, total cholesterol and LDL-C were significantly lower in familial breast cancer cases compared to sporadic cases. Triglycerides were not significantly different between the two groups. The results of our study and Boyd's two studies [Boyd et al., 1989;Boyd et al., 1995c] suggest that gene-environment interactions may moderate both lipids and breast cancer risk through similar pathways. Mammographic density is also positively associated with family history of breast cancer and is influenced by genetic factors [Boyd et al., 2005]. This indicates that genetic factors may be important in the association between serum lipids and mammographic density. This topic will be further explored in the Future Directions section.

In summary, obesity and family history of breast cancer are associated with both serum cholesterol and mammographic density. Exploring these factors in detail may help to elucidate the mechanisms that guide the association between cholesterol and

mammographic density. Dietary fat and physical activity, variables that are highly associated with serum lipids, do not have strong associations with mammographic density, suggesting that cholesterol may not work through these factors to affect mammographic density.

5.4 Study strengths

This study's strength rests in the superior methods used for measuring all variables that were used in this analysis, particularly the outcome variable of mammographic density and the main predictor variables of serum lipids. From previous literature, we know that a continuous measure of mammographic density is more accurate in the prediction of breast cancer risk [Byng et al., 1994]. Although we did not examine breast cancer directly in this study, we recognize that any investigation of mammographic density should use the more precise measure of mammographic density to examine relations with other risk factors. The reader of mammographic density for this study, Dr. Norman Boyd is arguably the most experienced reader using Cumulus®, an interactive density-thresholding software program. His measures of mammographic density have been reliable in the past, with observed ICCs of 0.912 for dense area and 0.897 for percent density [Boyd et al., 1995a]. In our study the ICCs ranged from 0.94 to 0.95 for the measures of percent density and absolute dense area indicating that these measures were reliable.

Serum lipids were measured using standard procedures at reputable laboratories in both Calgary and Edmonton. Our sample fasted for a mean of 12.9 hours which is well

within the acceptable range of the standard fasting times of 10-16 hours [Calgary Laboratory Services, 2002]. Since serum lipids are affected by recent food consumption, fasting serum levels were needed to ensure the validity of the serum cholesterol results. Not only were fasting times recorded by the participant and the phlebotomist, the standard lab procedure was to refuse blood collection in any woman if she had not been fasting. In addition, the ALPHA Trial Study Coordinators were contacted when any participant had arrived without fasting for at least 10 hours in order to facilitate rapid rescheduling of these participants. Therefore, even though fasting times were not recorded for all of the participants (226/302), there is a low probability that the participants had not fasted, ensuring that the serum cholesterol values were not affected by recent food consumption.

All blood was collected and analyzed within 12 hours of collection, which is within proper laboratory procedures for serum lipids [Calgary Laboratory Services, 2002]. Out of 303 participants, only one had extreme values that could not be explained and this participant was removed from all analyses of serum lipids in this project. This measure was deemed a laboratory recording error or a processing error. Therefore, blood processing did not affect the validity of our results.

Another strength of this study was the thorough collection of covariate data with which to control for confounding and gain insight into biological mechanisms. Of particular strength was the measurement of percent body fat, subcutaneous fat and intra-abdominal fat using dual x-ray absorptiometry and computed tomography scans. Other

studies [Boyd et al., 1989;Boyd et al., 1995b] relied on skin-caliper measures of body fat and have not had the detailed information on the differing layers of abdominal obesity. In our study we found that sub-cutaneous fat was highly correlated with waist circumference (r=0.77), and was correlated with percent density (r=-0.49). A previous analysis, using these data, examined the association between various anthropometric variables and mammographic density and found that total abdominal area, as measured by the CT scan, was the variable that explained most of the variance in percent density [Woolcott, 2006]. In order to elucidate the mechanisms by which adiposity affects mammographic density, it is important to understand which types of fat are most relevant to the association. In this analysis we found that waist circumference was the largest confounder of the relation between cholesterol and mammographic density and explained the most amount of variance in mammographic density. However, sub-cutaneous fat was also highly associated with mammographic density, whereas intra-abdominal fat was not as strongly associated. The difference in strength of relation between these two markers of central adiposity and mammographic density may be important for understanding the pathways through which adiposity effects breast cancer risk and mammographic density patterns. More importantly for this analysis, having many central adiposity measures allowed for specific selection of confounding variables in the linear regression models. Being able to analyze and select the adiposity measure that confounds the relation the most minimizes the chance of residual confounding.

The detailed collection of medication data was another strength of this study. All previous studies examining this relation did not collect information on medication use.

Of importance to this analysis were those women taking cholesterol lowering medications, such as statins, that may have modified the association between cholesterol and mammographic density. We assessed statin use as both a modifier and a confounder of the relation between serum cholesterol and mammographic density and found no effect of these medications on this relation. Having had the ability to analyze these effects helps to ensure the validity of our results.

This study had several methodological strengths that enhance the study's internal validity and reliability. Both measures of mammographic density and serum cholesterol were performed following standard procedures. Mammographic density was measured by an expert who produced very reliable measures. Serum cholesterol was measured after a minimum 10 hour fast, ensuring consumption of food did not confound the results. This study also collected detailed information on covariate data that was considered thoroughly in the analysis, thereby reducing the influence of residual confounding on the final results.

5.5 Study limitations

5.5.1 Study design

When considering these findings, the methodological limitations of this study must also be taken into consideration. The first potential weakness was the cross-sectional design. It is known that both cholesterol and mammographic density change over time and are influenced by numerous factors through out the aging process. Consequently, a cross-sectional design may not be the most appropriate study design

since temporal relations cannot be considered with this type of design. It may be more appropriate to examine this biologic relation in a longitudinal study that would permit repeated measurements of both cholesterol levels and mammographic density over time. All five studies that have examined this relation have been cross-sectional designs and only three were specifically designed to examine the relation between cholesterol, other factors, and mammographic density [Boyd et al., 1989;Boyd et al., 1995b;Maskarinec et al., 2001]. However, the agreement and relative consistency of the results across these studies still adds insight into this relatively novel association. For example, with the addition of our study, it is becoming apparent that exogenous hormone use may modify the association between mammographic density and serum lipids. This emerging pattern may warrant conducting a larger study to examine this relation in a sample that includes both pre- and post-menopausal women, with post-menopausal women being stratified by HRT use. Given the small number of studies that have examined this relation it would be premature to recommend that a prospective cohort be designed to examine this association specifically, however, on-going cohort studies that could combine serial measurements of cholesterol and mammographic density could be considered as a next option. Another, arguably more reasonable next step, would be to conduct a larger cross-sectional study with a more heterogeneous sample that would permit a more statistically powerful examination of this relation. Therefore, even though this study was a cross-sectional design, it still added information about this relation particularly since the results were consistent with some of the previous findings.

5.5.2 Study sample

A potential limitation of using this study sample to examine the association between cholesterol measures and mammographic density was the overall good health of the participants. The sample was originally selected for an aerobic exercise intervention in which women were randomized to a one-year long moderate-vigorous intensity exercise group or to a control group. In order to be eligible, the women had to be without any major co-morbidities, not have any prior breast disease, and healthy enough to participate in a year long exercise intervention. It is possible that this particular study population was healthy and somehow resistant to the negative effects of a sedentary lifestyle. Therefore, it may be reasonable to propose that a relation between cholesterol levels and mammographic density would be overlooked in this population of women. This phenomenon could be classified as a form a healthy volunteer bias. This type of bias would attenuate our results and lead us to conclude incorrectly that cholesterol and mammographic density are unrelated. The impact of this type of bias is difficult to assess. One method of assessing the presence of healthy volunteer bias is to compare the sample characteristics to population characteristics, like those recorded in the Canadian Community Health Survey [Government of Canada, 2004]. In 2004, it was estimated 35.7%, 32.1% and 31.1% of women between the ages of 55-64 years were normal, overweight (BMI: 25-29.9 kg/m^2) and obese ($\geq$30kg/m^2), respectively. Women between the ages of 65-74 years had similar body mass distributions. In our study, the distribution of body mass in participants was slightly higher but paralleled this distribution with respective frequencies of 20.8%, 41.3% and 37.9%. Rates of diabetes in Alberta are estimated to be between 10-15% [Johnson and Vermeulen, 2007], for this age group of

women. Breast cancer prevalence for this age group in 2004 was also estimated to be around 1% of the population [Canadian Cancer Society and National Cancer Institute of Canada, 2008]. All of these disease rates are relatively low, therefore with a sample of 320 women, it is conceivable that these women are representative of healthy postmenopausal women and that the results of this study have internal validity. The issue of a homogeneous sample would affect the external validity of our results however, and will be discussed in the Generalizability section.

The homogeneity of this sample contributed to the relatively small range of mammographic density values that were observed. In Boyd's previous two studies [Boyd et al., 1989;Boyd et al., 1995b] conducted with premenopausal women, the ranges of mammographic density were much wider. In his first study, he compared women with densities of less than 25 percent to women with densities greater than 75 percent [Boyd et al., 1989]. In his second study, the mean percent density in the lowest quintile was 7.3 percent, and in the highest it was 84.3 percent [Boyd et al., 1995b]. In our study, percent density ranged from zero to 56 percent. This smaller variation in the outcome variable could have affected the power to detect a difference. It is also possible that stronger associations between these variables are only seen in the higher ranges of percent density in this population. These two reasons could explain why we did not observe statistically significant associations. The homogeneity of this sample could have also contributed to the lack an association between physical activity and serum cholesterol measures. Since the study sample was sedentary at enrolment into the study, the range of past year physical activity levels was small. A small range of physical activity measures makes it

difficult to detect a linear relation with other variables and could have led to the null correlation between physical activity and serum cholesterol.

5.5.3 Measurement bias

Measurement bias would be a factor in our study if the measurement of lipids depended on the measure of mammographic density, or vice versa. Concerns have been raised around the quality of mammograms in obese women [Guest et al., 2000]. Fatter breasts tend to be compressed less than breasts with less adipose tissue which leads to poor image quality. The resulting decrease in geometric sharpness and decrease in contrast between tissues [Guest et al., 2000] could blur out dense tissue which usually shows up as a white mass. This may result in an underestimation of dense tissue in the breast. In the literature, this effect has been shown to have detrimental implications for the detection of breast tumours [Guest et al., 2000]. Since the majority of our sample was overweight to obese (79.2 percent), this type of measurement error could have been a factor in our results. More importantly, since HDL-C and TG were associated with adiposity this could imply that mammographic density measures were systematically different between the higher and lower levels of HDL-C and TG, respectively. For example, at higher values of TG, women were more likely to be overweight or obese, and therefore, their mammographic density was more likely to be inaccurately measured. These mammographic density measures would differ systematically from the ones taken in women of normal weight or lower TG levels. However, normal weight women made up only 20% of our study sample, therefore this bias, if it exists, would have only a minimal effect on the validity of our results. Also, serum lipids were only moderately

associated with measures of body composition and therefore the bias in measurement of mammographic density due to obesity may not carry over to serum lipids. Therefore, it is concluded that measurement bias, due to differential accuracy of mammograms by body composition, is of minimal concern for the results of this study.

All dietary variables could have been underreported through the use of the Diet History Questionnaire. This questionnaire asked participants to record their average consumption of foods over the past year. Previous studies, that have compared food frequency questionnaires and 24-hour recall questionnaires to unbiased biomarkers of dietary intake, have noted substantial underreporting of total energy intake and protein [Hill and Davies, 2001;Subar et al., 2003;Trabulsi and Schoeller, 2001]. Subar and colleagues [2003] performed the largest study of this kind and compared both 24-hour dietary recalls and the US version of our questionnaire, the DHQ, to doubly labelled water and urinary nitrogen levels [Subar et al., 2003]. They reported an average underreporting of total energy intake of 14 percent for women and noted that underreporting was slightly greater for total energy intake than for protein [Subar et al., 2003]. They suggest that this underreporting of total caloric intake will bias the results toward underreporting of fat, carbohydrates and alcohol. This study also reported that higher body mass index was associated with greater underreporting. Based on these results, it is probable that there was underreporting of total caloric intake and protein in our study. This underreporting may have caused an attenuation of our results in the analyses that included dietary cholesterol as a main predictor variable, although there is no evidence to suggest that underreporting of total calories and protein would affect

dietary cholesterol levels. The lack of association that our study observed between dietary cholesterol and mammographic density is consistent with other cross-sectional studies that have examined this relation [Boyd et al., 1989;Vachon et al., 2000]. These studies used a questionnaire to assess dietary intake and therefore the lack of association found in these studies, and ours, could be attributed to either, a true absence of any biological relation between dietary cholesterol and mammographic density, or to the inaccuracy of dietary measurement tools. There is no reason to believe that the measurement of dietary variables would differ systematically by measures of mammographic density, therefore, although this error could still affect our ability to detect significant associations, it would not affect the validity of our results.

Misclassification of variables is another form of measurement bias that could have affected the results of our study. However, both the main predictor variables and the main outcome variables were kept in a continuous format, hence the relative ranking of the measures were maintained. Misclassification of participants could have occurred in the exploratory analysis of effect modification by alcohol consumption. Alcohol consumption was measured using the DHQ, and as previously stated, underreporting of total calories may bias the results toward underreporting of alcohol consumption [Subar et al., 2003]. Also, participants in research trials tend to underreport alcohol consumption because of perceived social norms. This type of underreporting is labelled social desirability bias [Sackett, 1979]. In our study, these biases may have lead to an attenuation of effect modification by alcohol consumption. We observed effect modification in the relation between TG and percent density, but in the regression models

with other serum cholesterol measures and mammographic density, no statistically significant interaction was observed. More accurate reporting of alcohol consumption would have increased the sample size in the highest alcohol consumption group. This therefore could have either 1) attenuated the results further by increasing the range of TG values and decreasing the slope of the line, or 2) strengthened the association between TG and percent density in this group if the range of TG values did not increase. Since this result is the first occurrence of this type of effect modification, our findings need to be confirmed with further research. However, overall, misclassification of variables had little, if any, impact on the validity of our results.

5.5.4 Confounding

Confounding occurs when there is a mixing of effects between an exposure and disease outcome by a third extraneous variable. In the analyses with serum lipids and mammographic density, both waist circumference and age were controlled for in the regression models. Waist circumference is highly associated with mammographic density [Boyd et al., 2006] and with serum lipid levels, especially HDL-C and TG [Sirtori and Vega, 1997]. Age is also associated with mammographic density [Boyd et al., 2005] and associated with changes in serum lipids [Fletcher et al., 2005]. In the univariate analysis, age was significantly associated with increases in cholesterol and TG and decreases in percent density, but was not significantly associated with HDL-C, LDL-C or dense breast area. Age did modify the linear regression coefficients, however, not to a significant amount, based on the definition used in this analysis. Despite the lack of significance, age was included in all of the models to reduce any residual confounding

because of the known biological relations to both the outcome and predictor variables. No other variables were included in the models since no other variable modified the relation between cholesterol and mammographic density to a significant degree and no other variable was known to have strong biological relation with both the outcome and predictor variables. Maintaining a parsimonious model helps maximize the model stability and improve interpretation of the coefficients [Kleinbaum et al., 2007]. As previously mentioned, this study had thorough and accurate collection of covariate data, therefore, there is little probability that residual confounding caused by inaccurate measurement of covariates or missing covariate data could have influenced these results.

The limitations of this study did not affect the internal validity of the study results. Although the study design was cross-sectional and could not provide information about the temporal relations between the main variables under study, this study still added information about this novel relation, by being the fifth study to examine this association. The cross-sectional design may have limited the ability of this study to detect an association between dietary cholesterol and mammographic density, however it is assumed that food frequency questionnaires, like the DHQ, capture habitual patterns of dietary consumption, and therefore may reflect a biologically relevant exposure. A narrow range of mammographic density and the healthy nature of the sample may have limited our ability to observe significant results. Lastly, underreporting of alcohol consumption could indicate that the modification by alcohol in the relation between TG and mammographic density, as well as with other lipids, may be stronger than what was observed, or on the contrary, it may indicate that this modification was due to chance.

Since this study is the first report of this effect, it is important to repeat these analyses in other studies to confirm the results.

5.6 Generalizability of findings

This study used a highly selected volunteer sample of women to examine the relation between cholesterol and mammographic density. Based on the assessment of biases above it is concluded that this study was internally valid, therefore it is appropriate to discuss the implications of these results for other populations. The sample that was used for this analysis had no major co-morbidities, such as cardiovascular disease, diabetes, or previous cancers. The women were also sedentary and consumed less than two drinks of alcohol per day on average. The criteria for the selection of participants into this study were stringent to ensure that the participants would adhere to the one-year long exercise intervention and to ensure accurate measurement of important biological markers. Also, these criteria ensured that the women were healthy enough to complete the exercise intervention. After considering all of these factors, it is likely that this population is representative of sedentary, postmenopausal women that do not have any major co-morbidities.

In assessing the generalizability of this study it is important to consider HRT use. The results of Aeillo's study [Aiello et al., 2005] which found effect modification by HRT use, and the results of Maskarinec (2001) signify that our results may not be generalizable to postmenopausal women using HRT or who recently discontinued HRT.

It is concluded that the results of this study can be generalized to healthy, sedentary postmenopausal women who are not taking HRT.

5.7 Future recommendations

As was previously eluded to, the results of this study in combination with previous results provides justification for a larger cross-sectional examination of the relation between serum lipids and mammographic density in a more heterogeneous population that would have a wider range of exposures, socio-demographic and personal characteristics and medical profiles that might permit a more complete assessment of the role of serum lipids in development of dense breast tissue. An area of specific interest would be to investigate the role of HRT use on the relation between serum lipids and mammographic density amongst both premenopausal and postmenopausal women. A cross-sectional study that included a more diverse population and that would permit stratification by HRT use would add valuable information about the modifying effects of HRT on this relation.

Since both mammographic density and cholesterol have genetic influences, it may also be important to consider genetic components in a future study exploring the association between these variables. Boyd and colleagues (1995) attempted to do this type of study by comparing sporadic cases of breast cancer with familial breast cancer cases and their families [Boyd et al., 1995c]. In this study, he found significant differences in total cholesterol and LDL-C between these two groups. In our study and another study [Boyd et al., 1989], a negative association was also observed between TG

and family history of breast cancer. These observations suggest that genetic components may be important for informing this relation. Lipids have long been known to be influenced by genetic factors [Katan et al., 1986]. Mammographic density is also highly influenced by genetic factors [Boyd et al., 2005]. In two twin studies, it was estimated that genetic factors accounted for 63 percent of the residual variation in mammographic density, with the general environment and the individual's environment accounting for the remainder of the variance [Boyd et al., 2002a]. Exploring similarities in the genetic variation of serum lipids and mammographic density may provide more insight into this relation.

Lastly, despite the lack of association found between serum cholesterol and mammographic density in this study, it is possible that serum cholesterol plays an etiologic role in breast cancer which warrants further consideration. Studies have identified an association between serum cholesterol and breast cancer [Furberg et al., 2004;Manjer et al., 2001;Michalaki et al., 2005;Ray and Husain, 2001], and it is known that lipid profiles are altered throughout the cancer disease process [Potischman et al., 1991;Spiegel et al., 1982;Zielinski et al., 1988]; hence serum lipids are likely involved in the etiologic pathway for breast cancer. It may be more fruitful to explore alternate pathways through which serum cholesterol has an effect on breast cancer risk. One such possible pathway is adiponectin, an emerging biomarker in cancer research [Barb et al., 2007]. Adiponectin is produced by adipocytes, along with other adipokines such as tumor necrosis factor-alpha and leptin. It is thought that disregulation of adipokine production leads to metabolic and vascular disorders and cancer. Low adiponectin is

associated with obesity, cardiovascular disease, diabetes and breast cancer. It is also associated with a poor lipid profile and is lower with increasing levels of visceral fat [Barb et al., 2007]. Therefore, the metabolic pathway linking visceral fat with lipid metabolism and adiponectin production may elucidate biological mechanisms important in the etiology of breast cancer.

5.8 Conclusion

Our study indicates that serum lipids are not a good predictor of either percent density or dense tissue area in healthy, postmenopausal women. Central adiposity, measured by waist circumference, explained the majority of the variance in mammographic density measures indicating that central adiposity is a better predictor of mammographic density than serum cholesterol. The direction of our results, when compared to previous studies, suggests that HRT may modify the relation between mammographic density and serum lipids, and warrants future exploration of this analysis. In addition, although effect modification by alcohol consumption was observed in the relation between TG and percent density, future studies are necessary to confirm this effect.

Dietary cholesterol is a poor predictor of mammographic density measures. These findings are in agreement with previous literature and may be the result of the temporal design of the cross-sectional study or a true absence of association. Dietary cholesterol also did not predict serum cholesterol in this sample of women. Genetic

variants may be important to include in future analyses of dietary cholesterol since there is wide variation in how individuals respond to dietary cholesterol in the body.

Future studies of serum cholesterol and mammographic density should include samples of both pre- and post-menopausal women with the ability to stratify on HRT history. However, in order to further elucidate biological mechanisms associated with mammographic density and breast cancer, it may be more worthwhile to examine other mechanistic pathways. These pathways could include genetic variation or inflammatory markers such as adiponectin.

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A.1. Eligibility criteria for ALPHA participants

1. Female

2. Age 50-74 years at baseline

3. Postmenopausal

4. No previous diagnosis of breast cancer

5. No major co-morbidities (including diabetes, major heart or lung problems, previous cancers, severe arthritis, chronic fatigue syndrome, or liver problems)

6. No previous breast cancer diagnosis

7. Physically able to perform exercise (passes PAR-Q, obtains physician's approval, healthy heart and lung function assessed during fitness test)

8. Body mass index between 22.0-40.0

9. Breast tissue density ≥0%

10. Moderately sedentary lifestyle (no more than 3 days per week of recreational activity for a maximum of 30 minutes per session)

11. Not currently, previously (within last 12 months), or planning on taking hormone replacement therapy

12. Not a current smoker or excessive drinker (>2 drinks/day)

13. Not currently or planning on undertaking a weight loss program or taking weight loss medications

14. Lives in Calgary or Edmonton

15. English-speaking

16. Not planning on being out of the region for more than 4 weeks during the subsequent 18 months

A.2. Informed consent form

Alberta Cancer Board

DIVISION OF POPULATION HEALTH & INFORMATION

1331 - 29 Street N.W., Calgary, Alberta, Canada T2N 4N2 Tel: (403) 944-4862 Fax: (403) 270-8003

Website: www.cancerboard.ab.ca

ALPHA Trial: Alberta Physical Activity and Breast Cancer Prevention Trial

Principal Investigators: Dr. Christine Friedenreich, Alberta Cancer Board
Dr. Kerry Courneya, University of Alberta

Sponsor: Canadian Breast Cancer Research Initiative

Consent Form

This consent form, a copy of which has been given to you, is only part of the process of informed consent. It should give you the basic idea of what the research project is about and what your participation will involve. If you would like more details about something mentioned here, or information not included here, you should ***feel free to ask****. Please take the time to read this carefully and to understand any accompanying information.*

Description of Study

The Division of Epidemiology, Prevention, and Screening of the Alberta Cancer Board is conducting a study to understand how increased physical activity may be related to decreased risk of breast cancer. Participants in this study will be healthy women ages 50-74, who have never had breast cancer, and who do not currently exercise regularly. Half the participants will be assigned to a physical activity group for 12 months, and half will be asked to maintain their usual activities for that time. We will be measuring a number of breast cancer risk factors in both groups before and after the 12-month period. We are now asking you to join our study, and hope that you will be able to help us in our efforts to learn more about cancer causes and prevention of breast cancer.

If you decide to join the study, your participation would include the following:

- Use of your mammograms taken at *Screen Test* to measure the density of your breast tissue.
- Self-administered questionnaires that ask about your current health status including your menstrual, reproductive and hormone use history, your family history of cancer, your physical activity in the last twelve months, your usual diet in the last twelve months, and your ability and willingness to participate in the study. These questionnaires will take approximately 2-2½ hours to complete in total.
- Measurements of your height, weight, hips and waist.
- A small blood sample (60 ml = approximately 4 tablespoons) that will be taken at a laboratory convenient to you.

Participant's Initials: _____

- A fitness test taken on a treadmill. Under the supervision of trained personnel you will be asked to walk briskly on a treadmill while your heart rate is measured. The treadmill test takes approximately 20 minutes. The test will be stopped immediately if you experience pain or health problems.

- A CT (computed tomography) scan taken to assess abdominal fat. The scan is painless, quick, and safe and requires lying down for about 5 minutes while a single image is taken of the abdomen at the level of the belly button.

- A DXA (dual x-ray absorpitometry) scan taken to assess body composition throughout your entire body. The scan is painless and safe, and involves lying still for about 10 minutes on a padded table. DXA is a painless, non-invasive test. The x-ray dose you will be exposed to is extremely low, similar to what you would receive on a long distance airplane flight.

- The above tests and questionnaires will all be given twice; once at the beginning and once at the end of the study, except the blood tests, which will be taken an additional time halfway through the study.

- If you are randomly assigned to the physical activity group, you will be asked to increase your physical activity to 60 minutes of physical activity (including warm-up and cool-down), 5 days a week for 12 months. The physical activity will increase your heart rate, and may decrease your body fat. Examples include brisk walking, swimming, or stationary cycling. The physical activity program will be based at a fitness facility for three days a week, and will be home-based two days a week. Trained exercise physiologists will work closely with you throughout the 12 months to help you gradually improve your ability to exercise, and to help you design an individual program that suits your abilities and interests. You will be able to drop into the fitness facility at flexible times that suit your schedule.

- If you are not randomly assigned to the physical activity group, you will be asked to maintain your usual level of physical activity for a period of 12 months. At the end of the 12 months, to thank you for your participation, you will have the option of taking advantage of one of several types of activities (a one-month fitness facility membership, a series of yoga classes, sessions with a personal trainer, etc.) that will be offered to you.

Risks and Benefits

There may be some discomfort associated with the mammogram, or the needle prick used to obtain blood. There can rarely be complications such as infection, blood clots, or inflammation of the vein. There is radiation (x-ray) exposure involved in the mammogram; however, you will not be given any more radiation exposure than you would normally receive in your mammograms. The CT, DXA and mammography scans also involve x-ray radiation exposure. The dose of radiation is extremely small, and is about the same as the radiation exposure you receive on a long-distance airplane flight. All tests performed in this study are safe, and are performed under the supervision of trained personnel and physicians.

Your fitness level may improve if you participate in an exercise program; however, you may have no direct benefit from participation in this study.

Voluntary Participation

Your participation in this study is strictly **voluntary**. You may refuse participation without prejudice, penalty or loss of benefits to which you are otherwise entitled. If you decide to participate, you have the right to ask the Study Coordinator any questions concerning this study at any time. You have the right to agree to participate or refuse participation in any aspect of this study. You also have the right to withdraw from the study at any time without prejudice, penalty or loss of benefits to which you are otherwise entitled.

Participant's Initials: _____

Confidentiality

If you participate, the information that we obtain from your interview and from your blood samples will be kept strictly confidential and will be used for research purposes only. No record bearing your name will be provided to anyone else except the investigators involved in this study. You will not be identified as an individual in any report coming from this study. All material and data obtained from this study will be stored and may be used for future analysis without obtaining further consent from you. However, subsequent studies arising as a result of information obtained in this study will be submitted for ethical approval.

Laboratory Testing

As mentioned, we would like to test a small sample of your blood in order to look for specific blood hormone levels that may be related to breast cancer.

1. There are several options that we would like you to consider. You can choose all, some, or none of them (please put a check mark on the corresponding line) that would allow us to:

   a) Conduct analysis of your blood samples at the Alberta Cancer Board.
   Yes _____ No _____

   b) Contact you in the future for additional research purposes directly related to the present project.
   Yes _____ No _____

   c) If research with your blood reveals some other medical condition relating to you,
   (i) Do you wish to be informed? Yes _____ No _____
   (ii) Do you wish your family doctor to be informed? Yes _____ No _____

   d) List any specific wishes or restrictions you have regarding the use of your blood (be as specific as possible).
   _____
   _____
   _____
   _____

2. ____ You, _____ understand to your satisfaction the information regarding your participation in the *ALPHA Trial* and ***agree*** to participate as a subject.

   OR

   ____ You, _____ ***do not agree*** to participate as a subject.

3. You, _____ understand to your satisfaction that the research team may need to *re-contact* you in the future to and *agree* to be contacted if the need so arises. If however, you are not able to respond, you appoint_____(Tel:(_____) __ __ __ - __ __ __ __) to answer any questions on your behalf; or No, I_____ do not wish to be contacted or to appoint anyone for future contact.

Compensation

In the event that you suffer an injury as a result of participating in this research no compensation will be provided for you by the Canadian Breast Cancer Research Initiative, the Alberta Cancer Board, the University of Alberta, or by the research team. You still have all your legal rights. Nothing said here about treatment of compensation in any way alters your right to recover damages.

Participant's Initials: _____

Signatures

Your signature on this form indicates that you have understood to your satisfaction the information regarding your participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time without jeopardizing your health care. Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation. If you have further questions concerning matter related to this research, please contact:

Dr. Christine Friedenreich, Division of Epidemiology, Prevention and Screening, Alberta Cancer Board at: (403) 944-1841 (daytime) or (403) 282-3744 (evenings).

If you are calling long distance, you may call collect.

If you have any questions regarding your legal rights as a possible participant in this research please contact Pat Evans, Associate Director, Research Services, University of Calgary at (403) 220-3782.

Name of Participant	Signature of Participant	Date
Name of Witness	Signature of Witness	Date
Dr. Christine Friedenreich Name of Principal Investigator	Signature of Principal Investigator	Date

A copy of this consent form has been given to you for your records and reference.

Participant's Initials: _____

A.3. Participant Blood Questionnaire

Alberta Cancer Board

ALPHA Trial

Study ID

Participant Blood Questionnaire

It is important that we collect some information from you on the day you have your blood drawn. Please answer the following questions before you go the laboratory. Thank-you for your help.

---

1. Please record today's date: Day / Month / Year 2 0

2. Prior to having your blood drawn today, when was the last time you had anything to eat or drink other than plain water? Day / Month / Year 2 0 24 Hour :

3. Prior to having your blood drawn today, when was the last time you exercised? Day / Month / Year 2 0 24 Hour :

4. If you have taken any medications, vitamins or herbal supplements **in the past 24 hours** (prior to having your blood drawn), please fill out the following table (continue on the back of sheet if required).

Write numbers in boxes like . 5 5 or like this: 5 . 5

PLEASE PRINT CLEARLY

Name of Medication, Vitamin or Herbal Supplement	**Dose Taken** mg or units listed on container	**Date of Last Dose** (Day/Month/Year)	**Time of Last Dose** (24 hour)
	○ ug ○ mg ○ other → _____ other	/ /	:
	○ ug ○ mg ○ other → _____ other	/ /	:
	○ ug ○ mg ○ other → _____ other	/ /	:

51874

Name of Medication, Vitamin or Herbal Supplement	**Dose Taken** mg or units listed on container	**Date of Last Dose** (Day/Month/Year)	**Time of Last Dose** (24 hour)
	○ ug ○ mg ○ other ↳ _____ other	/ /	:
	○ ug ○ mg ○ other ↳ _____ other	/ /	:
	○ ug ○ mg ○ other ↳ _____ other	/ /	:
	○ ug ○ mg ○ other ↳ _____ other	/ /	:
	○ ug ○ mg ○ other ↳ _____ other	/ /	:
	○ ug ○ mg ○ other ↳ _____ other	/ /	:
	○ ug ○ mg ○ other ↳ _____ other	/ /	:
	○ ug ○ mg ○ other ↳ _____ other	/ /	:
	○ ug ○ mg ○ other ↳ _____ other	/ /	:

51874

A.4. Diet History Questionnaire

ALPHA Trial

Alberta Cancer Board

A research initiative of the Alberta Cancer Board
Adapted from the National Institutes of Health Diet History Questionnaire

DIET HISTORY QUESTIONNAIRE

GENERAL INSTRUCTIONS

- Answer each question as best you can. If you are not sure, please estimate. A guess is better than leaving a blank.
- **Shade** bubbles like this: ●
- Please use a pencil or ball point pen, not a felt pen.
- If you make a mistake put an X through the incorrect bubble.
- If you fill **NEVER** or **NO** for a question, please follow any arrows or instructions that direct you to the next question.

The questions in the Diet History Questionnaire use measurements like cups, ounces, tablespoons and teaspoons. Refer below to convert these measurements to their metric equivalents.

1 cup	= 240 mL	1 tablespoon	= 15 mL
1 ounce	= 30 mL	1 teaspoon	= 5 mL

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1. Over the past 12 months, how often did you drink **tomato juice** or **vegetable juice**?

O NEVER (GO TO QUESTION 2)

O 1 time per month or less
O 2-3 times per month
O 1-2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2-3 times per day
O 4-5 times per day
O 6 or more times per day

1a. Each time you drank **tomato juice** or **vegetable juice**, how much did you usually drink?

O Less than 3/4 cup (6 ounces)
O 3/4 to 1 1/4 cups (6 to 10 ounces)
O More than 1 1/4 cups (10 ounces)

2. Over the past 12 months, how often did you drink **orange juice** or **grapefruit juice**?

O NEVER (GO TO QUESTION 3)

O 1 time per month or less
O 2-3 times per month
O 1-2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2-3 times per day
O 4-5 times per day
O 6 or more times per day

2a. Each time you drank **orange juice** or **grapefruit juice**, how much did you usually drink?

O Less than 3/4 cup (6 ounces)
O 3/4 to 1 1/4 cups (6 to 10 ounces)
O More than 1 1/4 cups (10 ounces)

2b. How often was the juice fortified with **Calcium**?

O Almost never or never
O About 1/4 of the time
O About 1/2 the time
O About 3/4 of the time
O Almost always or always

3. Over the past 12 months, how often did you drink **other 100% fruit juice** or **100% fruit juice mixtures** (such as apple, grape, pineapple, or others)?

O NEVER (GO TO QUESTION 4)

O 1 time per month or less
O 2-3 times per month
O 1-2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2-3 times per day
O 4-5 times per day
O 6 or more times per day

Question 4 appears in the next column.

Over the past 12 months...

3a. Each time you drank **other fruit juice** or **fruit juice mixtures**, how much did you usually drink?

O Less than 3/4 cup (6 ounces)
O 3/4 to 1 1/2 cups (6 to 12 ounces)
O More than 1 1/2 cups (12 ounces)

4. How often did you drink other **fruit drinks** (such as cranberry cocktail, fruit punch, lemonade, or Kool-Aid, diet or regular)?

O NEVER (GO TO QUESTION 5)

O 1 time per month or less
O 2-3 times per month
O 1-2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2-3 times per day
O 4-5 times per day
O 6 or more times per day

4a. Each time you drank **fruit drinks**, how much did you usually drink?

O Less than 1 cup (8 ounces)
O 1 to 2 cups (8 to 16 ounces)
O More than 2 cups (16 ounces)

4b. How often were your fruit drinks **diet** or **sugar-free drinks**?

O Almost never or never
O About 1/4 of the time
O About 1/2 the time
O About 3/4 of the time
O Almost always or always

5. How often did you drink **milk as a beverage** NOT in coffee, NOT in cereal? (Please include chocolate milk and hot chocolate.)

O NEVER (GO TO QUESTION 6)

O 1 time per month or less
O 2-3 times per month
O 1-2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2-3 times per day
O 4-5 times per day
O 6 or more times per day

5a. Each time you drank **milk as a beverage**, how much did you usually drink?

O Less than 1 cup (8 ounces)
O 1 to 1 1/2 cups (8 to 12 ounces)
O More than 1 1/2 cups (12 ounces)

Question 6 appears on the next page.

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Over the past 12 months...

5b. What kind of **milk** did you usually drink?

- O Whole milk
- O 2% fat milk
- O 1% fat milk
- O Skim, nonfat, or 1/2% fat milk
- O Soy milk
- O Rice milk
- O Other

6. How often did you drink **meal replacement**, **energy**, or **high-protein beverages** such as Instant Breakfast, Ensure, Slimfast, Boost or others?

- O NEVER (GO TO QUESTION 7)
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

6a. Each time you drank **meal replacement beverages**, how much did you usually drink?

- O Less than 1 cup (8 ounces)
- O 1 to 1 1/2 cups (8 to 12 ounces)
- O More than 1 1/2 cups (12 ounces)

7. Over the past 12 months, did you drink **soft drinks** or **pop**?

- O NO (GO TO QUESTION 8)
- O YES

7a. How often did you drink **soft drinks** or **pop IN THE SUMMER**?

- O NEVER
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

7b. How often did you drink **soft drinks** or **pop DURING THE REST OF THE YEAR**?

- O NEVER
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

Question 8 appears in the next column.

7c. Each time you drank **soft drinks** or **pop**, how much did you usually drink?

- O Less than 12 ounces or less than 1 can or bottle
- O 12 to 16 ounces or 1 can or bottle
- O More than 16 ounces or more than 1 can or bottle

7d. How often were these soft drinks or pop **diet** or **sugar-free**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

7e. How often were these soft drinks or pop **caffeine-free**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

8. Over the past 12 months, did you drink **beer**? (Please do not include non-alcoholic beer.)

- O NO (GO TO QUESTION 9)
- O YES

8a. How often did you drink **beer IN THE SUMMER**?

- O NEVER
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

8b. How often did you drink **beer DURING THE REST OF THE YEAR**?

- O NEVER
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

Question 9 appears on the next page.

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Over the past 12 months...

8c. Each time you drank **beer**, how much did you usually drink?

- O Less than 5 ounces or less than 1 glass
- O 5 to 12 ounces or 1 to 2 glasses
- O More than 12 ounces or more than 2 glasses

9. How often did you drink **wine** or **wine coolers**?

- O NEVER (GO TO QUESTION 10)
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

9a. Each time you drank **wine** or **wine coolers**, how much did you usually drink?

- O Less than 5 ounces or less than 1 glass
- O 5 to 12 ounces or 1 to 2 glasses
- O More than 12 ounces or more than 2 glasses

10. How often did you drink **liquor** or **mixed drinks**?

- O NEVER (GO TO QUESTION 11)
- O 1 time per month or less
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

10a. Each time you drank **liquor** or **mixed drinks**, how much did you usually drink?

- O Less than 1 shot of liquor
- O 1 to 3 shots of liquor
- O More than 3 shots of liquor

11. Over the past 12 months, did you eat **oatmeal**, **cream of wheat** or **other cooked cereal**?

- O NO (GO TO QUESTION 12)
- O YES

Question 11a appears at top of the next column.

Question 12 appears in the next column.

11a. How often did you eat **oatmeal, cream of wheat** or **other cooked cereal IN THE WINTER**?

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

11b. How often did you eat **oatmeal, cream of wheat** or **other cooked cereal DURING THE REST OF THE YEAR**?

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

11c. Each time you ate **oatmeal, cream of wheat** or **other cooked cereal** how much did you usually eat?

- O Less than 3/4 cups
- O 3/4 to 1 1/4 cups
- O More than 1 1/4 cups

12. How often did you eat **cold cereal**?

- O NEVER (GO TO QUESTION 13)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

12a. Each time you ate **cold cereal**, how much did you usually eat?

- O Less than 1 cup
- O 1 to 2 1/2 cups
- O More than 2 1/2 cups

12b. How often was the cold cereal you ate **All Bran, Fiber One, 100% Bran,** or **Bran Buds**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 13 appears on the next page.

16336

Over the past 12 months...

12c. How often was the cold cereal you ate **some other bran** or **fiber cereal** (such as Cheerios, Shredded Wheat, Raisin Bran, Bran Flakes, Grape Nuts, Granola or Mini-Wheats)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

12d. How often was the cold cereal you ate any **other type of cold cereal** (such as Corn Flakes, Rice Krispies, Frosted Flakes, Special K, Froot Loops, Cap'n Crunch, or others)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

12e. Was **milk** added to your cold cereal?

- O NO (GO TO QUESTION 13)
- O YES

12f. What kind of **milk** was usually added?

- O Whole milk
- O 2% fat milk
- O 1% fat milk
- O Skim, nonfat, or 1/2 % fat milk
- O Soy milk
- O Rice milk
- O Other

12g. Each time **milk was added to your cold cereal**, how much was usually added?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

13. How often did you eat **applesauce**?

- O NEVER (GO TO QUESTION 14)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 14 appears in the next column.

13a. Each time you ate **applesauce**, how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

14. How often did you eat **apples**?

- O NEVER (GO TO QUESTION 15)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

14a. Each time you ate **apples**, how many did you usually eat?

- O Less than 1 apple
- O 1 apple
- O More than 1 apple

15. How often did you eat **pears** (fresh, canned, or frozen)?

- O NEVER (GO TO QUESTION 16)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

15a. Each time you ate **pears**, how many did you usually eat?

- O Less than 1 pear
- O 1 pear
- O More than 1 pear

16. How often did you eat **bananas**?

- O NEVER (GO TO QUESTION 17)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 17 appears on the next page.

16336

Over the past 12 months...

16a. Each time you ate **bananas**, how many did you usually eat?

- O Less than 1 banana
- O 1 banana
- O More than 1 banana

17. How often did you eat **dried fruit**, such as prunes or raisins (not including dried apricots)?

- O NEVER (GO TO QUESTION 18)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

17a. Each time you ate **dried fruit**, how much did you usually eat (not including dried apricots)?

- O Less than 2 tablespoons
- O 2 to 5 tablespoons
- O More than 5 tablespoons

18. Over the past 12 months, did you eat **peaches, nectarines** or **plums**?

- O NO (GO TO QUESTION 19)
- O YES

18a. How often did you eat **fresh peaches, nectarines,** or **plums** WHEN IN SEASON?

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

18b. How often did you eat **peaches, nectarines,** or **plums** (fresh, canned or frozen) **DURING THE REST OF THE YEAR**?

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 19 appears in the next column.

18c. Each time you ate **peaches, nectarines,** or **plums,** how much did you usually eat?

- O Less than 1 fruit or less than 1/2 cup
- O 1 to 2 fruits or 1/2 to 3/4 cup
- O More than 2 fruits or more than 3/4 cup

19. How often did you eat **grapes**?

- O NEVER (GO TO QUESTION 20)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

19a. Each time you ate **grapes**, how much did you usually eat?

- O Less than 1/2 cup or less than 10 grapes
- O 1/2 to 1 cup or 10 to 30 grapes
- O More than 1 cup or more than 30 grapes

20. Over the past 12 months, did you eat **cantaloupe**?

- O NO (GO TO QUESTION 21)
- O YES

20a. How often did you eat **fresh cantaloupe WHEN IN SEASON**?

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

20b. How often did you eat **fresh or frozen cantaloupe DURING THE REST OF THE YEAR**?

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 21 appears on the next page.

16336

Over the past 12 months...

20c. Each time you ate **cantaloupe,** how much did you usually eat?

- O Less than 1/4 melon or less than 1/2 cup
- O 1/4 melon or 1/2 to 1 cup
- O More than 1/4 melon or more than 1 cup

21. Over the past 12 months, did you eat **melon, other than cantaloupe** (such as watermelon or honeydew)?

- O NO (GO TO QUESTION 22)
- O YES

21a. How often did you eat **fresh melon, other than cantaloupe** (such as watermelon or honeydew) **WHEN IN SEASON**?

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

21b. How often did you eat **fresh** or **frozen melon, other than cantaloupe, DURING THE REST OF THE YEAR**?

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

21c. Each time you ate **melon other than cantaloupe,** how much did you usually eat?

- O Less than 1/2 cup or 1 small wedge
- O 1/2 to 2 cups or 1 medium wedge
- O More than 2 cups or 1 large wedge

Question 22 appears in the next column.

22. Over the past 12 months, did you eat **strawberries**?

- O NO (GO TO QUESTION 23)
- O YES

22a. How often did you eat **fresh strawberries WHEN IN SEASON**?

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

22b. How often did you eat **fresh or frozen strawberries, DURING THE REST OF THE YEAR** ?

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

22c. Each time you ate **strawberries,** how much did you usually eat?

- O Less than 1/4 cup or less than 3 berries
- O 1/4 to 3/4 cup or 3 to 8 berries
- O More than 3/4 cup or more than 8 berries

23. Over the past 12 months, did you eat **oranges, tangerines,** or **tangelos**?

- O NO (GO TO QUESTION 24)
- O YES

23a. How often did you eat **oranges, tangerines,** or **tangelos WHEN IN SEASON**?

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 24 appears on the next page.

16336

Over the past 12 months...

23b. How often did you eat **oranges, tangerines,** or **tangelos** (fresh or canned) **DURING THE REST OF THE YEAR** ?

O NEVER

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

23c. Each time you ate **oranges, tangerines,** or **tangelos,** how many did you usually eat?

O Less than 1 fruit
O 1 fruit
O More than 1 fruit

24. Over the past 12 months, did you eat **grapefruit**?

O NO (GO TO QUESTION 25)
O YES

24a. How often did you eat **fresh grapefruit WHEN IN SEASON**?

O NEVER

O 1-6 times per season
O 7-11 times per season
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

24b. How often did you eat **grapefruit** (fresh or canned) **DURING THE REST OF THE YEAR**?

O NEVER

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

24c. Each time you ate **grapefruit,** how much did you usually eat?

O Less than 1/2 grapefruit
O 1/2 grapefruit
O More than 1/2 grapefruit

Question 25 appears in the next column.

25. How often did you eat **other kinds of fruit**?

O NEVER (GO TO QUESTION 26)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

25a. Each time you ate **other kinds of fruit,** how much did you usually eat?

O Less than 1/4 cup
O 1/4 to 3/4 cup
O More than 3/4 cup

26. How often did you eat **COOKED greens** (such as spinach, chard, or kale)?

O NEVER (GO TO QUESTION 27)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

26a. Each time you ate **COOKED greens,** how much did you usually eat?

O Less than 1/2 cup
O 1/2 to 1 cup
O More than 1 cup

27. How often did you eat **RAW greens** (such as spinach, chard, or kale)? *(We will ask about lettuce later.)*

O NEVER (GO TO QUESTION 28)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

27a. Each time you ate **RAW greens,** how much did you usually eat?

O Less than 1/2 cup
O 1/2 to 1 cup
O More than 1 cup

Question 28 appears on the next page.

16336

Over the past 12 months...

28. How often did you eat **coleslaw**?

- O NEVER (GO TO QUESTION 29)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

28a. Each time you ate **coleslaw,** how much did you usually eat?

- O Less than 1/4 cup
- O 1/4 to 3/4 cup
- O More than 3/4 cup

29. How often did you eat **cabbage** or **sauerkraut** (other than coleslaw)?

- O NEVER (GO TO QUESTION 30)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

29a. Each time you ate **cabbage** or **sauerkraut,** how much did you usually eat?

- O Less than 1/4 cup
- O 1/4 to 1 cup
- O More than 1 cup

30. How often did you eat **carrots** (fresh, canned, or frozen)?

- O NEVER (GO TO QUESTION 31)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

30a. Each time you ate **carrots,** how much did you usually eat?

- O Less than 1/4 cup or less than 2 baby carrots
- O 1/4 to 1/2 cup or 2 to 5 baby carrots
- O More than 1/2 cup or more than 5 baby carrots

Question 31 appears in the next column.

31. How often did you eat **string beans** or **green beans** (fresh, canned, or frozen)?

- O NEVER (GO TO QUESTION 32)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

31a. Each time you ate **string beans** or **green beans,** how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

32. How often did you eat **peas** (fresh, canned or frozen)?

- O NEVER (GO TO QUESTION 33)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

32a. Each time you ate **peas,** how much did you usually eat?

- O Less than 1/4 cup
- O 1/4 to 3/4 cup
- O More than 3/4 cup

33. Over the past 12 months, did you eat **corn**?

- O NO (GO TO QUESTION 34)
- O YES

33a. How often did you eat **fresh corn WHEN IN SEASON**?

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 34 appears on the next page.

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Over the past 12 months...

33b. How often did you eat **corn** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR?**

O NEVER

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

33c. Each time you ate **corn,** how much did you usually eat?

O Less than 1 ear or less than 1/2 cup
O 1 ear or 1/2 to 1 cup
O More than 1 ear or more than 1 cup

34. Over the past 12 months how often did you eat **broccoli** (fresh or frozen)?

O NEVER (GO TO QUESTION 35)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

34a. Each time you ate **broccoli,** how much did you usually eat?

O Less than 1/4 cup
O 1/4 to 1 cup
O More than 1 cup

35. How often did you eat **cauliflower** or **Brussels sprouts** (fresh or frozen)?

O NEVER (GO TO QUESTION 36)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

35a. Each time you ate **cauliflower** or **Brussels sprouts,** how much did you usually eat?

O Less than 1/4cup
O 1/4 to 1/2 cup
O More than 1/2 cup

Question 36 appears in the next column.

36. How often did you eat **mixed vegetables**?

O NEVER (GO TO QUESTION 37)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

36a. Each time you ate **mixed vegetables,** how much did you usually eat?

O Less than 1/2 cup
O 1/2 to 1 cup
O More than 1 cup

37. How often did you eat **onions**?

O NEVER (GO TO QUESTION 38)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

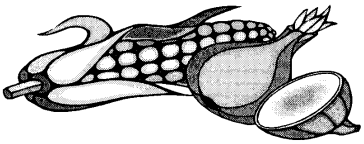
37a. Each time you ate **onions,** how much did you usually eat?

O Less than 1 slice or less than 1 tablespoon
O 1 slice or 1 to 4 tablespoons
O More than 1 slice or more than 4 tablespoons

38. Now think about all the **cooked vegetables** you ate in the past 12 months and how they were prepared. How often were your vegetables **COOKED WITH** some sort of **fat**, including oil spray? *(Please do not include potatoes.)*

O NEVER (GO TO QUESTION 39)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day



Question 39 appears on the next page.

16336

Over the past 12 months...

38a. Which fats were usually added to your vegetables **DURING COOKING?** *(Please do not include potatoes.* ***Mark as many as apply.)***

- O Margarine (including low-fat)
- O Butter (including low-fat)
- O Lard, or bacon fat
- O Olive oil
- O Corn oil
- O Canola or rapeseed oil
- O Oil spray, such as Pam or others
- O Other kinds of oils
- O None of the above

39. Now, thinking again about all the **cooked vegetables** you ate in the past 12 months, how often was some sort of fat, sauce, or dressing added **AFTER COOKING OR AT THE TABLE**? *(Please do not include potatoes.)*

- O NEVER (GO TO QUESTION 40)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1-2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 times per day
- O 3 or more times per day

39a. Which fats, sauces, or dressings were usually added **AFTER COOKING OR AT THE TABLE**? *(Please do not include potatoes.* ***Mark as many as apply.)***

- O Margarine (including low-fat)
- O Butter (including low-fat)
- O Lard, or bacon fat
- O Salad dressing
- O Cheese sauce
- O White sauce
- O Other

39b. If margarine, butter, lard, fatback, or bacon fat was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE,** how much did you usually add?

- O Did not usually add these
- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

39c. If salad dressing, cheese sauce, or white sauce was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE,** how much did you usually add?

- O Did not usually add these
- O Less than 1 tablespoon
- O 1 to 3 tablespoons
- O More than 3 tablespoons

Question 40 appears in the next column.

40. Over the past 12 months how often did you eat **sweet peppers** (green, red, or yellow)?

- O NEVER (GO TO QUESTION 41)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

40a. Each time you ate **sweet peppers,** how much did you usually eat?

- O Less than 1/8 pepper
- O 1/8 to 1/4 pepper
- O More than 1/4 pepper

41. Over the past 12 months did you eat **fresh tomatoes** (including those in salads)?

- O NO (GO TO QUESTION 42)
- O YES

41a. How often did you eat **fresh tomatoes** (including those in salads) **WHEN IN SEASON**?

- O NEVER
- O 1-6 times per season
- O 7-11 times per season
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

41b. How often did you eat **fresh tomatoes** (including those in salads) **DURING THE REST OF THE YEAR**?

- O NEVER
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

41c. Each time you ate **fresh tomatoes,** how much did you usually eat?

- O Less than 1/4 tomato
- O 1/4 to 1/2 tomato
- O More than 1/2 tomato

Question 42 appears on the next page.

16336

Over the past 12 months...

42. How often did you eat **lettuce salads** (with or without other vegetables)?

- O NEVER (GO TO QUESTION 43)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

42a. Each time you ate **lettuce salads,** how much did you usually eat?

- O Less than 1/4 cup
- O 1/4 to 1 1/4 cups
- O More than 1 1/4 cups

43. How often did you eat **salad dressing** (including low-fat) on salads?

- O NEVER (GO TO QUESTION 44)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

43a. Each time you ate **salad dressing** on salads, how much did you usually eat?

- O Less than 2 tablespoons
- O 2 to 4 tablespoons
- O More than 4 tablespoons

44. How often did you eat **sweet potatoes** or **yams**?

- O NEVER (GO TO QUESTION 45)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

44a. Each time you ate **sweet potatoes** or **yams,** how much did you usually eat?

- O 1 small potato or less than 1/4 cup
- O 1 medium potato or 1/4 to 3/4 cup
- O 1 large potato or more than 3/4 cup

Question 45 appears in the next column.

45. How often did you eat **French fries, home fries, hash browned potatoes,** or **tater tots**?

- O NEVER (GO TO QUESTION 46)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

45a. Each time you ate **French fries, home fries, hash browned potatoes,** or **tater tots** how much did you usually eat?

- O Less than 10 fries or less than 1/2 cup
- O 10 to 25 fries or 1/2 to 1 cup
- O More than 25 fries or more than 1 cup

46. How often did you eat **potato salad**?

- O NEVER (GO TO QUESTION 47)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

46a. Each time you ate **potato salad,** how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

47. How often did you eat **baked, boiled,** or **mashed potatoes**?

- O NEVER (GO TO QUESTION 48)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

47a. Each time you ate **baked, boiled,** or **mashed potatoes,** how much did you usually eat?

- O 1 small potato or less than 1/2 cup
- O 1 medium potato or 1/2 to 1 cup
- O 1 large potato or more than 1 cup

Question 48 appears on the next page.

16336

Over the past 12 months...

47b. How often was **sour cream** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- O Almost never or never (GO TO QUESTION 47d)
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

47c. Each time **sour cream** was added to your potatoes, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 3 tablespoons
- O More than 3 tablespoons

47d. How often was **margarine** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

47e. How often was **butter** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

47f. Each time **margarine** or **butter** was added to your potatoes, how much was usually added?

- O Never added
- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

47g. How often was **cheese** or **cheese sauce** added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- O Almost never or never (GO TO QUESTION 48)
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 48 appears in the next column.

47h. Each time **cheese or cheese sauce** was added to your potatoes, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 3 tablespoons
- O More than 3 tablespoons

48. How often did you eat **salsa**?

- O NEVER (GO TO QUESTION 49)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

48a. Each time you ate **salsa,** how much did you usually eat?

- O Less than 1 tablespoon
- O 1 to 5 tablespoons
- O More than 5 tablespoons

49. How often did you eat **ketchup**?

- O NEVER (GO TO QUESTION 50)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

49a. Each time you ate **ketchup,** how much did you usually eat?

- O Less than 1 teaspoon
- O 1 to 6 teaspoons
- O More than 6 teaspoons

50. How often did you eat **stuffing, dressing,** or **dumplings**?

- O NEVER (GO TO QUESTION 51)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

50a. Each time you ate **stuffing, dressing,** or **dumplings,** how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

Question 51 appears on the next page.

16336

Over the past 12 months...

51. How often did you eat **chili**?

- O NEVER (GO TO QUESTION 52)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

51a. Each time you ate **chili,** how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 3/4 cups
- O More than 1 3/4 cups

52. How often did you eat **Mexican foods** (such as tacos, tostados, burritos, tamales, fajitas, enchiladas, quesadillas, and chimichangas)?

- O NEVER (GO TO QUESTION 53)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

52a. Each time you ate **Mexican foods,** how much did you usually eat?

- O Less than 1 taco, burrito, etc.
- O 1 to 2 tacos, burritos, etc.
- O More than 2 tacos, burritos, etc.

53. How often did you eat **cooked dried beans** (such as baked beans, pintos, kidney, blackeyed peas, lima, lentils, soybeans, or refried beans)? *(Please don't include bean soups or chili.)*

- O NEVER (GO TO QUESTION 54)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

53a. Each time you ate **beans,** how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

Question 54 appears in the next column.

53b. How often were the beans you ate **refried beans, beans prepared with any type of fat,** or **with meat added**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

54. How often did you eat **other kinds of vegetables**?

- O NEVER (GO TO QUESTION 55)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

54a. Each time you ate **other kinds of vegetables,** how much did you usually eat?

- O Less than 1/4cup
- O 1/4 to 1/2 cup
- O More than 1/2 cup

55. How often did you eat **rice** or **other cooked grains** (such as bulgur, cracked wheat, or millet)?

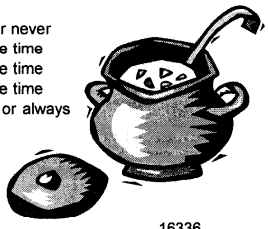
- O NEVER (GO TO QUESTION 56)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

55a. Each time you ate **rice** or **other cooked grains,** how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 1/2 cups
- O More than 1 1/2 cups

55b. How often was **butter, margarine,** or **oil** added to your rice **IN COOKING OR AT THE TABLE**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always



Question 56 appears on the next page.

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Over the past 12 months...

56. How often did you eat **pancakes, waffles,** or **French toast**?

- O NEVER (GO TO QUESTION 57)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

56a. Each time you ate **pancakes, waffles,** or **French toast,** how much did you usually eat?

- O Less than 1 medium piece
- O 1 to 3 medium pieces
- O More than 3 medium pieces

56b. How often was **margarine** (including low-fat) added to your pancakes, waffles, or French toast, **AFTER COOKING OR AT THE TABLE**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

56c. How often was **butter** (including low-fat) added to your pancakes, waffles, or French toast, **AFTER COOKING OR AT THE TABLE**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

56d. Each time **margarine** or **butter** was added to your pancakes, waffles or French toast, how much was usually added?

- O Never added
- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

56e. How often was **syrup** added to your pancakes, waffles, or French toast?

- O Almost never or never *(GO TO QUESTION 57)*
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 57 appears in the next column.

56f. Each time **syrup** was added to your pancakes, waffles, or French toast, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 4 tablepoons
- O More than 4 tablespoons

57. How often did you eat **lasagna, stuffed shells, stuffed manicotti, ravioli,** or **tortellini**? (Please do not include spaghetti or other pasta.)

- O NEVER (GO TO QUESTION 58)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

57a. Each time you ate **lasagna, stuffed shells, stuffed manicotti, ravioli,** or **tortellini,** how much did you usually eat?

- O Less than 1 cup
- O 1 to 2 cups
- O More than 2 cups

58. How often did you eat **macaroni and cheese**?

- O NEVER (GO TO QUESTION 59)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

58a. Each time you ate **macaroni and cheese**, how much did you usually eat?

- O Less than 1 cup
- O 1 to 1 1/2 cups
- O More than 1 1/2 cups

59. How often did you eat **pasta salad** or **macaroni salad?**

- O NEVER (GO TO QUESTION 60)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 60 appears on the next page.

16336

Over the past 12 months...

59a. Each time you ate **pasta salad** or **macaroni salad,** how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

60. Other than the pastas listed in Questions 57, 58, and 59, how often did you eat **pasta, spaghetti,** or **other noodles?**

- O NEVER (GO TO QUESTION 61)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

60a. Each time you ate **pasta, spaghetti,** or **other noodles,** how much did you usually eat?

- O Less than 1 cup
- O 1 to 3 cups
- O More than 3 cups

60b. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce** or **spaghetti sauce made WITH meat**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

60c. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce** or **spaghetti sauce made WITHOUT meat**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

60d. How often did you eat your pasta, spaghetti, or other noodles with **margarine, butter, oil,** or **cream sauce**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 61 appears in the next column.

61. How often did you eat **bagels** or **English muffins**?

- O NEVER (GO TO INTRODUCTION TO QUESTION 62)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

61a. Each time you ate **bagels** or **English muffins,** how much did you usually eat?

- O Less than 1 bagel or English muffin
- O 1 bagel or English muffin
- O More than 1 bagel or English muffin

61b. How often was **margarine** (including low-fat) added to your bagels or English muffins?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

61c. How often was **butter** (including low-fat) added to your bagels or English muffins?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

61d. Each time **margarine** or **butter** was added to your bagels or English muffins, how much was usually added?

- O Never added
- O Less than 1 teaspoon
- O 1 to 2 teaspoons
- O More than 2 teaspoons

61e. How often was **cream cheese** (including low-fat) added to your bagels or English muffins?

- O Almost never or never (GO TO INTRODUCTION TO QUESTION 62)
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 62 appears on the next page.

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Over the past 12 months...

61f. Each time **cream cheese** was added to your bagels or English muffins, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 2 tablespoons
- O More than 2 tablespoons

The next questions ask about your intake of bread other than bagels or English muffins. First, we will ask about bread you ate as part of sandwiches only. Then we will ask about all other bread you ate.

62. How often did you eat **bread** or **buns AS PART OF SANDWICHES** (including burger and hot dog buns)?

- O NEVER (GO TO QUESTION 63)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

62a. Each time you ate **bread** or **buns AS PART OF SANDWICHES,** how much did you usually eat?

- O 1 slice or 1/2 bun
- O 2 slices or 1 bun
- O More than 2 slices or more than 1 bun

62b. How often were the bread or buns that you used for your sandwiches **white** (including burger and hot dog buns)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

62c. How often was **mayonnaise** or **mayonnaise-type dressing** (including low-fat) added to your sandwich bread or buns?

- O Almost never or never (GO TO QUESTION 62e)
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 62e appears in the next column.

Question 63 appears in the next column.

62d. Each time **mayonnaise** or **mayonnaise-type dressing** was added to your sandwich breads or buns, how much was usually added?

- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

62e. How often was **margarine** (including low-fat) added to your sandwich bread or buns?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

62f. How often was **butter** (including low-fat) added to your sandwich breads or buns?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

62g. Each time **margarine** or **butter** was added to your sandwich breads or buns, how much was usually added?

- O Never added
- O Less than 1 teaspoon
- O 1 to 2 teaspoons
- O More than 2 teaspoons

63. How often did you eat **bread** or **dinner rolls NOT AS PART OF SANDWICHES** ?

- O NEVER (GO TO QUESTION 64)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

63a. Each time you ate **bread** or **dinner rolls NOT AS PART OF SANDWICHES,** how much did you usually eat?

- O 1 slice or 1 dinner roll
- O 2 slices or 2 dinner rolls
- O More than 2 slices or 2 dinner rolls

Question 64 appears on the next page.

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Over the past 12 months...

63b. How often were the bread or rolls you ate **white**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

63c. How often was **margarine** (including low-fat) added to your bread or rolls?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

63d. How often was **butter** (including low-fat) added to your bread or rolls?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

63e. Each time **margarine** or **butter** was added to your breads or rolls, how much was usually added?

- O Never added
- O Less than 1 teaspoon
- O 1 to 2 teaspoons
- O More than 2 teaspoons

63f. How often was **cream cheese** (including low-fat) added to your bread or rolls?

- O Almost never or never *(GO TO QUESTION 64)*
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

63g. Each time **cream cheese** was added to your bread or rolls, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 2 tablespoons
- O More than 2 tablespoons

Question 64 appears in the next column.

64. How often did you eat **jam, jelly,** or **honey** on bagels, muffins, bread, rolls, or crackers?

- O NEVER (GO TO QUESTION 65)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

64a. Each time you ate **jam, jelly** or **honey,** how much did you usually eat?

- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

65. How often did you eat **peanut butter** or **other nut butter**?

- O NEVER (GO TO QUESTION 66)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

65a. Each time you ate **peanut butter** or **other nut butter,** how much did you usually eat?

- O Less than 1 tablespoon
- O 1 to 2 tablespoons
- O More than 2 tablespoons

66. How often did you eat **roast beef** or **steak IN SANDWICHES**?

- O NEVER (GO TO QUESTION 67)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

66a. Each time you ate **roast beef** or **steak IN SANDWICHES,** how much did you usually eat?

- O Less than 1 slice or less than 2 ounces
- O 1 to 2 slices or 2 to 4 ounces
- O More than 2 slices or more than 4 ounces

Question 67 appears on the next page.

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Over the past 12 months...

67. How often did you eat **turkey** or **chicken COLD CUTS** (such as loaf, luncheon meat, turkey ham, turkey salami, or turkey pastrami)? *(We will ask about other turkey or chicken later.)*

O NEVER (GO TO QUESTION 68)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

67a. Each time you ate **turkey,** or **chicken COLD CUTS,** how much did you usually eat?

O Less than 1 slice
O 1 to 3 slices
O More than 3 slices

68. How often did you eat **luncheon** or **deli-style ham**? *(We will ask about other ham later.)*

O NEVER (GO TO QUESTION 69)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

68a. Each time you ate **luncheon** or **deli-style ham,** how much did you usually eat?

O Less than 1 slice
O 1 to 3 slices
O More than 3 slices

68b. How often was the luncheon or deli-style ham you ate **light, low-fat,** or **fat-free**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

Question 69 appears in the next column.

69. How often did you eat **other cold cuts** or **luncheon meats** (such as bologna, salami, corned beef, pastrami, or others, including low-fat)? *(Please do not include ham, turkey, or chicken cold cuts.)*

O NEVER (GO TO QUESTION 70)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

69a. Each time you ate **other cold cuts** or **luncheon meats,** how much did you usually eat?

O Less than 1 slice
O 1 to 3 slices
O More than 3 slices

69b. How often were the other cold cuts or luncheon meats you ate **light, low-fat,** or **fat-free**? *(Please do not include ham, turkey, or chicken cold cuts.)*

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

70. How often did you eat **canned tuna** *(including in salads, sandwiches, or casseroles)?*

O NEVER (GO TO QUESTION 71)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

70a. Each time you ate **canned tuna,** how much did you usually eat?

O Less than 1/4 cup or less than 2 ounces
O 1/4 to 1/2 cup or 2 to 3 ounces
O More than 1/2 cup or more than 3 ounces

70b. How often was the canned tuna you ate **water-packed tuna**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

Question 71 appears on the next page.

Over the past 12 months...

70c. How often was the canned tuna you ate **prepared with mayonnaise** or **other dressing** (including low-fat)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

71. How often did you eat **GROUND chicken or turkey**? *(We will ask about other chicken and turkey later.)*

- O NEVER (GO TO QUESTION 72)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

71a. Each time you ate **GROUND chicken** or **turkey,** how much did you usually eat?

- O Less than 2 ounces or less than 1/2 cup
- O 2 to 4 ounces or 1/2 to 1 cup
- O More than 4 ounces or more than 1 cup

72. How often did you eat **beef hamburgers** or **cheeseburgers**?

- O NEVER (GO TO QUESTION 73)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

72a. Each time you ate **beef hamburgers** or **cheeseburgers,** how much did you usually eat?

- O Less than 1 patty or less than 2 ounces
- O 1 patty or 2 to 4 ounces
- O More than 1 patty or more than 4 ounces

72b. How often were the beef hamburgers or cheeseburgers you ate made with **lean ground beef**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 73 appears in the next column.

73. How often did you eat **ground beef in mixtures** (such as meatballs, casseroles, chili, or meatloaf)?

- O NEVER (GO TO QUESTION 74)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

73a. Each time you ate **ground beef in mixtures,** how much did you usually eat?

- O Less than 3 ounces or less than 1/2 cup
- O 3 to 8 ounces or 1/2 to 1 cup
- O More than 8 ounces or more than 1 cup

74. How often did you eat **hot dogs or frankfurters**? *(Please do not include sausages or vegetarian hot dogs.)*

- O NEVER (GO TO QUESTION 75)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

74a. Each time you ate **hot dogs** or **frankfurters,** how many did you usually eat?

- O Less than 1 hot dog
- O 1 to 2 hot dogs
- O More than 2 hot dogs

74b. How often were the hot dogs or frankfurters you ate **light** or **low-fat hot dogs**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 75 appears on the next page.

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Over the past 12 months...

75. How often did you eat beef mixtures such as **beef stew, beef pot pie, beef and noodles,** or **beef and vegetables**?

O NEVER (GO TO QUESTION 76)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

75a. Each time you ate **beef stew, beef pot pie, beef and noodles,** or **beef and vegetables,** how much did you usually eat?

O Less than 1 cup
O 1 to 2 cups
O More than 2 cups

76. How often did you eat **roast beef** or **pot roast**? *(Please do not include roast beef or pot roast in sandwiches.)*

O NEVER (GO TO QUESTION 77)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

76a. Each time you ate **roast beef** or **pot roast,** (including in mixtures) how much did you usually eat?

O Less than 2 ounces
O 2 to 5 ounces
O More than 5 ounces

77. How often did you eat **steak** (beef)? *(Do not include steak in sandwiches.)*

O NEVER (GO TO QUESTION 78)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

77a. Each time you ate **steak** (beef), how much did you usually eat?

O Less than 3 ounces
O 3 to 7 ounces
O More than 7 ounces

Question 78 appears in the next column.

77b. How often was the steak you ate **lean steak**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

78. How often did you eat **pork** or **beef spareribs**?

O NEVER (GO TO QUESTION 79)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

78a. Each time you ate **pork** or **beef spareribs**, how much did you usually eat?

O Less than 4 ribs
O 4 to 12 ribs
O More than 12 ribs

79. How often did you eat **roast turkey, turkey cutlets,** or **turkey nuggets** (including in sandwiches)?

O NEVER (GO TO QUESTION 80)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

79a. Each time you ate **roast turkey, turkey cutlets,** or **turkey nuggets**, how much did you usually eat? *(Please note: 4-8 turkey nuggets=3 ounces.)*

O Less than 2 ounces
O 2 to 4 ounces
O More than 4 ounces

80. How often did you eat **chicken** as part of **salads, sandwiches, casseroles, stews,** or **other mixtures**?

O NEVER (GO TO QUESTION 81)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

Question 81 appears on the next page.

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Over the past 12 months...

80a. Each time you ate **chicken** as part of **salads, sandwiches, casseroles, stews,** or **other mixtures**, how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 1/2 cups
- O More than 1 1/2 cups

81. How often did you eat **baked, broiled, roasted, stewed,** or **fried chicken** (including nuggets)? *(Please do not include chicken in mixtures.)*

- O NEVER (GO TO QUESTION 82)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

81a. Each time you ate **baked, broiled, roasted, stewed,** or **fried chicken** (including nuggets), how much did you usually eat?

- O Less than 2 drumsticks or wings, 1 breast or thigh, or less than 4 nuggets
- O 2 drumsticks or wings, 1 breast or thigh, or 4 to 8 nuggets
- O More than 2 drumsticks or wings, 1 breast or thigh, or more than 8 nuggets

81b. How often was the chicken you ate **fried chicken** (including deep fried) or **chicken nuggets**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

81c. How often was the chicken you ate **WHITE meat**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

81d. How often did you eat chicken **WITH skin**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 82 appears in the next column.

82. How often did you eat **baked ham** or **ham steak**?

- O NEVER (GO TO QUESTION 83)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

82a. Each time you ate **baked ham** or **ham steak**, how much did you usually eat?

- O Less than 1 ounce
- O 1 to 3 ounces
- O More than 3 ounces

83. How often did you eat **pork** (including chops, roasts, and in mixed dishes)? *(Please do not include ham, ham steak, or sausage.)*

- O NEVER (GO TO QUESTION 84)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

83a. Each time you ate **pork**, how much did you usually eat?

- O Less than 2 ounces or less than 1 chop
- O 2 or 5 ounces or 1 chop
- O More than 5 ounces or more than 1 chop

84. How often did you eat **gravy** on meat, chicken, potatoes, rice, etc?

- O NEVER (GO TO QUESTION 85)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

84a. Each time you ate **gravy** on meat, chicken, potatoes, or rice, etc., how much did you usually eat?

- O Less than 1/8 cup
- O 1/8 to 1/2 cup
- O More than 1/2 cup

Question 85 appears on the next page.

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Over the past 12 months...

85. How often did you eat **liver** (all kinds) or **liverwurst**?

- O NEVER (GO TO QUESTION 86)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

85a. Each time you ate **liver** or **liverwurst**, how much did you usually eat?

- O Less than 1 ounce
- O 1 to 4 ounces
- O More than 4 ounces

86. How often did you eat **bacon** (including low-fat)?

- O NEVER (GO TO QUESTION 87)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

86a. Each time you ate **bacon**, how much did you usually eat?

- O Fewer than 2 slices
- O 2 to 3 slices
- O More than 3 slices

86b. How often was the bacon you ate **light, low-fat,** or **lean bacon**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

87. How often did you eat **sausage** (including low-fat)?

- O NEVER (GO TO QUESTION 88)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 88 appears in the next column.

87a. Each time you ate **sausage**, how much did you usually eat?

- O Fewer than 1 patty or 2 links
- O 1 to 3 patties or 2 to 5 links
- O More than 3 patties or 5 links

87b. How often was the sausage you ate **light, low-fat,** or **lean sausage**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

88. How often did you eat **fish sticks** or **fried fish** (including fried seafood or shellfish)?

- O NEVER (GO TO QUESTION 89)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

88a. Each time you ate **fish sticks** or **fried fish**, how much did you usually eat?

- O Less than 2 ounces or less than 1 fillet
- O 2 to 7 ounces or 1 fillet
- O More than 7 ounces or more than 1 fillet

89. How often did you eat **fish** or **seafood that was NOT FRIED** (including shellfish)?

- O NEVER (GO TO THE INTRODUCTION TO QUESTION 90)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

89a. Each time you ate **fish** or **seafood that was not fried**, how much did you usually eat?

- O Less than 2 ounces or less than 1 fillet
- O 2 to 5 ounces or 1 fillet
- O More than 5 ounces or more than 1 fillet

Question 90 appears on the next page

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Over the past 12 months...

Now think about all the meat, poultry, and fish you ate in the past 12 months and how they were prepared.

90. How often was **oil, butter, margarine,** or **other fat used to FRY, SAUTE, BASTE, OR MARINATE** any meat, poultry, or fish you ate? *(Please do not include deep frying.)*

O NEVER (GO TO QUESTION 91)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

90a. Which of the following **fats** were regularly used to prepare your meat, poultry, or fish? ***(Mark all that apply.)***

O Margarine (including low-fat)
O Butter (including low-fat)
O Lard, fatback, or bacon fat
O Olive oil
O Corn oil
O Canola or rapeseed oil
O Oil spray, such as Pam or others
O Other kinds of oils
O None of the above

91. How often did you eat **tofu, soya burgers,** or **soy meat-substitutes**?

O NEVER (GO TO QUESTION 92)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

91a. Each time you ate **tofu, soy burgers, or soy meat-substitutes,** how much did you usually eat?

O Less than 1/4 cup or less than 2 ounces
O 1/4 to 1/2 cup or 2 to 4 ounces
O More than 1/2 cup or more than 4 ounces

Question 92 appears in the next column.

92. Over the past 12 months, did you eat **soups**?

O NO (GO TO QUESTION 93)

O YES

92a. How often did you eat **soup DURING THE WINTER**?

O NEVER

O 1-6 times per winter
O 7-11 times per winter
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

92b. How often did you eat **soup DURING THE REST OF THE YEAR**?

O NEVER

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

92c. Each time you ate **soup,** how much did you usually eat?

O Less than 1 cup
O 1 to 2 cups
O More than 2 cups

92d. How often were the soups you ate **bean soups**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

92e. How often were the soups you ate **cream soups** (including chowders)?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

Question 93 appears on the next page.

Over the past 12 months...

92f. How often were the soups you ate **tomato** or **vegetable soups**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

92g. How often were the soups you ate **broth soups** (including chicken) **with** or **without noodles** or **rice**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

93. How often did you eat **pizza**?

O NEVER (GO TO QUESTION 94)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

93a. Each time you ate **pizza,** how much did you usually eat?

- O Less than 1 slice or less than 1 mini pizza
- O 1 to 3 slices or 1 mini pizza
- O More than 3 slices or more than 1 mini pizza

93b. How often did you eat pizza with **pepperoni, sausage,** or **other meat**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

94. How often did you eat **crackers**?

O NEVER (GO TO QUESTION 95)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

Question 95 appears in the next column.

94a. Each time you ate **crackers,** how many did you usually eat?

- O Fewer than 4 crackers
- O 4 to 10 crackers
- O More than 10 crackers

95. How often did you eat **corn bread** or **corn muffins**?

O NEVER (GO TO QUESTION 96)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

95a. Each time you ate **corn bread** or **corn muffins,** how much did you usually eat?

- O Less than 1 piece or muffin
- O 1 to 2 pieces or muffins
- O More than 2 pieces or muffins

96. How often did you eat **baking powder biscuits**?

O NEVER (GO TO QUESTION 97)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

96a. Each time you ate **baking powder biscuits,** how many did you usually eat?

- O Fewer than 1 biscuit
- O 1 to 2 biscuits
- O More than 2 biscuits

97. How often did you eat **potato chips, tortilla chips,** or **corn chips** (including low-fat, fat-free, or low-salt)?

O NEVER (GO TO QUESTION 98)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

Question 98 appears on the next page

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Over the past 12 months...

97a. Each time you ate **potato chips, tortilla chips,** or **corn chips,** how much did you usually eat?

- O Fewer than 10 chips or less than 1 cup
- O 10 to 25 chips or 1 to 2 cups
- O More than 25 chips or more than 2 cups

97b. How often were the chips you ate **low-fat,** or **fat-free chips**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

98. How often did you eat **popcorn** (including low-fat)?

O NEVER (GO TO QUESTION 99)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

98a. Each time you ate **popcorn,** how much did you usually eat?

- O Less than 2 cups, popped
- O 2 to 5 cups, popped
- O More than 5 cups, popped

99. How often did you eat **pretzels**?

O NEVER (GO TO QUESTION 100)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

99a. Each time you ate **pretzels,** how many did you usually eat?

- O Fewer than 5 average twists
- O 5 to 20 average twists
- O More than 20 average twists

Question 100 appears in the next column.

100. How often did you eat **peanuts, walnuts, seeds,** or **other nuts**?

O NEVER (GO TO QUESTION 101)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

100a. Each time you ate **peanuts, walnuts, seeds,** or **other nuts,** how much did you usually eat?

- O Less than 1/4 cup
- O 1/4 to 1/2 cup
- O More than 1/2 cup

101. How often did you eat **energy, high-protein,** or **breakfast bars** such as **Power Bars, Balance, Clif, Boost** or **others**?

O NEVER (GO TO QUESTION 102)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

101a. Each time you ate **energy, high-protein,** or **breakfast bars,** how many did you usually eat?

- O Less than 1 bar
- O 1 bar
- O More than 1 bar

102. How often did you eat **yogurt** (NOT including frozen yogurt)?

O NEVER (GO TO QUESTION 103)

O 1-6 times per year	O 2 times per week
O 7-11 times per year	O 3-4 times per week
O 1 time per month	O 5-6 times per week
O 2-3 times per month	O 1 time per day
O 1 time per week	O 2 or more times per day

102a. Each time you ate **yogurt,** how much did you usually eat?

- O Less than 1/2 cup or less than 1 container
- O 1/2 to 1 cup or 1 container
- O More than 1 cup or more than 1 container

Question 103 appears on the next page.

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Over the past 12 months...

103. How often did you eat **cottage cheese** (including low-fat)?

O NEVER (GO TO QUESTION 104)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

103a. Each time you ate **cottage cheese,** how much did you usually eat?

O Less than 1/4 cup
O 1/4 to 1 cup
O More than 1 cup

104. How often did you eat **cheese** (including low-fat; including on cheeseburgers or in sandwiches or subs)?

O NEVER (GO TO QUESTION 105)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

104a. Each time you ate **cheese,** how much did you usually eat?

O Less than 1/2 ounce or less than 1 slice
O 1/2 to 1 1/2 ounces or 1 slice
O More than 1 1/2 ounces or more than 1 slice

104b. How often was the cheese you ate **light** or **low-fat cheese**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

104c. How often was the **cheese** you ate **fat-free cheese**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

Question 105 appears in the next column.

105. How often did you eat **frozen yogurt, sorbet,** or **ices** (including low-fat or fat-free)?

O NEVER (GO TO QUESTION 106)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

105a. Each time you ate **frozen yogurt, sorbet,** or **ices,** how much did you usually eat?

O Less than 1/2 cup or less than 1 scoop
O 1/2 to 1 cup or 1 to 2 scoops
O More than 1 cup or more than 2 scoops

106. How often did you eat **ice cream, ice cream bars,** or **sherbet** (including low-fat or fat-free)?

O NEVER (GO TO QUESTION 107)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

106a. Each time you ate **ice cream, ice cream bars, or sherbet,** how much did you usually eat?

O Less than 1/2 cup or less than 1 scoop
O 1/2 to 1 1/2 cups or 1 to 2 scoops
O More than 1 1/2 cups or more than 2 scoops

106b. How often was the ice creamor sherbet you ate **light, low-fat,** or **fat-free**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

107. How often did you eat **cake** (including low-fat or fat-free)?

O NEVER (GO TO QUESTION 108)

O 1-6 times per year
O 7-11 times per year
O 1 time per month
O 2-3 times per month
O 1 time per week
O 2 times per week
O 3-4 times per week
O 5-6 times per week
O 1 time per day
O 2 or more times per day

Question 108 appears on the next page.

Over the past 12 months...

107a. Each time you ate **cake,** how much did you usually eat?

- O Less than 1 medium piece
- O 1 medium piece
- O More than 1 medium piece

107b. How often was the cake you ate **light, low-fat,** or **fat-free cake**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

108. How often did you eat **cookies** or **brownies** (including low-fat or fat-free)?

- O NEVER (GO TO QUESTION 109)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

108a. Each time you ate **cookies** or **brownies,** how many did you usually eat?

- O Less than 2 cookies or 1 small brownie
- O 2 to 4 cookies or 1 medium brownie
- O More than 4 cookies or 1 large brownie

108b. How often were the cookies or brownies you ate **light, low-fat,** or **fat-free**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

109. How often did you eat **doughnuts, sweet rolls, Danish,** or **pop tarts**?

- O NEVER (GO TO QUESTION 110)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

Question 110 appears in the next column.

109a. Each time you ate **doughnuts, sweet rolls, Danish,** or **pop tarts,** how much did you usually eat?

- O Less than 1 piece
- O 1 to 2 pieces
- O More than 2 pieces

110. How often did you eat **sweet muffins** or **dessert bread** (including low-fat or fat-free)?

- O NEVER (GO TO QUESTION 111)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

110a. Each time you ate **sweet muffins** or **dessert bread,** how much did you usually eat?

- O Less than 1 medium piece
- O 1 medium piece
- O More than 1 medium piece

110b. How often were the sweet muffins or dessert bread you ate **light, low-fat,** or **fat-free**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

111. How often did you eat **fruit crisp, cobbler,** or **strudel**?

- O NEVER (GO TO QUESTION 112)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

111a. Each time you ate **fruit crisp, cobbler, or strudel,** how much did you usually eat?

- O Less than 1/2 cup
- O 1/2 to 1 cup
- O More than 1 cup

Question 112 appears on the next page.

Over the past 12 months...

112. How often did you eat **pie**?

- O NEVER (GO TO QUESTION 113)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

112a. Each time you ate **pie,** how much did you usually eat?

- O Less than 1/8 of a pie
- O About 1/8 of a pie
- O More than 1/8 of a pie

The next four questions ask about the kinds of pie you ate. Please read all four questions before answering.

112b. How often were the pies you ate **fruit pie** (such as apple, blueberry, others)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

112c. How often were the pies you ate **cream, pudding, custard,** or **meringue pie**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

112d. How often was the pie you ate **pumpkin pie**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

112e. How often was the pie you ate **pecan pie**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 113 appears in the next column.

113. How often did you eat **chocolate**?

- O NEVER (GO TO QUESTION 114)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

113a. Each time you ate **chocolate,** how much did you usually eat?

- O Less than 1 average bar or less than 1 ounce
- O 1 average bar or 1 to 2 ounces
- O More than 1 average bar or more than 2 ounces

114. How often did you eat **other candy**?

- O NEVER (GO TO QUESTION 115)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

114a. Each time you ate **other candy,** how much did you usually eat?

- O Fewer than 2 pieces
- O 2 to 9 pieces
- O More than 9 pieces

115. How often did you eat **eggs, egg whites,** or **egg substitutes** (NOT including eggs in baked goods and desserts)? *(Please include eggs in salads, quiche, and souffles.)*

- O NEVER (GO TO QUESTION 116)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

115a. Each time you ate **eggs,** how many did you usually eat?

- O 1 egg
- O 2 eggs
- O 3 or more eggs

Question 116 appears on the next page.

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Over the past 12 months...

115b. How often were the eggs you ate **egg substitutes**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

115c. How often were the eggs you ate **egg whites only**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

115d. How often were the eggs you ate **regular whole eggs**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

115e. How often were the eggs you ate **cooked in oil, butter, or margarine**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

115f. How often were the eggs you ate part of **egg salad**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

116. How many cups of **coffee**, caffeinated or decaffeinated, did you drink?

- O NONE (GO TO QUESTION 117)
- O Less than 1 cup per month
- O 1-3 cups per month
- O 1 cup per week
- O 2-4 cups per week
- O 5-6 cups per week
- O 1 cup per day
- O 2-3 cups per day
- O 4-5 cups per day
- O 6 or more cups per day

116a. How often was the coffee you drank **decaffeinated**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 117 appears in the next column.

117. How many glasses of **ICED tea**, caffeinated or decaffeinated, did you drink?

- O NONE (GO TO QUESTION 118)
- O Less than 1 cup per month
- O 1-3 cups per month
- O 1 cup per week
- O 2-4 cups per week
- O 5-6 cups per week
- O 1 cup per day
- O 2-3 cups per day
- O 4-5 cups per day
- O 6 or more cups per day

117a. How often was the iced tea you drank **decaffeinated** or **herbal tea**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

118. How many cups of **HOT tea**, caffeinated or decaffeinated, did you drink?

- O NONE (GO TO QUESTION 119)
- O Less than 1 cup per month
- O 1-3 cups per month
- O 1 cup per week
- O 2-4 cups per week
- O 5-6 cups per week
- O 1 cup per day
- O 2-3 cups per day
- O 4-5 cups per day
- O 6 or more cups per day

118a. How often was the hot tea you drank **decaffeinated** or **herbal tea**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

119. How often did you add **sugar** or **honey** to your coffee or tea?

- O NEVER (GO TO QUESTION 120)
- O Less than 1 time per month
- O 1-3 times per month
- O 1 time per week
- O 2-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

119a. Each time **sugar** or **honey** was added to your coffee or tea, how much was usually added?

- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

Question 120 appears on the next page.

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Over the past 12 months...

120. How often did you add **artificial sweetener** to your coffee or tea?

- O NEVER (GO TO QUESTION 121)
- O Less than 1 time per month
- O 1-3 times per month
- O 1 time per week
- O 2-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

120a. What kind of **artificial sweetener** do you usually use?

- O Equal or aspartame
- O Sweet N Low or saccharin
- O Splenda

121. How often was **non-dairy creamer** added to your coffee or tea?

- O NEVER (GO TO QUESTION 122)
- O Less than 1 time per month
- O 1-3 times per month
- O 1 time per week
- O 2-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

121a. Each time **non-dairy creamer** was added to your coffee or tea, how much was usually added?

- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

121b. What kind of **non-dairy creamer** did you usually use?

- O Regular powdered
- O Low-fat or fat-free powdered
- O Regular liquid
- O Low-fat or fat-free liquid

122. How often was **cream** or **half and half** added to your coffee or tea?

- O NEVER (GO TO QUESTION 123)
- O Less than 1 time per month
- O 1-3 times per month
- O 1 time per week
- O 2-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

Question 123 appears in the next column.

122a. Each time **cream** or **half and half** was added to your coffee or tea, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 2 tablespoons
- O More than 2 tablespoons

123. How often was **milk** added to your coffee or tea?

- O NEVER (GO TO QUESTION 124)
- O Less than 1 time per month
- O 1-3 times per month
- O 1 time per week
- O 2-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2-3 times per day
- O 4-5 times per day
- O 6 or more times per day

123a. Each time **milk** was added to your coffee or tea, how much was usually added?

- O Less than 1 tablespoon
- O 1 to 3 tablespoons
- O More than 3 tablespoons

123b. What kind of **milk** was usually added to your coffee or tea?

- O Whole milk
- O 2% fat milk
- O 1 % fat milk
- O Skim, nonfat, or 1/2% milk
- O Evaporated or condensed (canned) milk
- O Soy milk
- O Rice milk
- O Other

124. How often was **sugar** or **honey** added to foods you ate? (Please do not include sugar in coffee, tea, other beverages, or baked goods).

- O NEVER (GO TO INTRODUCTION TO QUESTION 125)
- O 1-6 times per year
- O 7-11 times per year
- O 1 time per month
- O 2-3 times per month
- O 1 time per week
- O 2 times per week
- O 3-4 times per week
- O 5-6 times per week
- O 1 time per day
- O 2 or more times per day

124a. Each time **sugar** or **honey** was added to foods you ate, how much was usually added?

- O Less than 1 teaspoon
- O 1 to 3 teaspoons
- O More than 3 teaspoons

Question 125 appears on the next page.

16336

The following questions are about the kinds of margarine, mayonnaise, sour cream, cream cheese, and salad dressing that you eat. If possible, please check the labels of these foods to help you answer.

125. Over the past 12 months, did you eat **margarine**?

O NO (GO TO QUESTION 126)
O YES

125a. How often was the margarine you ate **regular-fat margarine** (stick or tub)?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

125b. How often was the margarine you ate **light** or **low-fat margarine** (stick or tub)?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

125c. How often was the margarine you ate **fat-free margarine**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

126. Over the past 12 months, did you eat **butter**?

O NO (GO TO QUESTION 127)
O YES

126a. How often was the butter you ate **light** or **low-fat** butter)?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

Question 127 appears in the next column.

127. Over the past 12 months, did you eat **mayonnaise** or **mayonnaise-type dressing**?

O NO (GO TO QUESTION 128)
O YES

127a. How often was the mayonnaise you ate **regular-fat mayonnaise**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

127b. How often was the mayonnaise you ate **light** or **low-fat mayonnaise**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

127c. How often was the mayonnaise you ate **fat-free mayonnaise**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

128. Over the past 12 months, did you eat **sour cream**?

O NO (GO TO QUESTION 129)
O YES

128a. How often was the sour cream you ate **regular-fat sour cream**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

128b. How often was the sour cream you ate **light, low-fat** or **fat-free sour cream**?

O Almost never or never
O About 1/4 of the time
O About 1/2 of the time
O About 3/4 of the time
O Almost always or always

Question 129 appears on the next page.

16336

Over the past 12 months...

129. Over the past 12 months, did you eat **cream cheese**?

- O NO (GO TO QUESTION 130)
- O YES

129a. How often was the cream cheese you ate **regular-fat cream cheese**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

129b. How often was the cream cheese you ate **light, low-fat** or **fat-free cream cheese**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

130. Over the past 12 months, did you eat **salad dressing**?

- O NO (GO TO INTRODUCTION TO QUESTION 131)
- O YES

130a. How often was the salad dressing you ate **regular-fat** (including oil and vinegar dressing)?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

130b. How often was the salad dressing you ate **light** or **low-fat**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

130c. How often was the salad dressing you ate **fat-free**?

- O Almost never or never
- O About 1/4 of the time
- O About 1/2 of the time
- O About 3/4 of the time
- O Almost always or always

Question 131 appears in the next column.

The following two questions ask you to summarize your usual intake of vegetables and fruits. Please do not include salads, potatoes, or juices.

131. Over the past 12 months, how many servings of **vegetables** (not including salad or potatoes) did you eat per week or per day?

- O Less than 1 per week
- O 1-2 per week
- O 3-4 per week
- O 5-6 per week
- O 1 per day
- O 2 per day
- O 3 per day
- O 4 per day
- O 5 or more per day

132. Over the past 12 months, how many servings of **fruit** (not including juices) did you eat per week or per day?

- O Less than 1 per week
- O 1-2 per week
- O 3-4 per week
- O 5-6 per week
- O 1 per day
- O 2 per day
- O 3 per day
- O 4 per day
- O 5 or more per day

133. Over the past month, which of the following foods did you eat **AT LEAST THREE TIMES?** ***(Mark as many as apply.)***

- O Avocado, guacamole
- O Cheesecake
- O Chocolate, fudge, or butterscotch toppings or syrups
- O Chow mein noodles
- O Croissants
- O Dried apricots
- O Egg rolls
- O Granola bars
- O Hot peppers
- O Jello, gelatin
- O Milkshakes or ice-cream sodas
- O Olives
- O Oysters
- O Pickles or pickled vegetables or fruit
- O Plantains
- O Pork neckbones, hock, head, feet
- O Pudding or custard
- O Veal, venison, lamb
- O Whipped cream, regular
- O Whipped cream, substitute
- O NONE

134. Over the past 12 months, have you followed any type of **vegetarian diet**?

- O NO (GO TO INTRODUCTION TO QUESTION 135)
- O YES

134a. Which of the following food did you **TOTALLY EXCLUDE** from your diet? *(Mark all that apply.)*

- O Meat (beef, pork, lamb, etc.)
- O Poultry (chicken, turkey, duck)
- O Fish and seafood
- O Eggs
- O Dairy products (milk, cheese, etc.)

Question 135 appears in the next column.

16336

The next questions are about your use of fiber supplements or vitamin pills.

135. Over the past 12 months, did you take any of the following types of **fiber** or **fiber supplements** on a regular basis (more than once per week for at least 6 of the last 12 months)? ***(Mark all that apply.)***

- O NO, didn't take any fiber supplements on a regular basis (GO TO QUESTION 136)
- O YES, psyllium products (such as Metamucil, Prodiem, Correctol)
- O YES, Bran (such as wheat bran, oat bran, or bran wafers)

136. Over the past 12 months, did you take any **multivitamins,** such as One-a-Day-, or Centrum-type multivitamins (as pills, liquids, or packets)?

- O NO (GO TO INTRODUCTION TO QUESTION 138)
- O YES

137. How often did you take **One-a-Day-, or Centrum-type** multivitamins?

- O Less than 1 day per month
- O 1-3 days per month
- O 1-3 days per week
- O 4-6 days per week
- O Every day

137a. Does your **multivitamin** usually contain **minerals** (such as iron, zinc, etc.)?

- O NO
- O YES
- O Don't know

137b. For how many years have you taken **multivitamins**?

- O Less than 1 year
- O 1-4 years
- O 5-9 years
- O 10 or more years

Question 138 appears in the next column.

These last questions are about the vitamins, minerals, or herbal supplements you took that are NOT part of a One-a-Day- or Centrum-type of multivitamin. Please include vitamins taken as part of an antioxidant supplement.

138. How often did you take **Beta-carotene (NOT** as part of a multivitamin in Question 137)?

- O NEVER (GO TO QUESTION 139)
- O Less than 1 day per month
- O 1-3 days per month
- O 1-3 days per week
- O 4-6 days per week
- O Every day

138a. When you took **Beta-carotene**, about how much did you take in one day?

- O Less than 10,000 IU
- O 10,000 -14,999 IU
- O 15,000 - 19,999 IU
- O 20,000 - 24,999 IU
- O 25,000 IU or more
- O Don't know

138b. For how many years have you taken **Beta-carotene**?

- O Less than 1 year
- O 1-4 years
- O 5-9 years
- O 10 or more years

139. How often did you take **Vitamin A** (**NOT** as part of a multivitamin in Question 137)?

- O NEVER (GO TO QUESTION 140)
- O Less than 1 day per month
- O 1-3 days per month
- O 1-3 days per week
- O 4-6 days per week
- O Every day

139a. When you took **Vitamin A**, about how much did you take in one day?

- O Less than 8,000 IU
- O 8,000 - 9,999 IU
- O 10,000 - 14,999 IU
- O 15,000 - 24,999 IU
- O 25,000 IU or more
- O Don't know

Question 140 appears on the next page.

16336

Over the past 12 months...

139b. For how many years have you taken **Vitamin A**?

- O Less than 1 year
- O 1-4 years
- O 5-9 years
- O 10 or more years

140. How often did you take **Vitamin C (NOT** as part of a multivitamin in Question 137)?

- O NEVER (GO TO QUESTION 141)
- O Less than 1 day per month
- O 1-3 days per month
- O 1-3 days per week
- O 4-6 days per week
- O Every day

140a. When you took **Vitamin C**, about how much did you take in one day?

- O Less than 500 mg
- O 500-999 mg
- O 1,000-1,499 mg
- O 1,500-1,999 mg
- O 2,000 mg or more
- O Don't know

140b. For how many years have you taken **Vitamin C**?

- O Less than 1 year
- O 1-4 years
- O 5-9 years
- O 10 or more years

141. How often did you take **Vitamin E (NOT** as part of a multivitamin in Question 137)?

- O NEVER (GO TO QUESTION 142)
- O Less than 1 day per month
- O 1-3 days per month
- O 1-3 days per week
- O 4-6 days per week
- O Every day

Question 142 appears in the next column.

141a. When you took **Vitamin E**, about how much did you take in one day?

- O Less than 400 IU
- O 400-799 IU
- O 800-999 IU
- O 1,000 IU or more
- O Don't know

141b. For how many years have you taken **Vitamin E**?

- O Less than 1 year
- O 1 - 4 years
- O 5 - 9 years
- O 10 or more years

142. How often did you take **calcium supplements** or **calcium containing antacids (NOT** as part of a multivitamin in Question 137)?

- O NEVER (GO TO QUESTION 143)
- O Less than 1 day per month
- O 1-3 days per month
- O 1-3 days per week
- O 4-6 days per week
- O Every day

142a. When you took **calcium supplements** or **calcium containing antacids**, about how much elemental calcium did you take in one day? *(If possible, please check label for elemental calcium.)*

- O Less than 500 mg
- O 500-599 mg
- O 600-999mg
- O 1,000 mg or more
- O Don't know

142b. For how many years have you taken **calcium supplements** or **calcium-containing antacids** ?

- O Less than 1 year
- O 1 - 4 years
- O 5 - 9 years
- O 10 or more years

Question 143 appears on the next page.

16336

Over the past 12 months...

143. How often did you take **Vitamin D (NOT** as part of a multivitamin in Question 137)?

- O NEVER (GO TO QUESTION 144)
- O Less than 1 day per month
- O 1-3 days per month
- O 1-3 days per week
- O 4-6 days per week
- O Every day

143a. When you took **Vitamin D**, about how much did you take in one day?

- O Less than 125 IU
- O 125-249 IU
- O 250-399 IU
- O 400 IU or more
- O Don't know

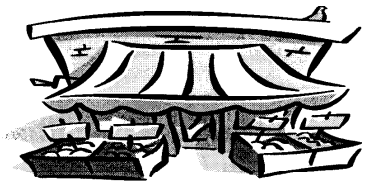
143b. For how many years have you taken **Vitamin D**?

- O Less than 1 year
- O 1 - 4 years
- O 5 - 9 years
- O 10 or more years

These last two questions ask about other supplements you took more than once per week.

144. Please mark any of the following **single supplements** you took more than once per week (**NOT** as part of a multivitamin):

- O B-6
- O B-Complex
- O Brewer's yeast
- O Cod liver oil
- O Coenzyme Q
- O Fish oil (Omega-3 fatty acids)
- O Folic acid/folate
- O Glucosamine
- O Hydroxytryptophan (HTP)
- O Iron
- O Niacin
- O Selenium
- O Zinc



145. Please mark any of the following **herbal** or **botanical supplements** you took more than once per week:

- O Aloe Vera
- O Astragalus
- O Bilberry
- O Cascara sagrada
- O Cat's claw
- O Cayenne
- O Cranberry
- O Dong Kuai (Tangkwei)
- O Echinacea
- O Evening primrose oil
- O Feverfew
- O Garlic
- O Ginger
- O Ginko biloba
- O Ginseng (American or Asian)
- O Goldenseal
- O Grapeseed extract
- O Kava, kava
- O Milk thistle
- O Saw palmetto
- O Siberian ginseng
- O St. John's wort
- O Valerian
- O Other

146. Is there anything else you eat at least once a month? Please write name of food, frequency and amount.

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Thank you very much for completing this questionnaire!

Before returning the questionnaire to us, please check that you did not accidentally skip any questions or pages.

16336

A.5. Baseline Health Questionnaire

		/			/	2	0		
Day			Month			Year			

ALPHA Trial

Baseline Health Questionnaire

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Directions:
Answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

Alberta Cancer Board

A. DEMOGRAPHIC INFORMATION

A1. When were you born? ☐☐ / ☐☐ / ☐☐☐☐
Day Month Year

A2. What is your current marital status?

- ○ Married
- ○ Common-law
- ○ Separated
- ○ Divorced
- ○ Widowed
- ○ Never Married
- ○ Other _____ specify

A3. What is the highest level of school that you have completed?

- ○ Did not complete grade school
- ○ Grade school
- ○ High school
- ○ College or trade school
- ○ University undergraduate degree
- ○ University graduate degree

A4. To which ethnic or cultural groups did you, or most of your ancestors belong, on first coming to North America?

- ○ British
- ○ Western European
- ○ Eastern European
- ○ French
- ○ Northern European
- ○ Southern European
- ○ Aboriginal
- ○ East and Southeast Asian
- ○ Southern Asian
- ○ Western Asian
- ○ Pacific Islands
- ○ Arab
- ○ Latin, Central and South American
- ○ Caribbean
- ○ African
- ○ Other _____ (specify)

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B. MENSTRUAL HISTORY

B1. How old were you when your menstrual periods started?

☐☐ (years)

B2. What statement best describes your menstrual status one month before today?

- ○ Still having periods and not going through menopause or change of life.
- ○ Still having periods and possibly going through change of life.
- ○ Going through menopause or change of life.
- ○ Periods stopped by themselves or natural menopause.
- ○ Periods stopped by surgical removal of the uterus or both ovaries.
- ○ Periods stopped by radiation or chemotherapy.
- ○ On hormone eplacement therapy and still having periods.
- ○ Other (specify) _____
- ○ Don't know

B3. How old were you when had your last menstrual period? (After your menstrual periods stopped, you may have started taking hormones that caused you to start having periods again. We are interested in when your menstrual periods stopped before you started taking these hormones.)

☐☐ (years)

B4. Have you had a hysterectomy? A hysterectomy is an operation to remove your uterus or womb.

- ○ Yes
- ○ No

If yes, how old were you when you had the operation? ☐☐ (years)

B5. Have you had both ovaries removed?

- ○ Yes
- ○ No

If yes, how old were you when you had the operation?
(However if both have been removed in two seperate operations, provide the age at which you had the second operation) ☐☐ (years)

C. REPRODUCTIVE HISTORY

C1. Have you ever been pregnant?

○ Yes If no, go to section D
○ No

C2. How many pregnancies have you had?

☐☐ Total number of pregnancies including miscarriages, abortions, still births and live births

C3. How many live births have you had?

☐☐ Total number of pregnancies that resulted in live births

C4. How old were you when your first child was born?

☐☐ years

D. MEDICAL HISTORY

D1. Have you ever been diagnosed with any of the following conditions?

High cholesterol or triglycerides	○ Yes	○ No
Heart attack (myocardial infarction)	○ Yes	○ No
Cardiac chest pains (angina pectoris)	○ Yes	○ No
Stroke	○ Yes	○ No
Arthritis (rheumatoid arthritis or osteoarthritis)	○ Yes	○ No
Osteoporosis	○ Yes	○ No
Blood clots in the veins of your legs or pelvis	○ Yes	○ No
Blood clot in your lungs	○ Yes	○ No
Thyroid problems	○ Yes	○ No
Any other medical conditions	○ Yes	○ No

If yes, what conditions?

61063

D2. **Have any of your mother, daughters or sisters had breast cancer? Do not include any stepsisters, half sisters or adopted sisters.**

○ **Yes** ○ **No**

D3. **Have you ever been told by a doctor that you have benign breast disease, i.e., a breast condition or disorder that is not cancerous? The doctor may have referred to it as fibrocystic breast disease or breast cysts, lumps etc.**

○ **Yes** ○ **No** (If **No**, go to section E)

If yes, was a breast biopsy taken? *A breast biopsy is a small operation that involves removing a piece of your breast tissue in order to diagnose a breast problem.*

○ **Yes** ○ **No**

E. ANTHROPOMETRIC HISTORY

E1. **What is the tallest you have ever been without shoes on?**

☐ **feet** ☐☐ . ☐ **inches** **or** ☐☐☐ . ☐ **cm**

E2. **What was your body weight at the following ages? (use your usual body weight, when you were not pregnant.)**

20 years	☐☐☐	**lbs**
30 years	☐☐☐	**lbs**
40 years	☐☐☐	**lbs**
50 years	☐☐☐	**lbs**
60 years	☐☐☐	**lbs**
70 years	☐☐☐	**lbs**

F. MENOPAUSAL HORMONE USE HISTORY

F1. Have you ever used any hormone medications, in the form of a pill, shot, implant, skin patch, body gel, vaginal cream, or suppository for the purpose of hormone replacement therapy?

○ **Yes (If yes, please complete table)** ○ **No (go to Section G)**

Table of Menopausal Hormone Use

Please tell us when you started using menopausal hormones, the type, the dose, the number of days in a 28 cycle that you used the hormones, and the length of time that you used these hormones. Please refer to the pictures of these hormones to help you remember the type and dose.

Type of hormone brand name or description	Age started	Age stopped	Duration of use	Dose	Units	Mode
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ______	○ pill ○ patch ○ cream/gel ○ injection ○ other
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ______	○ pill ○ patch ○ cream/gel ○ injection ○ other
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ______	○ pill ○ patch ○ cream/gel ○ injection ○ other
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ______	○ pill ○ patch ○ cream/gel ○ injection ○ other

Type of hormone **brand name or description**	**Age started**	**Age stopped**	**Duration of use**	**Dose**	**Units**	**Mode**
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ________	○ pill ○ patch ○ cream/gel ○ injection ○ other
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ________	○ pill ○ patch ○ cream/gel ○ injection ○ other
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ________	○ pill ○ patch ○ cream/gel ○ injection ○ other
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ________	○ pill ○ patch ○ cream/gel ○ injection ○ other
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ________	○ pill ○ patch ○ cream/gel ○ injection ○ other
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ________	○ pill ○ patch ○ cream/gel ○ injection ○ other
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ________	○ pill ○ patch ○ cream/gel ○ injection ○ other
	☐☐ years	☐☐ years	☐☐ ○ month(s) ○ year(s)	☐☐☐☐☐ ○ don't know	○ ug ○ mg ○ grams ○ other ________	○ pill ○ patch ○ cream/gel ○ injection ○ other

G. MEDICATION USE

The next section is about **prescription and over-the-counter** *medications that you have taken in the past year.*

During the past 12 months, did you take any medications (prescription or over the counter) on a regular basis? ***"Regular"*** *is defined as at least 3 times a week for at least 1 month.* ***Do not include HRT medications.***

○ **Yes (If yes, please complete table)** ○ **No (Go to Section H)**

Name of medication	What dose do/did you take regularly	Units of dose	How often do/did you take it?	How long have/had you been taking it?
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know

Name of medication	What dose do/did you take regularly	Units of dose	How often do/did you take it?	How long have/had you been taking it?
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
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H. Vitamin and Supplement Use

The next section is about ***vitamins and supplements*** *that you have taken in the past year.*

During the past 12 months, did you take any vitamins or supplements, on a regular basis? ***"Regular"*** *is defined as at least 3 times a week for at least 1 month.* ***Do not include medications.***

○ Yes (If yes, please complete table) **○ No (Go to Section H)**

Name of vitamin or supplement	**What dose do/did you take regularly**	**Units of dose**	**How often do/did you take it?**	**How long have/had you been taking it?**
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know

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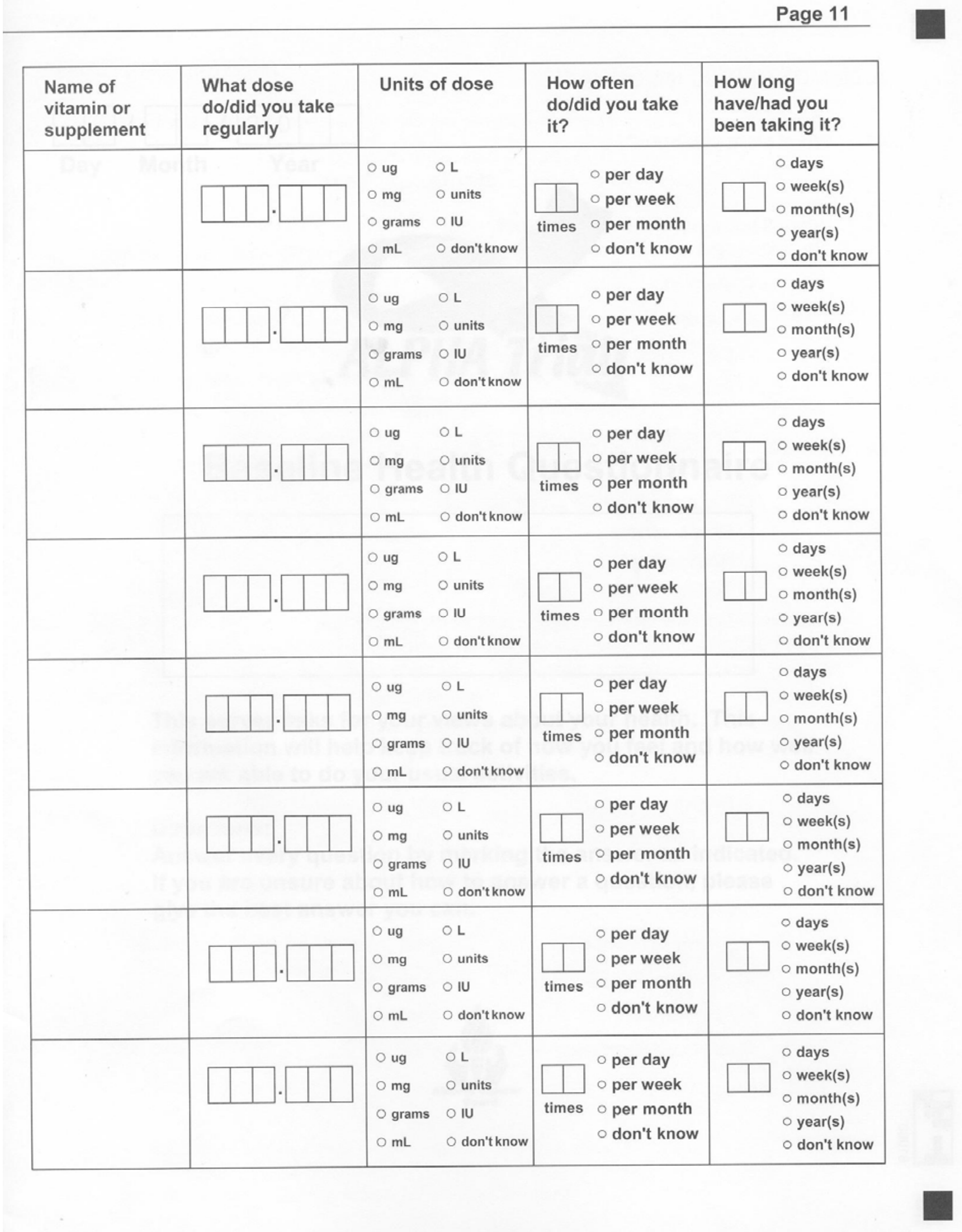

Name of vitamin or supplement	What dose do/did you take regularly	Units of dose	How often do/did you take it?	How long have/had you been taking it?
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
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	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know
	☐☐☐.☐☐☐	○ ug ○ L ○ mg ○ units ○ grams ○ IU ○ mL ○ don't know	☐☐ times ○ per day ○ per week ○ per month ○ don't know	☐☐ ○ days ○ week(s) ○ month(s) ○ year(s) ○ don't know

A.6. Ethics Approval

FEB-28-2007 16:07 U OF C OFF. MED BIOETHICS 403 283 8524 P.03/03

Alberta Cancer Board

DIVISION OF POPULATION HEALTH & INFORMATION

1331 - 29 Street N.W., Calgary, Alberta, Canada T2N 4N2 Tel: (403) 521-3862 Fax: (403) 270-8003

Website: www.cancerboard.ab.ca

October 19, 2006

RECEIVED OCT 20 2006 OFFICE OF MEDICAL

Office of Medical Bioethics
Faculty of Medicine
Heritage Medical Research Building, Rm 93
3330 Hospital Drive NW
Calgary, Alberta, Canada T2N 4N1

Dear Dr. Godlovitch:

RE: Alberta Physical Activity and Breast Cancer Prevention Trial (ALPHA Trial)

Ethics ID: 16649 PI: Dr. Christine Friedenreich

I am writing to request ethics approval for a modification to my study, the ALPHA Trial. Specifically, I would like to include Ms. Ame-Lia Tamburrini's Master's Thesis proposal as a substudy of the ALPHA Trial. Her project is entitled: *The relation between dietary and serum cholesterol and mammographic density as a risk factor for breast cancer.*

The aim of this cross-sectional study is to understand the nature of the association between cholesterol and mammographic density, a known risk factor for breast cancer, among sedentary, postmenopausal women. The specific objectives of the proposed project are to determine: 1) the association between serum cholesterol and mammographic density; 2) the association between dietary cholesterol and mammographic density; and 3) the association between dietary and serum cholesterol. A cross-sectional analysis will be performed using baseline data from 320 sedentary, postmenopausal women between the ages of 50-74 years who voluntarily enrolled in the ALPHA Trial. All of the data required for these analyses were collected to address the main objectives of the ALPHA trial (1 - To examine the effect of an exercise intervention on estrone, estradiol and adiposity; 2 - To examine the effect of an exercise intervention on mammographic density, insulin-like growth factors and insulin resistance.). Since the objectives of Ame-Lia's project are related to the main goals of the ALPHA Trial, but were not specified in the original proposal, I would like to add them as a modification to the ALPHA Trial.

Thank you very much. If you have further questions please do not hesitate to contact me.

Yours sincerely,

Christine M. Friedenreich, PhD
Adjunct Professor, Department of Community Health Sciences;
Research Scientist, Division of Population Health and Information

TOTAL P.03

JCenH

FILE COPY

FACULTY OF MEDICINE | UNIVERSITY OF CALGARY

December 14, 2006

OFFICE OF MEDICAL BIOETHICS
Room 93, Heritage Medical Research Bldg
3330 Hospital Drive NW
Calgary, AB, Canada T2N 4N1
Telephone: (403) 220-7990
Fax: (403) 283-8524
Email: omb@ucalgary.ca

Dr. C. Friedenreich
Division of Epidemiology, Prevention and Screening
Alberta Cancer Board
Tom Baker Cancer Centre

Dear Dr. Friedenreich:

Re: ALPHA Trial: Alberta Physical Activity and Breast Cancer Prevention Trial

Grant ID: 16649

Your request to modify the above-named research protocol and the related informed consent form has been reviewed and approved by the Conjoint Health Research Ethics Board on December 14, 2006.

I am pleased to advise you that it is permissible for you to use the revised protocol adding Ms. Ame-Lia Tamburrini's Master's Thesis Proposal entitled "The relation between dietary and serum cholesterol and mammographic density as a risk factor for breast cancer" as a sub study to the ALPHA Trial, based on the information contained in your correspondence of October 19, 2006.

A progress report concerning this study is required annually, from the date of the original approval 2002-08-27. The report should contain information concerning:

(i) the number of subjects recruited;
(ii) a description of any protocol modification;
(iii) any unusual and/or severe complications, adverse events or unanticipated problems involving risks to subjects or others, withdrawal of subjects from the research, or complaints about the research;
(iv) a summary of any recent literature, finding, or other relevant information, especially information about risks associated with the research;
(v) a copy of the current informed consent form;
(vi) the expected date of termination of this project;

Thank you for the attention which I know you will bring to these matters.

Yours sincerely,

Glenys Godlovitch, BA(Hons) LLB, PhD.
Chair, Conjoint Health Research Ethics Board
GG/eb

c.c. Adult Research Committee Ms. Anora Beckie