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Alcohol and cancer: Relationship with site-specific cancers and steps toward a biochemical marker for alcohol intake

Gapstur, Susan Mary, Ph.D.

University of Minnesota, 1993

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ALCOHOL AND CANCER: RELATIONSHIP WITH SITE-SPECIFIC CANCERS AND STEPS TOWARD A BIOCHEMICAL MARKER FOR ALCOHOL INTAKE

A THESIS

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL

OF THE UNIVERSITY OF MINNESOTA

BY

SUSAN MARY GAPSTUR

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

JANUARY 1993

UNIVERSITY OF MINNESOTA

This is to certify that I have examined this bound copy of a doctoral thesis by

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Name of Faculty Advisor

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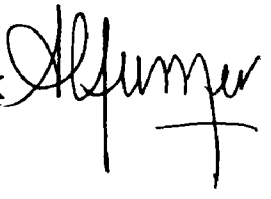

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PREFACE

Traditionally, epidemiology has been used to explore the occurrence of cancer and risk factors associated with cancer in populations; and laboratory sciences have extensively studied the molecular biology/biochemistry of cancer. Recently there has been an interest in merging these two disciplines (molecular epidemiology) to describe more accurately the etiology of this multifaceted disease. The epidemiology of cancer associated with certain environmental risk factors has been well established. Conversely, for other factors such as diet, these associations are not as clear. Molecular epidemiology has potential for characterizing relationships between some dietary exposures and initiation, promotion and/or progression of cancer. A better understanding of the biology and better methods of measuring exposure, through the development of biomarkers, would enhance our knowledge of these complex relationships.

Alcohol consumption is one dietary factor that has been associated consistently with aerodigestive cancers and liver cancer in epidemiologic studies. However, the evidence supporting an association with other sites, such as the colon, rectum and endometrium, often tend to be weaker. These epidemiologic studies may be limited by design, and/or methods of assessing alcohol exposure. Ideally, alcohol exposure would be determined objectively and related, forward in time, to the incidence of cancer. However, accurate information on exposure, especially past exposure, is not always available; self-report data on recent consumption is often the only measure relied on. Measurement of alcohol-exposure in epidemiologic studies may be greatly enhanced by the use of an objective biomarker or set of markers.

In Chapter 1 of this thesis, the existing literature on the epidemiology of the relationships

between alcohol consumption and the incidence of colon, rectal and endometrial cancer is reviewed. Associations between alcohol consumption and the incidence of colon cancer and rectal cancer are examined in a cohort of postmenopausal women. The incidence of each cancer across categories of alcohol-consuming participants of the Iowa Women's Health Study are compared to the incidence of each cancer in nondrinking participants. Associations between alcohol consumption and proximal and distal colon cancers were explored separately. These results are reported in Chapter 2. In addition, the incidence of endometrial cancer across categories of alcohol-consuming participants of the Iowa Women's Health Study are compared to the incidence of endometrial cancer in nondrinking participants; these results are reported in Chapter 3 of this thesis. The effect of family history of cancer, age at menarche, age at menopause, body mass index, noncontraceptive estrogen use and other risk factors on this association are described.

Chapter 4 discusses the literature regarding biomarkers and existing measures of alcohol consumption. There are several steps in developing a biomarker, which accurately reflects alcohol exposure, that must be carried out prior to implementing it in population based studies. One possible biomarker of alcohol intake is the resulting product from the reaction between acetaldehyde and hemoglobin . The development of an *in vitro* model system and the effects of acetaldehyde concentration and incubation time on the amount of acetaldehyde-modified hemoglobin are described in Chapter 5.

A summary is presented in Chapter 6.

CHAPTER I

THE ASSOCIATIONS BETWEEN ALCOHOL AND COLON AND RECTAL CANCER, AND ENDOMETRIAL CANCER: A LITERATURE REVIEW.

INTRODUCTION

People have been consuming alcoholic beverages since before fermentation processes and beer were first discovered by the ancient Greeks. Since this time, many adverse health effects of heavy drinking including cirrhosis of the liver, and diseases of the heart, nervous, immune and endocrine systems as well as of the gastrointestinal tract, have been identified. It is difficult to assess the impact of alcohol on mortality; this is partly due to underreporting of alcohol related conditions on death certificates and partly due to the fact that acute intoxication is often associated with deaths from motor vehicle accidents, homicide and suicide (1). According to self-reported data, approximately 35 percent of the US population abstain from alcohol and 35, 22 and 8 percent are light, moderate and heavy drinkers, respectively, (light= less than one drink per day; moderate= 1-3 drinks per day; and heavy= more than three drinks per day) (2).

Although neoplastic tissue has been recognized in humans since at least 4000 B.C., when Hippocrates coined the term *carcinoma* (3), it was only during this century, in 1910, that a French physician first reported an association between alcohol, specifically absinth (an herbal liquor), and cancer (oesophagus). Since this report, epidemiologic studies have shown a consistent link between alcohol and many types of cancer. The strongest carcinogenic effect of alcohol is on cancers of the aerodigestive tract, such as the oral cavity, pharynx, larynx, oesophagus, and cancer of the liver (4). Although weak, there is also a consistent association between alcohol and breast cancer (5); this is an especially important observation given the high incidence of breast cancer and alcohol consumption in some countries. It is estimated that 2-4 percent of all cancer deaths may be attributable to alcohol consumption (6).

Colon and rectal cancers, among the most common cancers in the world, have correlated positively with alcohol consumption in geographic and time-trend studies. However, inconsistent associations have resulted from both cohort and case-control studies. The few prospective cohort studies that considered the relationship between colorectal cancer and alcohol have reported either positive or null associations. Not all of the studies have included women and some did not examine gender-specific associations. Both the occurrence of colon and rectal cancer and the amount and frequency of alcohol consumed varies between men and women according to age (7). Therefore, it seems prudent to explore the association between alcohol and these two cancers in women alone. The epidemiology of colon and rectal cancer, as well as the literature that examines the association between alcohol consumption and these two cancers are reviewed below.

As mentioned above, there appears to be a consistent, although moderately elevated risk of breast cancer in women who consume alcohol compared to women who abstain. The risk of breast cancer among moderate consumers of alcohol is about fifty percent higher than abstainers (6). Because breast cancer and endometrial cancer share many of the same risk factors and because at least one plausible mechanism for the association of alcohol with cancer involves hormone metabolism, it seem reasonable to explore whether alcohol may also be associated with endometrial cancer. The epidemiology of endometrial cancer is reviewed including a discussion of the few studies that have considered the association between alchol and this cancer.

BACKGROUND AND SIGNIFICANCE

Epidemiology of Colon and Rectal Cancer

Occurrence and Risk Factors:. Together, colon and rectal cancers are among the most

common cancers in both men and women. However, there is at least a twenty-fold difference in colon and rectal cancer mortality rates across the world (8). This wide variability across countries suggests that environmental factors, (including diet), may play a role in their etiology. Evidence for this hypothesis is supported by international variations in colon and rectal cancer death rates correlated with certain dietary factors (9, 10).

The incidence of colorectal cancer is second only to that of lung cancer in the United States, (Table 1) (2). Cancers of the colon and rectum are the third most common cause of cancer mortality for both males and females (8). The incidence of rectal cancer remained relatively stable from 1973-1988 in both men and women. However, the incidence of colon cancer increased in white men and black men and women, and remained relatively stable only in white women over the same period. Overall, the incidence of colon cancer is slightly higher in blacks than in whites, and the incidence of rectal cancer is slightly lower (2). Mortality rates for both colon and rectal cancers in women decreased by 16 and 36 percent respectively, between 1956-58 and 1986-88. Over this same period of time, colon cancer mortality rates increased by 21 percent in men but rectal cancer rates slightly decreased (5 percent).

Colon cancer is rare before age 30, after which the incidence slowly increases until about age 45 when there is a sharp increase in colon cancer which continues with increasing age. Although the overall incidence of rectal cancer is only about 20 percent that of colon cancer, the age-specific pattern of rectal cancer is similar to colon cancer until age 85, when the incidence of rectal cancer declines slightly (11).

Except among those aged 30-34, colon and rectal cancers are more common in females until about age 50, when the incidence of rectal cancer in males exceeds that in females.

Colon cancer is more common in females than in males until age 55. The sex-ratio differences across age-strata suggest possible hormonal associations with risk of colon cancer (12).

If the cancer is diagnosed when it is still localized the five-year survival for colorectal cancer is 88 percent in whites and 83 percent in blacks. The overall relative five-year survival for these cancers increased from 43 percent in 1960-1963 to 57 percent in 1981-1987 in whites and from 34 to 47 percent in blacks (8).

The major risk factors for colon and rectal cancers (not in order of importance) are personal history of cancer, intestinal polyps, ulcerative colitis, family history of colorectal cancer, a genetic predisposition (ie. familial polyposis), and high energy, high fat, high protein and/or a low fiber diet (13, 14). In women, colon cancer may be inversely associated with parity (12, 15) and positively associated with an older age at first birth, particularly with the ascending colon (16). However, the association with parity has not been consistently observed (17). These two reproductive factors do not appear to be associated with rectal cancer (15, 16). Oral contraceptive use may be protective against colon cancer (16), however, the risk of rectal cancer in oral contraceptive users may be elevated (15).

It has long been suspected that alcohol may be involved in the development of colorectal cancer. In 1957, Percy Stocks (18) first reported an elevated, though not statistically significant, risk of colorectal cancer among daily beer drinkers compared to abstainers (RR=1.4). Since this report, the association between alcohol and cancers of the large bowel have been explored in several ecologic and analytical epidemiology studies. Determining whether these associations are indeed causal has important implications given

the high prevalence of alcohol consumption and the high incidence of colorectal cancer in the United States. A review of the studies that have examined the effect of alcohol on colon and/or rectal cancers, focusing on gender-specific associations, is described below.

First, it is important to note that several different methods of assessing alcohol consumption have been used in these studies. For example, in some studies exposure status was defined as membership in certain groups such as a) those with a history of alcohol abuse or misuse (19, 20, 21, 22), or b) those employed in the beer industry (23, 24). In other studies, individual intake was assessed using dietary histories obtained by trained nutritionists, questionnaires that query participants regarding average number of monthly drinks of specific beverages, or food-frequency questionnaires asking average intake of beer, wine and spirits over the last year. From these assessment tools, alcohol intake has been computed and reported in terms of average grams of ethanol per day, kilograms per year, drinks per day or week, milliliters of ethanol per day, etc. The frequency of consumption (ie none, infrequently, occasionally, daily) rather than the amount has been used as a means for comparing cases to noncases in some studies. Not all studies considered abstainers as the reference group; some studies considered the lowest group of alcohol consumers such as the lowest half, tertile or quartile as the reference group. It is not entirely clear what consequences these methodologic differences may have on the consistency among studies.

Alcohol and Colon Cancer (Table 2). All five ecologic comparisons examining the relationship between alcohol and colon cancer have shown positive results (10a, 10b, 25, 26, 27). No differences between males and females were found in the studies that reported gender-specific associations (10, 25, 26) with one exception (27) where colon cancer mortality was significantly correlated with total alcohol and beer consumption in males but

only with beer in females. However, in ecologic studies where gender-specific outcome data were examined, there were no relevent gender-specific drinking data that allow for true gender-specific analysis. Geographic differences in cancer mortality were positively correlated with beer consumption (r=0.58-0.76) (10, 25, 27). In addition, changes in per capita beer consumption from 1950-52 to 1960-62 were compared to changes in colon cancer mortality rates from 1960-64 to 1970-74 (to allow for a latency period), in the United States, United Kingdom, Australia and New Zealand (26). There was a positive relationship between increased beer consumption and colon cancer mortality rates over time. A significant, positive correlation (r=0.4) between wine and cancer mortality rates across 41 U.S. states was found in one study (10a); however, a nonsignificant, negative correlation (r= -0.13 to -0.23) was found across 29 countries in the only other study that considered this association (27). Consumption of spirits across 41 states also correlated positively with colon cancer mortality rates (r=0.5-0.6) (10a).

Of the nine cohort studies that have investigated the association between alcohol and colon cancer in either alcoholics (19, 20a, 20b, 21, 22, 28, 29) or people employed in breweries (23, 24), none showed significant results. Only two of these studies included females. There was a nonsignificant 60 percent elevated risk of colon cancer mortality among alcoholic females when compared to the general population of the United Kingdom (22).

Two (30, 31) of the four general, population cohort studies (32, 33) that investigated the effect of alcohol on colon cancer reported significant results in either males and/or females. In a study of Japanese adults (31), there was a five-fold increase in the risk of sigmoid colon cancer mortality among males who drank alcohol daily compared to abstainers but no association with the proximal colon. Compared to abstainers, daily beer consumers had a 12-fold elevated risk of sigmoid colon cancer mortality. Daily sochu and sake consumption

were also significantly associated with colon cancer mortality. In females, the relative risk for any alcohol consumption was 1.9 (sigmoid colon cancer). Klatsky et al. (30) reported a dose-response relationship between alcohol and colon cancer in females and no association in males. These results were not specific to any particular colon subsite or alcoholic beverage.

In seven of at least 15 case-control studies conducted to date, a significant, positive association between alcohol and colon cancer has been reported. Of the three studies (34, 35, 36) that included only males, one found no association (34) and another reported a nonsignificant 60 percent elevation in risk for men who consume at least 70 grams of ethanol per day (35) (this was not specific to any type of alcoholic drink). Longnecker (36) reported a significant dose-response between colon cancer and self-reported alcohol intake five-years prior to diagnosis but not with consumption 20-years prior. Only self-reported beer consumption five-years prior to diagnosis was associated with colon cancer, but not with wine or spirits or with any drink consumed 20-years previously.

Three (37, 38, 39) of five (40, 41) case-control studies that combined males and females reported a positive association between colon cancer and alcohol. In a study to assess dietary factors associated with cancers of the colon and rectum in China, the relative risk of colon cancer for those who consume at least 10 kg of ethanol per year compared to those who drink less was 2.0 (39). In a Nebraska case-control study, commercial beer consumption was associated with at least a 2.7 fold-elevation in risk of colon cancer compared to abstainers, but no association with homemade beer or any type of wine (37). There was also a positive, dose-response relationship between beer and colon cancer in a Belgian case-control study (38).

Of the seven case-control studies that stratified on gender, 1 (14) reported a positive association between alcohol and colon cancer in females and 3 (14, 47, 48) in males. Four studies showed no association in either males or females (42, 43, 44, 45). One of the earlier case-control studies assessed environmental factors associated with cancers of the colon and rectum, (46), only beer was significantly associated in males. Williams and Horm (47), analyzed data from the Third National Cancer Survey and found a positive relationship between colon cancer and total alcohol as well as beer, wine, liquor and spirits in males; there was no relationship in females. Consumption of spirits, but not beer, wine, or total alcohol was positively associated in men and, slightly more so, in women in an Australian study (14); this association was specific to the proximal colon.

Alcohol and Rectal Cancer (Table 3). Rectal cancer has been positively correlated with alcohol consumption in all of the geographic and time-trend studies considered (10a, 10b, 25, 26, 27). No differences were reported in the four studies that investigated gender-specific correlations (10a, 25, 26, 27). Geographic differences in rectal cancer mortality rates were positively correlated with beer consumption (R=0.71-0.81) (10a, 10b, 25, 26, 27). Changes in per capita beer consumption from 1950-52 to 1960-62 were positively associated with changes in rectal cancer mortality rates from 1960-64 to 1970-74 (to allow for a latency period), in the United States, United Kingdom, Australia and New Zealand (26). There was also a significant correlation (r=0.5) between wine and rectal cancer mortality rates across 41 U.S. states in one study (10a); however a nonsignificant, negative correlation (r=-0.17 to -0.25) between wine consumption and mortality rates across 29 countries was observed in the only other study that considered this relationship (27). Spirits consumption across 41 states were also positively correlated (r=0.5) with differences in rectal cancer mortality rates (10a).

Seven cohort studies examined the association between alcohol and rectal cancer in either alcoholics (19, 21, 22, 28, 29) or people employed in the beer industry (23, 24). In the only study that found an association, there was an elevated rectal cancer mortality rate among male brewery workers in Dublin compared to that of skilled and unskilled workers in Dublin (SMR=1.6) (23). This is an especially intriguing observation given that these employees were each allowed to consume two pints of stout or other beer daily free of charge. However, no association was found in the only other study of brewery workers (24). Although the daily free rationing of beer in this Danish study was more than the Dublin study, (6 pints vs. 2 pints) it consisted of a lighter lager-type of beer. The conflicting results from these two studies suggest that there may be congeners in stout beers that lead to elevated risks of rectal cancer that are not present in lighter beers.

A dose-response relationship between alcohol and rectal cancer was observed in all three cohort studies of the general population (30, 31, 33). There was no difference between males and females in the only study that included both genders, nor was the association specific to either wine, beer or spirits (30). Beer was significantly associated with rectal cancer in men who consumed 15 liters of beer per month compared to abstainers (RR=3) in an earlier report of the Japanese-Hawaiian cohort (48, 33).

Alcohol was positively associated with rectal cancer in nine of at least 18 case-control studies. Two (34, 36) of the three studies (35) that included only males found a significant, positive association between rectal cancer and at least one category of total alcohol intake. Longnecker et al. (36) assessed consumption both five and twenty-years prior to diagnosis and found an elevated risk of rectal cancer with both total alcohol and beer consumption at both times (RR ranged from 1.5-1.9). In a Korean case-control study (34), there was a five-fold elevated risk of rectal cancer in heavy alcohol consumers

compared to abstainers.

Gender-specific associations were not assessed in five (37, 38, 40, 41, 49) of the fifteen case-control studies that included both males and females. There was no association between rectal cancer and either total alcohol consumption (40, 41) or any type of alcoholic beverage (37, 38, 49) in any of these studies.

Two of the ten case-control studies that stratified on gender found no association between alcohol and rectal cancer in either males or females (42, 43). Six studies reported a significant association with at least one type of alcoholic beverage in males (14, 39, 45, 46, 50, 51) and three reported a significant association in females (14, 47, 51). The study by Miller et al. (44) reported a nonsignificant inverse association association with alcohol consumption in both males and females. Odd-ratios for rectal cancer in males ranged 0.5 in men who consumed <47.7 grams of ethanol (excluding beer) per day (44) to 2.1 in men who consumed at least 6 kg of ethanol per year (39). In females, odds ratios were approximately 2.0 for the highest level of consumers compared to abstainers (47, 51). There was a positive association between beer and rectal cancer in 3 (45, 46, 50) of five studies (44, 47) for males. Beer was not significantly associated with rectal cancer in females in any of these five studies; however, the association was inverse in at least two studies (44, 50). There was a positive relationship between wine and rectal cancer only in the Australian study by Potter et al. (14) and this was confined to females. However, inverse associations with wine were reported for males and females by Williams et al. (47). There was a significant positive association with spirits (14), and an inverse association between spirits and rectal cancer in the study by Kune et al. (45); in both of these studies the finding was confined to males.

Alcohol and Colorectal Cancer (Table 4). No ecologic studies investigating per capita alcohol consumption and colorectal cancer (as an entity) have been reported to date. However, two (52, 53) of three cohort studies found no association with alcohol consumption in men. The third study, a cohort of residents living in a retirement community, showed a significant dose-response relationship with daily alcohol intake for men (p-trend=0.004) but not women (54).

Two (49, 55) of four case-control studies found no relationship between colorectal cancers and any alcoholic beverage for males and females combined. One of the earliest case-control studies that examined dietary factors and cancer only in men reported a nonsignificant association between beer consumption and cancers of the large intestine (18). Slattery et al. (56) found a significant dose-response in males between colorectal cancer and total alcohol consumption that disappeared after adjusting for the confounding effects of religion, body mass index, calories, crude fiber, pipe use and caffeine consumption.

Summary Colon, Rectal and Colorectal Cancer and Alcohol. Some studies considered potential modifying effects of other risk factors, specifically diet, on the association between alcohol and colon cancer. Longnecker (36) reported that, in males, the association between consumption of 5 or more drinks per day (five-years in the past) and cancer of the rectum or cancer of the right colon was confined to those with low dietary calcium intake versus those with a high intake (RR=2.2 right colon; 1.5 rectum). He also notes the same effect with vitamin D (RR=2.0 right colon; 1.7 rectum). There was also evidence of an interaction with folate intake and alcohol intake on risk of rectal cancer, specific to males, but not colon cancer (51).

Although cross-cultural, ecologic comparisons appear to suggest a positive association between alcohol consumption (mostly beer) and colon and rectum cancers, cautious interpretation of these results is warranted. Correlational studies are unable to control effectively for other characteristics that may also correlate with both alcohol consumption and disease (ie. cigarette smoking, poor dietary habits). In addition, ecologic studies are limited by differences in the methods of collecting data and in the quality of the data collected among countries. Finally, ecologic studies compare group means of exposure, with an inherent high degree of misclassification.

In studies of alcoholics or brewery workers, the risk of colon cancer was elevated (nonsignificant) in three of nine studies (RR ranged from 1.0-1.8). The risk of rectal cancer was elevated in four of seven studies (RR ranged from 1.0-3.3), although only the study of brewery workers from Dublin showed a significant association (RR=1.6). There may not have been enough power to detect a significant association in many of the studies, given the small number of cases.

Of the 15 general population studies that examined alcohol and colon cancer, 7 reported a positive association, and 12 of the 21 alcohol-rectal cancer studies showed a positive association. Beer appeared to be positively related to cancer of the colon and even more so cancer of the rectum in men more often than in women, in whom there were nonsignificant inverse associations in only two of ten studies. Inconsistencies in these studies may have resulted from the few number of cases in some case-control studies, differences in control groups, in methods of assessing consumption, and differences in preferred beverages across countries and between men and women.

Epidemiology of Endometrial Cancer:

Occurrence and Risk Factors:. The uterus consists of the cervix, and the body (corpus). The corpus contains two layers; the internal layer is the endometrium, the external layer, is the myometrium. Approximately 93 percent of corpus uteri cancers in whites and 86 percent of corpus uteri cancers in blacks are of endometrial origin (57). The majority of corpus uteri cancers are endometrial and are not always discerned from other uterine cancers; therefore, the descriptive epidemiology may not be strictly comparable across geographic areas. Unless otherwise noted, cancers of the corpus uteri will be referred to as endometrial cancers from this point on.

Internationally, endometrial cancer accounts for approximately 5 percent of all female cancers. Argentina has the highest rates of endometrial cancer; it is also more common in the United States, Canada and Western Europe, and less common in Singapore, Japan, China and India. International (ecologic) studies have shown strong correlations between dietary, and reproductive factors and the incidence of endometrial cancer (9, 58).

In the United States, endometrial cancer is the fifth most commonly occurring cancer in women (Table 1) (2) and the eighth most common cause of cancer deaths (59). From 1960 to 1975 incidence rates for endometrial cancer nearly doubled (60), but have since gradually declined. It has been suggested that these secular changes in incidence followed the advent of noncontraceptive estrogen use. In the early 1970s, unopposed estrogens were commonly prescribed to relieve menopausal symptoms; by the late 1970s, progesterone combined with lower concentrations of estrogen had replaced the use of high concentrations of estrogen alone. The rise and fall of endometrial cancer have been attributed by some to these changes in hormone prescribing practices. However, some studies have attributed at least a portion of the increase in incidence of endometrial cancer in

the early 1970s to changing diagnostic criteria; abnormal tissue that was formerly called advanced endometrial hyperplasia, was later considered carcinoma. In addition, the number of hysterectomies, especially on older women increased in the 1960s and 70s, reducing considerably the number at risk of endometrial cancer, although, there has been an attempt to take into account hysterectomy rates in estimating the incidence rates of endometrial cancer. Endometrial cancer is very rare before age 25 and occurs most commonly in women age 60-80 (11). The incidence of endometrial cancer is almost two times higher in whites than blacks.

In 1956-58, endometrial cancer was the fifth most common cause of cancer mortality in American women. Since this time there has been a steady decline in endometrial cancer mortality rates. The decline in cancer mortality may be attributed to earlier detection with an increase in regular pelvic examinations in both younger and older women, or the changing nature of disease, or removal of uterus from those at higher risk.

The relative five-year survival rate for endometrial cancer ranges from 93 percent for localized to 27 percent for distant (59). Among whites, the overall five-year survival has improved from 73 percent in 1961-1963 to 84 percent in 1981-1987, and among blacks there was an increase from 31 percent to 56 percent. Whether the differences in survival rates between white and black women may be due to differences in seeking health care, regular examinations, or possible differences in hysterectomy rates is not clear.

The risk factors most strongly associated with endometrial cancer are related to hormonal levels, particularly estrogen. Increased risk of carcinoma of the endometrium has been consistently linked with increasing body mass index, early age at menarche (particularly among premenopausal women), late age at menopause, decreased parity, noncontraceptive

estrogen use, history of hypertension or diabetes, a personal history of breast, ovarian or colon cancer, and a family history of ovarian or endometrial cancer. Oral contraceptive estrogen use is associated with a lower risk of endometrial cancer, a phenomenon which may persist for up to 10 years after use has ended. Cigarette smoking has also been inversely associated with postmenopausal endometrial cancer in some studies (57, 60).

Although ecologic studies support the role of diet in the etiology of endometrial cancer, very few analytic studies have investigated this relationship. Studies investigating the association between alcohol and endometrial cancer are described below.

Alcohol and Endometrial cancer (Table 5). Only three epidemiologic studies, all case-control, have examined the association between alcohol and endometrial cancer to date. Two (Williams 1977, Webster 1986) studies were suggestive of an inverse association whereas the third study showed an increased risk (62).

Data from the Third National Cancer Survey (47) showed a nonsignificant inverse association between alcohol and endometrial cancer when cases were compared to controls with other cancers. The authors noted that the comparison group excluded anyone with cancers of the lung, larynx, mouth, esophagus and bladder cancer in order to limit possible exposure bias which otherwise may lead to false, inverse associations. Breast cancer cases, however, were included in the comparison group. Therefore, the risk of endometrial cancer associated with alcohol consumption may be biased in this study because breast cancer is positively associated with alcohol consumption.

In a case-control study of postmenopausal Italian women, (62), the relationships between endometrial cancer and several nutritional factors, including alcohol, were examined using

206 endometrial cancer cases and 206 hospital controls. Controls in this study were ineligible if admitted for gynecologic, hormonal, or neoplastic disease or who had a hysterectomy. There was a positive, dose-response relationship with daily alcohol consumption after controlling for several potential confounding factors.

In a third case-control study of alcohol and endometrial cancer, (61), cases were identified (age 20-54) from six areas of the National Cancer Institutes Surveillance, Epidemiology and End Results (SEER) program in which histologic confirmation was possible. Controls, frequency-matched on age, were selected from the general population. Women who had undergone a hysterectomy, had a history of endometrial cancer or did not live in one of the six SEER areas were excluded from analysis; 437 cases and 2247 control were included. An inverse association with alcohol consumption was observed. That is, non-drinkers and women who consumed 1-49 grams of alcohol per week (1-5 drinks) had a significantly elevated risk of endometrial cancer when compared to women who consumed more than 150 grams per week (10-15 drinks). This association appeared to be modified by obesity and parity; women who abstained and were overweight or had low parity were at highest risk. The authors noted that an inverse association between alcohol and endometrial cancer may result from differential recall between cases and noncases, and that if noncases tended to underreport their consumption these results would actually underestimate an increased risk in nondrinkers.

Biologic Plausibility of an Association Between Alcohol and Cancers

The formation of tumors is thought to be a multistep process where the primary step is an initiating event that leads to an irreversible change in DNA. To date, no evidence has yet supported a hypothesis that ethanol, by itself, is an initiating agent (4). However, ethanol has been shown to act as a cocarcinogen when administered in combination (either before

or at the same time) with known chemical carcinogens; ethanol also acts as a tumor promotor in some tissues if administered after initiation (63). Alternatively, under certain conditions, ethanol may inhibit the carcinogenic process by stimulating the activity of detoxifying enzymes (64). Most studies exploring the etiologic role of alcohol on cancer have focused primarily on those organs most often associated with alcohol consumption including the oesophagus, oral cavity, liver, pancreas and rectum, although, a few studies have considered both the proximal and distal colon.

Seitz and Simanowski (64) and others (65) have proposed several possible mechanisms by which alcohol may be involved with carcinogenesis. These include: enhanced activation of procarcinogens; altered metabolism of carcinogens; interference with DNA-repair and the immune response; stimulation of cell regeneration; and potentiation of problems associated with nutritional deficiencies.

The primary hypothesis for a carcinogenic effect of alcohol on the large intestine is via effects on the secretion and metabolism of bile acids (66, 67). Moderate alcohol consumption may increase bile acid concentrations in the gut (68) and decrease transit time (69). Thus alcohol may influence the formation of secondary bile acids, such as deoxycholate, which have been implicated in the formation of colon cancer in some studies (70); however, not all studies have supported this hypothesis (71).

Acetaldehyde, the highly toxic metabolic product of alcohol, is present in higher concentrations in the distal colon compared to the proximal colon of rats fed alcohol chronically (72). Acetaldehyde has been shown to inhibit activity of the DNA-repair enzyme O^6-methylguanine transferase in the liver of both rats and humans (65). Whether this explains differential tumor growth in the distal colon compared to the proximal colon is

not yet established.

Alternative hypotheses have also been proposed to explain the association between alcohol and cancers of the large intestine. Chronic alcohol consumption enhanced microsomal enzyme activation of procarcinogens, such as polycyclic hydrocarbons, in the gut (73). These activated compounds may initiate the carcinogenic process by binding to DNA, RNA or proteins. Ingestion of some nutritional anticarcinogens may be reduced and absorption may be altered in the presence of higher alcohol consumption; heavy alcohol use has correlated inversely with folate intake (74), and serum levels of selenium (75), and vitamin E (76). Alcohol may lead to increase exposure to both endogenously and exogenously formed carcinogens due to abnormal gut permeability, particularly in alcoholics (77). Finally, in the rectum, alcohol may have an indirect effect on increase cell regeneration. Simanowski et al. (78) observed increased cell proliferation and an increase in proliferative compartment size in the rectal mucosa of rats fed ethanol compared to controls. Altered cell proliferation rates may be predictive of increased susceptibility to carcinogens (64).

It is suspected that, if alcohol is involved with the development of hormone-sensitive cancers, such as endometrial, it does so by altering the metabolism of androgens and estrogens. Human and animal studies have shown that elevated levels of circulating estrogens, particularly free estrogens, are positively correlated with the incidence of tumors (79). In an epidemiologic study comparing alcohol consumption between endometrial cancer cases and controls, Webster et al. (61) hypothesized and observed an inverse association. This hypothesis is supported by evidence showing that premenopausal women who consumed excessively high amounts of alcohol were more likely to have irregular mentrual cycles and significantly less serum estradiol, androstenedione and sex hormone binding protein than controls (80). In addition, Cauley et al. (81) reported that

both estrone and estradiol levels decreased with increasing, self-reported alcohol consumption in normal, postmenopausal women. However, experimental studies of acute alcohol administration to normal premenopausal females have generally found no difference in serum estrogen or androgen levels between experimental and control groups (82). In another study of endometrial cancer cases and controls, self-reported alcohol consumption was positively associated with an increased risk of endometrial cancer (62). These results may also be related to alterations in serum levels of sex hormone binding protein and steroid hormone metabolism in alcohol consumers compared to abstainers. In a study comparing noncirrhotic, premenopausal women with a history of alcoholism to normal controls, Valimaki et al. (83) observed increased serum concentrations of prolactin, androstenedione and dehydroepiandrosterone in the alcoholic women, although there was no difference in either serum estrone or estradiol consentrations.

In females who do not consume alcohol excessively, and chronically, the association between alcohol and hormonally associated cancers is unclear. It is important to consider the possibility that other predisposing characteristics such as a family history of cancer, history of noncontraceptive estrogen use or high body mass index may modify this association.

SUMMARY

Because as much as 50 percent of female cancers may be related to nutritional factors, particularly tumors of the large intestine and hormonally-related tumors (Wynder 1976), identifying modifiable dietary factors most strongly and consistently associated with cancer is necessary to facilitate cancer prevention. Insofar as results of the only three epidemiologic studies examining the association between alcohol and endometrial cancer are inconsistent, and because there is still very little consistency among the abundance of

alcohol-colorectal cancer studies, especially for women, further research is needed.

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Table 1. Age-adjusted cancer incidence for the four most common cancers according to sex, race.*

Number of new cases per 100,000 population

	MALES		
	White		African-American
1.	Prostate	101.9	Prostate 136.0
2.	Lung & Bronchus	80.6	Lung & Bronchus 119.0
3.	Colon & Rectum	58.8	Colon & Rectum 56.6
	Colon	40.6	Colon 41.5
	Rectum	18.2	Rectum 15.0
4.	Urinary Bladder	32.4	Oral Cavity & Pharynx 21.5
	FEMALES		
	White		African-American
1.	Breast	112.9	Breast 96.5
2.	Lung & Bronchus	41.0	Colon & Rectum 44.8
			Colon 35.7
			Rectum 9.1
3.	Colon & Rectum	39.6	Lung & Bronchus 41.3
	Colon	28.9	
	Rectum	10.7	
4.	Corpus Uteri	21.3	Cervix Uteri 15.4
			(6. Corpus Uteri 13.1)

* Data are based on the National Cancer Institutes SEER program (1988)

Table 2. Alcohol and colon cancer

Reference, Population	Alcohol Consumption	Observation		Comments
ECOLOGIC STUIDES[a]				
Breslow & Enstrom (1974)				
a.USA, 41 states	Per capita consumption	Males	Females	Age-Adjusted
Cancer mortality rates	Beer(liters/capita)	0.73**	0.69**	Adjusting for % urban
during 1950-67 compared	Spirits	0.60**	0.54**	spirits and cigarettess,
to per capita	Wine	0.45**	0.37**	sex-specific association
consumption				between beer and colon
				cancer were significant.
b.24 Countries				
Average annual incidence	Per capita consumption	Males		
of cancer for males	Beer (liter/capita)	0.58**		
compared to per capita	Spirits	-0.16		
consumption of alcohol	Wine	0.05		
Enstrom (1977)				
USA, 47 states	Per capita consumption	Males	Females	
Per capita beer	Beer	0.76***	0.73***	
consumption compared				
to cancer mortality.				
McMichael et al. (1979)				
4 Countries	% change in beer		% Change in Cancer Mortality	
Changes in colon cancer	consumption per capita		Males	Females
mortality from 1960-64 to	New Zeal.	+28	+47	+12
1970-74 compared to	Australia	+19	+19	+ 3
changes in per capita beer	Eng. & Wales	+1	+16	- 3
consumption from	U.S.	-10	+ 6	- 10
1950-52 to 1960-62.				
Potter et al. (1982)				
29 countries	Per capita consumption	Males	Females	
Colon cancer mortality	Total Alcohol	0.42*	0.27	
rates in 1970-74	Beer	0.66*	0.64*	
compared to annual per	Wine	- 0.13	- 0.23	
capita consumption				
during 1970-72				

COHORT STUDIES[b]			
Sundby (1967) Norway Chronic alcoholics (9 colon cancer deaths)	Chronic Alcoholics	Males 1.00	Expected number of cases computed from Oslo's mortality rates.
Hakulinen (1974) Finland Alcohol misusers (82 cases of colon cancer)	Alcohol Misusers	Males 0.95 (p>0.1)	Expected number of cases computed from the Finnish Cancer Registry.
Hakulinen (1974) Finland Chronic alcoholics (3 cases of colon cancer)	Chronic Alcoholics	Males 1.84 (p>0.05)	Expected number of cases computed from the Finnish Cancer Registry.
Monson & Lyon (1975) USA, Mass. 1382 chronic alcoholics institutionalized in 1930,35 40 followed and until 1971. (7 deaths)	Chronic Alcoholics	Males & Females 0.6 (0.3, 1.3)	Expected number of deaths computed from cancer mortality rates for US whites.
Adelstein & White (1976) United Kingdom 2070 alcoholics hospitalized in 1953-7 and 1964. (9 colon cancer deaths)	Chronic Alcoholics	Males Female 1.2 1.6	Expected number of deaths computed from the general population.
Robinette (1979) USA US Veterans Alcoholics (7 colon cancer deaths)	Chronic Alcoholics	Males 0.9 (0.3-1.9)	
Dean et al. (1979) Ireland, Dublin 1626 Male brewery workers who died between 1954 and 1973 (32 colon cancer deaths)	Beer (stout)	Males 1.3	Expected deaths from age-standardized death rates of Dublin skilled and unskilled manual workers

Jensen (1980)				
Denmark 14,313 brewery workers followed for 29 years for mortality (67 colon cancer deaths)	Beer (pilsner)	Males 1.0 (0.8, 1.4)		Expected number of cases computed from rates in the general population.
Schmidt & Popham (1981)				
Canada 9889 chronic alcoholics (19 colon cancer deaths)	Chronic Alcoholics	Males 1.04		Expected number of deaths computed from death rates in Ontario
COHORT STUIDIES[c]				
Gordon & Kannel (1984)				
USA, Mass 5209 Framingham Cohort followed for cancer mortality	Total Alcohol	Males NA†	Females NA	Adjusted for age, SBP, relative weight no. of cigarettes per day and lipoprotein.
Klatsky et al. (1988)				
USA, California 106,203 health plan members followed from 1978-1984	Total Alcohol Never <1 (drinks/day) 1-2 ≥3 p-trend =	Males 1.0 0.9 1.2 1.2 0.23	Females 1.1 1.3 1.8 2.6* 0.01	Adjusted for age, race, smoking, coffee, BMI cholesterol and education using CPH No relationship with specific alcohol beverages No relationship with specific colon sites.
Hirayama (1989)				
Japan 17-year follow-up of 265,118 Japanese adults to determine cancer deaths (Association are for sigmoid colon cancer)	Total Alcohol Abstainers Infrequent Occasional Daily p=trend	Males 1.0 2.03 3.83* 5.42* <0.001	Females 1.0 1.9* (any)	Adjusted for age No association with proximal colon (males) SIgmoid colon cancer & daily intake of beer (male) RR=12.6 of sake (male) RR=4.6 of shochu (male)RR=6

Stemmermann et al. (1990)				
USA, Hawaii	Total Alcohol	Males		Adjust for age at exam
7572 Japanese-Hawaiian	Abstainers	1.0		1 & cigarette smoking
men followed from	<5 oz/month	0.7		using CPH.
mid 1960's-1989	5-14	1.0		Not specific to a type
	15-39	1.2		of alcoholic beverage
	≥40	1.4		Not specific to any
		p-trend=0.16		colon subsite.

CASE-CONTROL STUDIES[d]

Wynder & Shigematsu (1967)				
USA, New York		Male	Females	Compared frequencies
288 colon cancer cases	Heavy Drinkers	NA	NA	
273 age-hospital matched	Beer	+*	NA	
controls	Wine	NA	NA	
	Liquor	NA	NA	
Wynder et al. (1969)				
Japan		Males	Females	Compared frequencies
Colon cancer cases	Total Alcohol	NA	NA	
Hospital controls				
Williams & Horm (1977)				
USA, Multicenter	Total Alcohol	Males	Females	Adjust for age, race,
653 colon cancer cases	Abstainer	1.0	1.0	cigarette smoking
4020 other cancer controls	<51 oz-year	1.4	1.2	Specific type of
	≥51	1.5*	1.4	alcohol drinkers were
	Beer ≥51 can-year	1.7*	1.3	compared to total
	Wine ≥51 glass-year	2.1*	1.5	alcohol abstainers
	Liquor ≥51 jigger-year	1.6*	1.2	
Graham et al. (1978)				
USA, New York		Males	Females	
256 colon cancer cases	Total Alcohol	NA	NA	
1222 hospital controls	Beer	NA		
	Wine	NA		
	Liquor	NA		
Tuyns et al. (1982)				
France, Calvados	Total Alcohol	Males & Females		Adjusted for sex & age
142 colon cancer cases	Abstainers	1.0		
1976 population controls	Drinker	1.4 (0.3, 5.7)		

Miller et al. (1983)				
Canada	Alcohol (no Beer)	Males	Females	Adjusted for age and
348 colon cancer cases	None	1.0	1.0	saturated fat using
Age-sex-hospital and	<47.7 (grams/day)	1.2	1.0	unconditional logistic
neighborhood frequency	≥47.7	1.4	1.0	regression
matched controls.	p-trend =	0.10	0.41	
	Beer			
	None	1.0	1.0	
	<143 (grams/day)	1.1	0.6	
	≥144	1.1	0.9	
	p-trend =	0.28	0.22	
Pickle et al. (1984)				
USA, Nebraska	Beer	Males & Females		Adjusted for gender,
58 colon cancer cases	commercial	2.7* (1.3, 5.5)		pipe & cigarette
176 age-sex-race matched	homemade	0.8 (0.2, 3.8)		smoking by logistic
176 hospital controls	Wine			regression
	commercial	1.2 (0.3, 2.3)		
	homemade	0.5 (0.2, 1.4)		
Potter & McMichael (1986)				
Australia, Adelaide	Total Alcohol	Males	Females	Relative risk estimated
220 incident colon cancer	0 (g/day)	1.0	1.0	from matched
cases	≤0.1	0.6	1.4	univaritae analysis
438 sex-age matched	0.2-4.0	0.4	1.2	
population based controls	4.1-12.8	0.8	2.0	
	12.9-31.8	1.0	2.0	
	Beer (glass/week)	1.0	1.01	
	Wine "	1.02	1.04	
	Spirits "	1.08*	1.13*	
Kune et al. (1987)				
Australia, Melbourne	Total Alcohol	Males	Females	Adjusted for diet
715 rectal or colon cancer	1 (quartile)	1.0	1.0	using unconditional
cases	2	1.4	1.1	logistic regression
727 age-sex-community	3	1.0	1.2	
matched controls	4	1.0	1.4	
	Beer	NA	NA	
	Wine	NA	NA	
	Spirits	NA	NA	
Tuyns et al. (1988				
Belgium	Beer	Males & Females		Adjusted for age, sex
453 colon cancer	1 (tertile)	1.0		& province
2851 population controls	2	1.1		
	3	1.4*		
	p-trend=	0.02		

Ferraroni (1989)				
Italy, Milan	Total Alcohol		Males & Females	Adjusted for age, sex,
455 colon cancer cases	<3 drinks/day		1.0	social class, education,
to 1944 hospital controls	3-6		1.1	marital status,
	>6		1.2	smoking & coffee
		p-trend =	0.7	using unconditional
	Beer		NA	logistic regression
	Wine		NA	
	Spirits		NA	
Peters et al. (1989)				
USA, California	Total Alcohol		Males	Adjusted for age and
147 male colon and rectal	0-9 grams/day		1.0	education using
cancer cases compared to	10-39		1.0	unconditional logistic
147 race-sex-age-	40-69		0.8	regression
neighborhood matched	70+		1.6	
controls	Beer		NA	
	Wine		NA	
	Liquor		NA	
Longnecker (1990)				
USA, New england	Total Alcohol		Males	Adjusted for age,
367 right colon cancers	0 drinks/day		1.0	income & smoking
Community controls	0.5		0.9	by logistic regression
	1		1.0	Results are for alcohol
	2		1.0	intake five-years ago
	3-4		1.7*	NA with total alcohol
	≥5		1.8	intake 20-years ago
		p-trend =	0.007	Beer intake 20-years
	Beer		+*	ago was significantly
	Wine		NA	associated.
	Spirits		NA	
Hu et al. (1991)				
China, Harbin City	Total Alcohol		Males&Females	Adjusted for diet using
111 incident colon cancers	Low		1.0	logistic regression
Age-sex-residential area	High		2.0*	Low and high alcohol
hospital matched controls				intake are <10 & ≥10
				kg/year respectively

Choi & Kahyo (1991)			
Korea	Total Alcohol	Males	Adjusted for age,
63 colon cancer cases	Nondrinker	1.0	marital status, diet
189 age-admission	Light	0.6	education & smoking
date matched controls	Moderate	1.1	status
	Medium-Heavy	1.0	
	Heavy	0.8	

a. Ecologic Studies present correlation coefficients, except the study by McMichael et al. presents percent change in mortality and percent change in alcohol consumption
b. Retrospective cohort studies present standardized mortality ratios
c. Prospective cohort studies present relative risks
d. Case-control studies present odds ratios
* estimates are significantly different from reference group ($p<0.05$)
** estimates are statististically significant ($p<0.01$)
*** estimates are statististically significant ($p<0.001$)
† NA, No association

Table 3. Alcohol and rectal cancer

Reference, Population	Alcohol Consumption	Observation		Comments
ECOLOGIC STUIDES[a]				
Breslow & Enstrom (1974)				
a.USA, 41 states	Per capita consumption	Males	Females	Age-Adjusted
Cancer mortality rates	Beer (liters/capita)	0.78**	0.71**	Adjusting for % urban,
during 1950-67 compared	Spirits	0.54**	0.53**	spirits and cigarettes,
to per capita consumption	Wine	0.52**	0.53**	the sex-specific association between beer and rectal cancer were significant.
b.24 Countries				
Average annual incidence	Per capita consumption	Males		
of cancer compared to	Beer (liter/capita)	0.83**		
per capita consumption	Spirits	-0.16		
of alcohol	Wine	0.04		
Enstrom (1977)				
USA, 47 states	Per capita consumption	Males	Females	
Per capita beer	Beer	0.81***	0.75***	
consumption compared to cancer mortality.				
McMichael et al. (1979)				
4 Countries	% change in beer		% Change in Cancer Mortality	
Changes in Rectal cancer	consumption per capita		Males	Females
mortality from 1960-64	New Zeal.	+28	+40	+ 2
to 1970-74 compared to	Australia	+19	+15	+19
changes in per capita beer	Eng. & Wales	+1	+ 4	- 10
consumption from	U.S.	-10	+26	- 37
1950-52 to 1960-62				
Potter et al. (1982)				
29 countries	Per capita consumption	Males	Females	
Rectal cancer mortality	Total Alcohol	0.47*	0.39*	
rates in 1970-74	Beer	0.77*	0.75*	
compared to annual per	Wine	- 0.17	- 0.25	
capita consumption during 1970-72				

COHORT STUDIES[b]			
Sundby (1967) Norway Chronic alcoholics (12 rectal cancer deaths)	Chronic Alcoholics	Males 1.9 2.9	 Expected=Oslo Expected=Norway
Monson & Lyon (1975) USA, Mass. 1382 chronic alcoholics institutuionalized in 1930,35 or 40 followed until 1971. (4 rectal cancer deaths)	Chronic Alcoholics	Males & Females 0.7	Expected deaths computed from cancer mortality rates for US whites
Adelstein & White (1976) United Kingdom 2070 alcoholics hospitalized in 1953-7 and 1964. (4 rectal cancer deaths)	Chronic Alcoholics	Males Females 1.2 NA	Expected deaths computed from the general population.
Robinette et al. (1979) USA, US Veterans Alcoholics (6 rectal cancer deaths)	Chronic Alcoholics	Males 3.3 (0.7- 22.4)	
Dean et al. (1979) Ireland, Dublin 1626 Male brewery workers who died between 1954 and 1973 (32 rectal cancer deaths)	Beer (stout)	Males 1.6* (1.1, 2.3)	Expected deaths from age-standardized death rates of Dublin skilled and unskilled manual workers
Jensen (1979) Denmark 14,313 brewery workers followed for 29 years for cancer morbidity (72 rectal cancer cases)	Beer (pilsner)	Males 1.0 (0.8, 1.3)	Expected number of cases computed from the general population.
Schmidt & Popham (1981) Canada 9889 chronic alcoholics (10 rectal cancer deaths)	Chronic Alcoholics	Males 1.02	Expected number of deaths computed from Ontario's death rates

COHORT STUIDIES[c]

Klatsky et al. (1988)			
USA, California	Total Alcohol	Males&Females	Adjusted for age, race,
106,203 health	Never	1.0	smoking, coffee,
plan members	<1 (drinks/day)	1.4	BMI, cholesterol &
followed from	1-2	2.3	education using CPH
1978-1984	≥3	3.2*	No relationship with
	p-trend = 0.03		specific alcohol beverages
			Gender-specific associations were not significant
Hirayama (1989)			
Japan	Total Alcohol	Males	Age-adjusted
17-year follow-up of	Abstainers	1.0	
265,118 Japanese adults	Infrequent	0.95	
	Occasional	1.14	
	Daily	1.39	
	p=trend<0.05		
Stemmermann et al. (1990)			
USA, Hawaii	Total Alcohol	Males	Adjusted for age at
7572 Japanese-Hawaiian	Abstainers	1.0	exam 1 and smoking
men followed from	<5 oz/month	0.9	using CPH.
mid 1960's-1989	5-14	1.7	Pollack et al (1984),
	15-39	1.5	reported an association
	≥40	1.9*	between rectal cancer
	p-trend=0.01		and consumption of 15 liters of beer per month RR=3.05

CASE-CONTROL STUDIES[d]

Wynder & Shigematsu (1967)				
USA, New York		Males	Females	Compared frequencies
204 rectal cancer cases	Heavy Drinkers	+*	NA	
273 age-hospital matched	Beer	+*	NA	
controls	Wine	NA	NA	
	Liquor	NA	NA	
Wynder et al. (1969)				
Japan		Males	Females	Compared frequencies
Rectal cancer cases	Total Alcohol	NA	NA	
Hospital controls				

Williams & Horm (1977)				
USA, Multicenter	Total Alcohol	Males	Females	Adjusted for age, race,
303 rectal cancer cases	Abstainer	1.0	1.0	cigarette smoking
4020 other cancer controls	<51 oz-year	0.8	0.8	Specific type of
	≥51	0.7	2.0*	alcohol drinkers were
	Beer ≥51 can-year	0.8	2.1	compared to abstainer
	Wine ≥51 glass-year	0.5	0.7	
	Liquor ≥51 jigger-year	0.9	1.5	
Graham et al. (1978)				
USA, New York		Males	Females	
330 rectal cancer cases	Total Alcohol	NA	NA	
1222 hospital controls	Beer	NA		
	Wine	NA		
	Liquor	NA		
Tuyns et al. (1982)				
France, Calvados	Total Alcohol	Males & Females		Adjusted for sex & age
198 rectal cancer cases	Abstainers	1.0		
1976 population controls	Drinker	1.6 (0.5, 5.5)		
Miller et al. (1983)				
Canada	Alcohol (no Beer)	Males	Females	Adjusted for age &
194 rectal cancer cases	None	1.0	1.0	saturated fat using
Age-sex-hospital and	<47.7 (g/day)	0.5*	1.3	unconditional logistic
neighborhood frequency	≥47.7	1.3	0.8	regression
matched controls.	p-trend =	0.43	0.34	
	Beer			
	None	1.0	1.0	
	<143 (g/day)	1.1	0.6	
	≥144	1.1	0.9	
	p-trend =	0.23	0.09	
Manousos et al. (1983)				
Greece		Males & Females		
35 rectal cancer cases	Total Alcohol	NA		Adjusted for age, sex,
age-sex matched hospital	Beer	NA		meat and vegetable
controls	Wine	NA		using logistic
	Hard Liquor	NA		regression
	Ouzo	NA		

Pickle et al. (1984)				
USA, Nebraska	Beer	Males & Females		Adjusted for gender,
28 rectal cancer cases	commercial	1.4 (0.5, 3.7)		pipe & cigarette
176 age-sex-rece	homemade	1.2 (0.2, 5.6)		smoking by logistic
matched hospital	Wine			regression
controls	commercial	0.9 (0.3, 2.3)		
	homemade	1.4 (0.5, 3.9)		
Kabat et al. (1984)				
USA, Nebraska	Beer	Males	Females	Adjusted for education
218 rectal cancers	Never	1.0	1.0	and religion by
585 age-sex-admission	<1 (oz/day)	1.6	0.5	conditional logistic
date matched controls	1-7.9	1.3	0.5	regression
	8-31.9	1.8	0.7	
	≥32	3.5*	-	
	p-trend<0.05		-	
	Wine	NA	NA	
	Spirits	NA	NA	
Potter & McMichael (1986)				
Australia, Adelaide	Total Alcohol	Males	Females	Odds ratios computed
199 rectal cancer cases	0 (g/day)	1.0	1.0	using matched
396 age-sex population	≤0.1	0.7	0.6	analysis (age, sex)
based controls	0.2-4.0	0.8	1.7	
	4.1-12.8	0.6	1.1	
	12.9-31.8	1.7	1.5	
	Beer (glass/week)	1.0	0.97	
	Wine "	0.98	1.11*	
	Spirits "	1.04*	1.05	
Kune et al. (1987)				
Australia, Melbourne	Total Alcohol	Males	Females	Adjusted for diet
715 large bowel cancer	1 (quartile)	1.0	1.0	using unconditional
cases	2	1.5	1.3	logistic regression
396 age-sex-community	3	1.1	1.5	
matched controls	4	1.5	0.9	
	Beer	+*	+	
	Wine	NA	NA	
	Spirits	-**	NA	
Tuyns et al. (1988)				
Belgium	Beer	Males & Females		Adjusted for age, sex &
368 rectal cancer cases	1 (tertile)	1.0		province
2851 population controls	2	1.0		
	3	1.2		
	p-trend=0.5			

Ferraroni (1989)				
Italy, Milan	Total Alcohol	Males & Females		Adjusted for age, sex,
295 rectal cancer cases	<3 drinks/day	1.0		SES, education,
1944 hospital controls	3-6	0.8		marital status,
	>6	0.9		smoking status &
	p-trend =0.5			coffee intake using
				unconditional logistic
				regression
Peters et al. (1989)				
USA, California	Total Alcohol	Males		Adjusted for age,
41 rectal cancer cases	0-9 g/day	1.0		and education using
147 race-sex-age-neighborhood	10-39	1.2		unconditional logistic
matched controls.	40-69	0.6		regression
	70+	1.4		
	Beer	NA		
	Wine	NA		
	Liquor	NA		
Longnecker (1990)				
USA, New england	Total Alcohol	Males		Adjusted for age income
251 rectal cancer cases	0 drinks/day	1.0		& smoking using
Community controls	0.5	1.1		logistic regression
	1	0.9		Results are for
	2	1.2		alcohol intake 5 years
	3-4	1.7*		ago but significant
	≥5	1.5		also for intake 20
	p-trend =0.007			years ago
	Beer	+*		
	Wine	NA		
	Liquor.	NA		
Freudenheim (1990, 1991)				
USA, New York	Totol Alcohol	Males	Females	RR computed using
277 male and 145 female	1 (quartile M)/(tertile F)	1.0	1.0	regression
rectal cancer cases	2	1.1	0.9	Significant associations
Age-sex-neighborhood	3	1.0	1.9	for male rectal cancer
matched controls	4	1.8*		after adjusting for
	p-trend=0.06		<0.05	and other nutrients
Hu et al. (1991)				
China, Harbin City	Total Alcohol	Males	Females	Adjusted for diet using
225 incident rectal cancers	Low	1.0	1.0	logistic regression
Age-sex-residential area	High	2.1*	NA	Low and high alcohol
hospital matched controls				intake are <6 and ≥6
				kg/year respectively

Choi and Kahyo (1991)			
Korea	Total Alcohol	Males	Adjusted for age,
133 rectal cancer cases	Nondrinker	1.0	marital status, diet
399 age-hospital admission	Light	2.2*	education & cigarette
date matched controls	Moderate	2.0	smoking
	Medium-Heavy	2.5*	
	Heavy	4.8*	

a. Ecologic Studies present correlation coefficients, except the study by McMichael et al. presents percent change in mortality and percent change in alcohol consumption
b. Retrospective cohort studies present standardized mortality ratios
c. Prospective cohort studies present relative risks
d. Case-control studies present odds ratios
* estimates are significantly different from reference group ($p<0.05$)
** estimates are statististically significant ($p<0.01$)
*** estimates are statististically significant ($p<0.001$)
† NA, No association

Table 4. Alcohol and colorectal cancer

Reference, Population	Alcohol Consumption	Relative Risk		Comments
COHORT STUDIES[a]				
Garland (1985)				
USA,		Males		
2107 men followed for 19 years	Total Alcohol	NA†		
Kono et al. (1986)				
Japan	Total Alcohol	Males		Adjusted for age
Cohort of 5135 male	Abstainer	1.0		and smoking
Japanese physicians	Ex-drinker	1.2 (0.4, 4.0)		using CPH
followed from 1965-83	Occasional Drinker	1.3 (0.5, 3.2)		
for cause specific mortality	<2 go/day	1.1 (0.4, 3.0)		
	≥2 go/day	1.4 (0.5, 4.0)		
Wu et al. (1987)				
USA, California	Total Alcohol	Males	Females	Age-adjusted
11,888 residents of	Non-daily	1.0	1.0	In males results
a retirement community	1-29 (ml/day)	2.2*	1.1	are not site-
followed from 1981-1985	≥30	2.4*	1.5	specific.
		p-trend =0.004	0.23	In females results are for left colon
CASE-CONTROL STUDIES[b]				
Stocks (1957)				
United Kingdom	Beer	Males		
166 male colorectal cases	< daily	1.0		
4630 controls	≥ daily	1.4 (0.9-2.1)		
Higginson (1966)				
USA, Kansas City		Males & Females		No Association
340 colorectal cancers		NA		with type of
1020 hospital controls.				alcohol preferred
Manousos et al. (1983)				
Greece		Males & Females		
100 incident large-	Total Alcohol	NA		Adjusted for age,
bowel cancer cases	Beer	NA		sex, meat and
age-sex matched	Wine	NA		vegetable intake
hospital controls	Hard Liquor	NA		using logistic
	Ouzo	NA		regression

Stattery et al. (1990)	Total Alcohol	Males	Females	Unadjusted
USA, Utah	0 grams/week	1.0	1.0	No association in
231 colon cancer cases	1-15	1.9*	1.1	males after
391 population based	>15	1.8*	0.6	adjusting for age,
controls		p-trend=0.03	0.6	pipe use, bmi, caffeine, religion crude fiber and calories (females not analyzed)
				Not site specific
				No interaction with pipe use or caffeine

a. Prospective Cohort studies present relative risks
b. Case-control studies present odds ratios
†NA, No association

Table 5. Alcohol and endometrial cancer

Reference, Population	Alcohol Consumption	Odds Ratios Endometrial Cancer	Comments
CASE-CONTROL STUDIES			
Williams & Horm (1977)	Total Alcohol		
USA, Multicenter	Abstainer	1.0	Adjusted for age,
345 endometrial cancer	<51 oz-year	0.7	race & smoking
cases	≥51 oz-year	0.6	
Other cancer controls			
	Beer ≥51 can-year	0.3	
	Wine ≥51 glass-year	0.5	
	Spirits ≥51 jigger-year	0.8	
LaVecchia et al. (1986)			
Italy, Milan	Total Alcohol		
206 Incident cases of	0 drinks/day	1.0	Adjusted for BMI
endometrial cancer	<2	1.6 (0.8, 3.2)	interviewer, parity
206 hospital matched	2-2.9	1.6 (0.8, 3.2)	history of diabetes
controls.	3-3.9	3.4 (1.0, 11.5)	or hypertension,
	≥4	4.3 (1.0, 18.4)	age at menarche &
	p-trend=-0.02		menopause and use
			of female hormones
Webster et al. (1989)			
USA	Total Alcohol		
351 endometrial cancer	non-drinkers	1.8 (1.1, 3.0)	Adjusted for age, race
cases	1-49	1.6 (1.0, 2.5)	history of OC use &
2247 population controls	50-149	1.1 (0.7, 1.8)	history of smoking.
	≥150 g/week	1.0	These findings did
			not vary by type of
			alcoholic beverage
			Association modified
			by BMI and Parity

CHAPTER II

ALCOHOL CONSUMPTION AND COLON AND RECTAL CANCER INCIDENCE IN POSTMENOPAUSAL WOMEN: THE IOWA WOMEN'S HEALTH STUDY

INTRODUCTION

Colon and rectal cancers are among the most common cancers in the United States, and throughout the world (1). However, there is at least a twenty-fold variation internationally in the occurrence of these cancers. A great deal of consideration and epidemiologic research has focused on determining the role of dietary differences among countries on this variation (2, 3). Alcohol is one component of the diet correlating positively with colon and rectal cancer incidence and mortality in both geographic (4, 5) and time-trend studies (6, 7).

Cohort and case-control studies have shown inconsistent associations between alcohol consumption and colon/rectal cancer. A significantly elevated risk (RR=1.6) of rectal cancer was observed in only one (8) of nine studies (9, 10, 11, 12, 13, 14, 15) comparing morbidity or mortality rates of colon or rectal cancer in high risk populations (chronic alcoholics or people employed in the beer industry) to those in the general population. Four (16, 17, 18, 19) of at least seven prospective cohort studies (20, 21, 22) and 12 (23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34) of at least 21 (35, 36, 37, 38, 39, 40, 41, 42, 43) case-control studies conducted to date exploring the association between alcohol and colon and/or rectal cancer have observed a positive association. Perhaps the most recognized finding is an elevated risk of rectal cancer with beer consumption (8, 23, 30, 31); however, every type of alcoholic beverages has been associated with an increased risk of these cancers in at least one study.

Both colon and rectal cancers occur more often in females than males until age 55, after which the incidence in males exceeds that in females (44). Female gut physiology differs significantly from male gut physiology (45); females have slower transit times, smaller fecal bulk, and lower output of bile acids. These gender differences suggest there may be

possible hormonal associations with the risk of colon cancer (46). In addition, ethanol appears to be metabolized more rapidly by females than males (47). Further studies of the association between alcohol and colon and rectal cancer in women are therefore justified.

The purpose of this study was to examine the association between alcohol consumption and the incidence of colon cancer and rectal cancer in a cohort of postmenopausal women. Special attention was given to the possible modifying effects of other potential risk factors on this association, including factors that may play a role in altering hormone levels in females. Associations between colon cancer and specific types of alcoholic beverages were explored separately, as were associations between total alcohol consumption and proximal and distal colon cancers.

METHODS

The Iowa Women's Health Study cohort

The IWHS is a longitudinal cohort study designed to examine the association between several personal, dietary, and lifestyle factors and the incidence of cancer in postmenopausal women, aged 55-69 at baseline. Study participants were selected randomly from Iowa's 1985 Department of Transportation's drivers license list, (which contained approximately 94 percent of all age-eligible women living in Iowa (48)). In January 1986, 99,826 selected women were mailed a questionnaire and a letter describing the purpose of the study. From the original sample, 1,796 were ineligible for participation (wrong age or gender); 41,837 eligible questionnaires were returned (a response rate of 42%). Respondents have been compared to nonrespondents based upon the available drivers license information (49). In general, respondent were slightly younger, had a

somewhat lower body mass index, and resided in more rural counties than nonrespondents.

The population at-risk of developing either colon or rectal cancer (n=38,006) excluded women who, at baseline, reported a history of malignancy other than skin cancer.

Measurement of alcohol intake and other risk factors

Information on the major cancer risk factors was ascertained by a 16-page questionnaire Self-reported items included birth date, race, education, reproductive and menstrual history, use of oral contraceptives and noncontraceptive estrogens, history of cancer in a female relative, and personal history of cancer. Participants also were asked to report their current height and weight, as well as weight at specified ages. To assess body fat distribution, a paper tape measure was enclosed along with detailed instructions for circumference measurements of the waist, hips, upper arm and lower leg. The validity and reliability of these data have reported elsewhere (50). Briefly, validity was assessed by comparing technician-measurements to self-measurements. Reliability was assessed by comparing self-measurements taken independently, six weeks apart. Intraclass correlation coefficients were greater than 0.85 for both validity and reliability.

Usual dietary and alcohol consumption were assessed using the Harvard semi-quantitative food frequency questionnaire developed by Willett et al. (51). Participants recorded their average daily intake over the last year according to one of nine possible frequency responses: never or less than one drink per month, 1-3 per month, 1 per week, 2-4 per week, 5-6 per week, 1 per day, 2-3 per day, 4-5 per day and 6+ per day. Frequencies were recorded separately for red wine, white wine, beer, and liquor. Intake, in grams per day, was computed with the aid of the Harvard Nutrient Data Base: the frequency with

which each beverage was consumed was multiplied by the ethanol content of the specific beverage, (10.8 grams of ethanol per 4 ounce glass of red or white wine, 13.2 grams per bottle or can of beer, and 15.1 grams per drink or shot of liquor). Average daily alcohol intake was computed by summing the contribution from each type of alcoholic beverage.

Willett et al. (52) and Giovannucci et al. (53) have reported that the semi-quantitative food frequency questionnaire is highly valid and reproducible for assessing average daily alcohol consumption. The accuracy and reproducibility of the questionnaire in the IWHS cohort has been reported elsewhere (54). Briefly, Pearson correlation coefficients of alcohol intake from the IWHS baseline food frequency questionnaire with second and third questionnaires were 0.99 and 0.98, respectively. The correlation between average daily alcohol intake measured by the third questionnaire and the average of five 24-hour recalls in 44 subjects was 0.32; this correlation is low possibly because five 24- hour periods is too few to characterize usual intake. Nevertheless, these data suggest that the food-frequency questionnaire is highly reliable.

Abstainers were defined as women whose reported usual daily alcohol intake was 0 grams. Of the baseline questionnaires returned, 3.8 percent of the women left intake information blank for all four alcohol beverages. For purposes of analysis, usual daily alcohol consumption for these women was considered 0 grams per day. Analyses were repeated in which alcohol intake for them was considered missing; the results were comparable.

Follow-up of cohort and identification of colon and rectal cancer cases

The cohort has been followed for 5 years to determine incident cancer cases. Two follow-up mail surveys for vital status and address-change were conducted in October, 1987 and August, 1989. The status of non-respondents to the follow-up surveys was determined by

the National Change of Address service to identify women who had moved out of Iowa, and by the National Death Index to identify out-of-state deaths through 1989.

Incident colon and rectal cancer cases were identified using the Health Registry of Iowa, part of the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program; identification involved matching cases from 1986-1990 with IWHS participants using a combination of first and last names, maiden name, zip code, birthdate, and Social Security number.

The risk of colon cancer was evaluated by specific sites in the large bowel: proximal colon cancers included those of the hepatic flexure, cecum and ascending colon (ICD-O codes 153.0, 153.4, and 153.6); distal colon cancers included those of the transverse, descending, and sigmoid colon and splenic flexure (ICD-0 codes 153.1, 153.2, 153.3 and 153.7). Total colon cancers cases included those of the proximal and distal as well as those not otherwise specified (ICD-0 code 153.9). Rectal cancer cases included cancers of the rectosigmoid junction and rectum (ICD-O codes 154.0 and 154.1). After five-years of follow-up, 237 colon cancer cases and 75 rectal cancer cases were identified from the at-risk cohort.

Statistical analysis

Person-years of follow-up for each individual was computed as the amount of time since completion of the baseline questionnaire to one of the following events: 1) colon, rectal cancer diagnosis, 2) death (if in Iowa), 3) a move out of Iowa (if known), 4) midpoint of interval between date of last contact and either date of next follow-up or December 31, 1990 (if date of move was unknown), or 5) midpoint of interval between date of last

contact and date of death (for non-Iowa deaths). For women without any of these events, follow-up was until December 31, 1990.

Women were classified *a priori* according to three levels of alcohol intake: 0 grams per day (abstainers), and two others based on a median split of drinkers (< 4 and ≥ 4 grams of ethanol per day). Cutpoints for quantiles of other risk factors were determined from the distribution of the total at-risk cohorts.

Incidence rates were calculated by dividing the number of events by the person-years of follow-up. Relative risks and their 95 percent confidence intervals (55), were computed within categories of potential risk factors with adjustment for five-year age categories: 55-59, 60-64, and 65-69. Tests for linear trend based on Mantel's extension were computed to examine dose-response (56).

Consideration was given to the following variables as potential confounders: education (as a measure of socioeconomic status), body mass index (kg/m^2), waist-to-hip ratio, age at menarche, age at menopause, parity, age at first birth, oral contraceptive use, noncontraceptive use, a personal history of colon/rectal polyps, physical activity, and cigarette smoking. Possible confounding was examined by comparing the proportion of women within categories of alcohol consumption (0, <4 and ≥4 grams of ethanol per day) across strata of other colon or rectal cancer risk factors. Pearson chi-square estimates were examined as a measure of the general association between two factors in an R x C table.

Multivariate analysis was performed using Cox proportional hazards regression to analyze the association between each cancer and alcohol consumption while simultaneously controlling for age, and other potential confounding factors. Models containing only age

and indicator variables for alcohol consumption were compared to models including each of the potential confounders; if the regression coefficient changed by more than 10 percent in the larger model, confounding was considered to be present. Cox proportional hazards regression also was used to test for multiplicative effect modification by comparing the -2 log likelihood chi-square estimates between a model containing the main effects and a model containing two-way interaction terms for alcohol and each risk factor separately. Analysis was performed using PROC PHREG of the SAS statistical package (57). The proportional hazards assumptions was tested and confirmed.

The associations between colon and rectal cancer and specific types of alcoholic beverage were examined using Cox proportional hazards regression. A single model was tested, incorporating indicator variables (one set for each type of alcoholic beverage: wine beer, liquor) to represent categories of consumption.

RESULTS

Among postmenopausal women in this cohort, 63 percent of the colon cancer cases, 55 percent of the rectal cancer cases and 56 percent of the noncases were abstainers. There were no statistically significant differences in the average grams of alcohol consumed per day between colon or rectal cancer cases and noncases (Table 1).

Table 2 shows Mantel-Haenszel age-adjusted relative risks of colon and rectal cancer associated with usual consumption of <4 and ≥ 4 g of alcohol per day compared to abstainers. These data were suggestive of an inverse association with colon cancer (p for trend=0.08) although the confidence intervals included 1.0. Usual alcohol intake

was not associated with rectal cancer.

To determine whether higher levels of alcohol consumption were significantly associated with colon or rectal cancer, drinkers were further stratified into 4 categories: <1.5, 1.5-5.0, 5.0-14.9, and ≥ 15 g per day. The Mantel-Haenzsel age-adjusted relative risks of colon cancer associated with each level of alcohol intake were 0.79, 0.76, 0.80, 0.75, respectively (p for trend= 0.09); all of the confidence intervals included 1.0. Compared to abstainers, the relative risks of rectal cancer for each level of alcohol consumption were 1.05, 1.24, 0.95, 1.49, respectively (p for trend=0.48). In the rectal cancer analysis, there were no more than 13 cases at any level, thus the confidence intervals were wide, and again, none of these estimates were significantly different from 1.0.

Mantel-Haenzsel age-adjusted relative risks of colon and rectal cancer associated with potential confounders are presented in Table 3. Colon cancer was positively associated with increasing body mass index and inversely associated with a late age at menopause. The risk of rectal cancer for women whose age at menarche was ≥ 14 was significantly decreased compared to those who were ≤ 11 years old. Women who reported a personal history of rectal or colon polyps were almost three times more likely to develop rectal cancer than women with no history of polyps. Age at first live birth, physical activity and cigarette smoking were not associated with colon or rectal cancer in this cohort.

To further evaluate possible confounding, Pearson chi-square tests suggest alcohol was significantly associated with each of the suspected confounders, with the exception of a history of colon or rectal polyps (Table 4).

In multivariate analysis, using Cox proportional hazards regression, there was no

confounding by any of the other suspected risk factors (data not shown). Therefore, consideration was given to the possible modifying effects of these risk factors on the association between alcohol and colon or rectal cancer. There was no multiplicative interaction between age and alcohol on the risk of either colon or rectal cancer (data not shown) or between alcohol and any of the other potential risk factors for colon cancer (Table 5).

For rectal cancer, women who consumed alcohol and had a history of colon/rectal polyps appeared to be at higher risk (Table 6) than abstainers with polyps or drinkers with no polyps; on the multiplicative scale, this interaction was not statistically significant although these data may provide evidence of an interaction on the additive scale. The only statistically significant multiplicative interaction was between alcohol and physical activity. In univariate analysis (Table 2), it was shown that vigorous physical activity conferred a greater risk of rectal cancer (not statistically significant). Using Cox proportional hazards regression, this increased risk with increased alcohol consumption may be confined to women who were vigorously active.

Finally, analyses exploring the association between alcohol and specific colon subsites (Table 7) showed a significant, inverse dose-response between usual alcohol intake and distal colon cancer incidence; however, there was no association with the incidence of proximal colon cancer. This inverse association with distal colon cancer was specific to wine consumption (Table 8). There was no evidence proximal colon cancer was associated with intake of any specific type of alcoholic beverage. There were nonsignificant increased risks of rectal cancer associated with beer and with liquor consumption compared to abstainers.

DISCUSSION

In this study of postmenopausal women, there appeared to be a weak, inverse association between usual alcohol consumption and the incidence of colon cancer, limited to the distal colon and most significant for wine consumption. In addition, there appeared to be a statistically significant multiplicative interaction between physical activity and alcohol intake on risk of rectal cancer, such that there was a positive association between alcohol intake and rectal cancer only among women who were vigorously active.

An inverse association between alcohol consumption and distal colon cancer is not consistant with results of other epidemiologic studies. Previous cohort studies of alcohol and colon cancer in women have found either null (20) or positive (17, 18) associations. Klatsky et al. showed a significant dose-response relationship between alcohol and colon cancer. The relative risk for women who reported $\geq$ 3 drinks per day compared to abstainers was 2.6 (17); there were no differences between specific colon subsites. Among female Japanese, there was a 90 percent elevated risk of sigmoid colon cancer associated with any alcohol consumption (18). The conflicting results between this and previous studies may reflect a difference in study populations. For example, females of all ages have been included in previous reports. The incidence of distal colon cancer is increasingly higher in men than women with increasing age, whereas, for proximal colon cancer, the incidence remains 10-20 percent higher in females compared to males throughout life (58). The association between alcohol and colon cancer in females may depend not only on colon subsite but also on age, and more specifically menopausal status.

In the only cohort study of alcohol and rectal cancer where an association in females was

explored, no gender-differences were observed; however, a significant positive dose-response between alcohol and rectal cancer was reported for males and females combined (RR=3.17, 95 percent CI 1.05 - 9.57) (17). In the present IWHS cohort study, there was a no association between alcohol and rectal cancer.

Potential effect modification was explored for several personal characteristics. The only remarkable interaction was increased rectal cancer among the most active women who drank ≥ 4 grams of ethanol per day compared to abstainers. Although this interaction was statistically significant, cautious interpretation is necessary given the number of interactions tested. Nevertheless, these results raise interesting questions regarding the effects of alcohol on gut motility.

Laboratory data indicate that alcohol by itself is not carcinogenic (59). Nevertheless, several mechanisms have been proposed for alcohol-associated colorectal carcinogenesis. Alcohol may be involved through the induction of microsomal cytochrome p-450 enzymes leading to enhanced procarcinogen activation. Alcohol also may alter carcinogen metabolism, enhance dietary deficiencies, increase cellular regeneration, interfere with DNA-repair and the immune response (60, 61). Although these mechanisms could explain a positive association between alcohol and colon or rectal cancer, they do not offer biologic support to the decreased risk of distal colon cancer observed in this study. Any explanation of this finding would be purely speculative.

It is known that alcohol is absorbed before it reaches the large intestine (62); therefore, any hypothesis would involve an indirect mechanism. Serum concentrations of hormones and growth factors, both involved in cell replication, may be affected by alcohol consumption. First, usual alcohol consumption has been inversely associated with serum concentrations

of female sex-steroid hormones among normal postmenopausal women (63). Second, recently, it has been hypothesized that fluctuations in the homeostasis of growth factors, caused by environmental factors such as alcohol consumption, may contribute to colonic carcinogenesis (64). Alcohol has been shown to inhibit the production and secretion of insulin-like growth factors from hepatocytes (65). Thus, it is possible that one long-term effect of chronic, low-level alcohol consumption would be to down-regulate the expression of growth factor receptors, thus inhibiting cell replication. However, different patterns of alcohol consumption such as binge-drinking, may have different effects on the role of growth factors in carcinogenesis. Whether these observation are relevant to the results of this study are uncertain.

Consideration must be given to potential limitations and sources of bias in this study. First, alcohol consumption was assessed by self-report using a food-frequency questionnaire. Although this has been shown to be a "valid" and reliable method for measuring alcohol intake, its limitation is that heavy drinkers may underreport consumption. With a limited range of exposure among drinkers and a large percent of nondrinkers in this cohort, the possibility of detecting a significant dose-response is reduced. Second, almost nothing is known about the duration of alcohol use on risk of either colon or rectal cancer. In this cohort study, women were asked to report their usual intake over the last year; changes in alcohol consumption throughout life could lead to considerable misclassification. Specifically, women classified as abstainers in this study may have been heavy drinkers at some point in the past and quit drinking due to gastrointestinal-associated symptoms prior to diagnosis of their cancer. Third, there was no attempt to control for potential confounding effects of dietary factors in these analyses; in the colon cancer study by Steinmetz (66), however, using dietary data from this cohort, alcohol was not correlated with any of the major nutrients (fat, protein, fiber). Recently,

Bostick et al. (67) have shown an inverse association between vitamin E intake and colon cancer in the Iowa Women's Health Study. This micronutrient was not a confounder of the associations between alcohol and colon or rectal cancer in multivarite analysis. Finally, data on family history of colon cancer were not ascertained; therefore, potential confounding by this risk factor could not be evaluated.

In summary, there are inconsistencies among studies on the association between alcohol and colon and rectal cancer. Findings from the present study for colon cancer are contrary to expectations. Given the high incidence of colorectal cancer (1) and the high prevalence of alcohol consumption (68) in the United Status, a better understanding of this association, focusing on personal characteristics is needed before the role of alcohol on the occurrence of colon and rectal cancer can be determined.

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Table 1. Average daily alcohol consumption for colon and rectal cancer cases compared to noncases.

	Cases			Noncases			
	N	mean g/day	(SEM*)	N	mean g/day	(SEM*)	p-value†
Colon	237	3.10	(0.52)	37,769	3.78	(0.05)	0.19
Rectum	75	3.19	(0.75)	37,931	3.78	(0.05)	0.44

* SEM, standard error of the mean

† p-value for an unpaired t-test assuming unequal variances.

TABLE 2. Age-adjusted relative risk of colon and rectal cancer associated with alcohol consumption among 38,006 postmenopausal women, Iowa Women's Health Study, 1986-1990.

	Colon (n=237)				Rectal (n=75)			
Alcohol Intake (g/day)	No. of cases	Total person-years	Age-adjusted RR†	95% CI‡	No. of cases	Total person-years	Age-adjusted RR†	95% CI‡
0	150	102,177	1.00		41	102,378	1.00	
< 4.0	46	41,111	0.77	0.56-1.08	17	41,154	1.07	0.61-1.89
≥ 4.0	41	37,319	0.78	0.55-1.10	17	37,354	1.27	0.72-2.24
p for trend §			0.08				0.46	

† Age-adjusted relative risk (RR) by 5-year groupings using the Mantel-Haenszel method.

‡ CI,Confidence Interval

§ Based on Mantel's extended test

TABLE 3. Age-adjusted relative risk of colon and rectal cancer associated with potential risk factors among 38,006 postmenopausal women, Iowa Women's Health Study, 1986-1990.

	Colon (n=237)				Rectal (n=75)			
Risk factor	No. of cases*	Total person-years	Age-adjusted RR†	95% CI‡	No. of cases*	Total person-years	Age-adjusted RR†	95% CI‡
Education								
< High school	44	35,034	1.0		10	35,069	1.0	
High school	111	75,385	1.21	0.85-1.72	32	75,517	1.58	0.76-3.22
> High school	80	69,689	0.94	0.65-1.37	33	69,794	1.82	0.89-3.70
p for trend §			0.49				0.13	
Body mass index (kg/m^2)								
< 22.90	36	35,690	1.0		12	35,725	1.0	
22.90-24.99	32	35,616	0.89	0.55-1.43	17	35,642	1.42	0.68-2.98
25.00-27.39	60	36,322	1.62	1.07-2.45	17	36,408	1.37	0.65-2.87
27.40-30.59	58	36,284	1.57	1.03-2.38	13	36,372	1.05	0.48-2.30
≥ 30.60	51	36,695	1.37	0.90-2.10	16	36,739	1.29	0.61-2.73
p for trend §			0.02				0.83	

Waist-to-hip ratio								
≤ 0.76	43	36,158	1.0		11	36,231	1.0	
0.77-0.80	43	36,311	0.97	0.63-1.48	19	36,349	1.66	0.79-3.49
0.81-0.85	37	35,336	0.86	0.55-1.34	12	35,377	1.05	0.46-2.40
0.86-0.90	50	35,878	1.14	0.75-1.71	20	35,934	1.72	0.82-3.63
> 0.90	62	36,137	1.33	0.90-1.97	13	36,200	1.04	0.46-2.36
p for trend §			0.07				0.99	
Age at menarche (years)								
≤ 11	41	27,721	1.0		15	27,762	1.0	
12	59	48,216	0.82	0.55-1.23	19	48,327	0.69	0.35-1.36
13	60	52,958	0.74	0.49-1.10	28	52,976	0.95	0.50-1.77
≥ 14	71	49,611	0.94	0.64-1.38	13	49,709	0.44	0.21-0.92
p for trend §			0.92				0.10	
Age at menopause (years)								
≤ 44	61	41,897	1.0		14	41,964	1.0	
45-49	60	45,117	0.92	0.64-1.31	17	45,191	1.13	0.56-2.29
50-54	90	66,987	0.92	0.67-1.28	31	67,114	1.41	0.75-2.65
≥ 55	14	18,501	0.49	0.27-0.87	7	18,506	0.98	0.39-2.43
p for trend §			0.08				0.52	

Parity								
Nulliparous	16	16,097	1.0		8	16,105	1.0	
1-2	72	57,514	1.26	0.73-2.17	21	57,585	0.75	0.33-1.68
3-4	103	71,295	1.56	0.92-2.66	33	71,429	1.07	0.49-2.33
> 4	44	34,564	1.43	0.80-2.55	12	34,628	0.89	0.36-2.21
p for trend §			0.24				0.91	
Age at first live birth (years)								
≤ 19	51	35,628	1.0		14	35,684	1.0	
20-24	107	81,386	0.90	0.65-1.26	28	81,517	0.86	0.45-1.63
25-29	46	35,077	0.87	0.58-1.30	21	35,127	1.34	0.68-2.67
≥ 30	14	10,252	0.85	0.47-1.55	3	10,283	0.62	0.17-2.24
p for trend §			0.51				0.80	
Oral contraceptive use								
Never	198	146,198	1.0		64	146,431	1.0	
Ever	37	33,704	0.89	0.62-1.28	11	33,749	0.99	0.52-1.91
Noncontraceptive estrogen use								
Never	151	110,770	1.0		50	110,934	1.0	
Ever	82	67,893	0.88	0.67-1.15	25	67,998	0.82	0.50-1.31
Rectal/colon polyps								
No	206	162,710	1.0		60	162,960	1.0	
Yes	21	12,143	1.33	0.85-2.09	14	12,155	2.90	1.62-5.20

Physical activity								
Low	115	83,687	1.0		32	83,823	1.0	
Moderate	61	48,895	0.90	0.66-1.22	19	48,972	1.00	0.57-1.76
High	55	44,397	0.88	0.64-1.23	23	44,453	1.31	0.77-2.23
p for trend §			0.44				0.35	
Cigarette smoking								
Never	157	117,773	1.0		48	117,986	1.0	
Ex-smoker	42	34,010	0.93	0.66-1.31	16	34,052	1.20	0.68-2.11
Current	37	25,988	1.12	0.78-1.60	8	26,014	0.81	0.38-1.72
p for trend §			0.73				0.85	

* number of cases may add up to less than 237 (colon cancer) or 75 (rectal cancer) due to missing data

† Age-adjusted relative risk (RR) by 5-year groupings using the Mantel-Haenszel method.

‡ CI,Confidence Interval

§ Based on Mantel's extended test

TABLE 4. Relationship between alcohol consumption and potential colon and rectal cancer risk factors among 38,006 postmenopausal women, Iowa Women's Health Study, 1986-1990.

	% With Risk Factor			
Risk factor	Abstain	<4 g/day	≥4 g/day	p-value*
Education				
< High school	22.6	16.7	13.9	
High school	42.4	41.9	39.7	
> High school	35.0	41.4	46.4	<0.000
Body mass index (kg/m^2)				
< 22.90	17.2	19.6	27.3	
22.90-24.99	17.5	21.3	24.1	
25.00-27.39	19.2	21.5	21.0	
27.40-30.59	21.4	20.3	16.1	
≥ 30.60	24.7	17.3	11.5	<0.000
Waist-to-hip ratio				
≤ 0.76	17.7	22.6	23.7	
0.77-0.80	18.8	21.2	23.1	
0.81-0.85	19.3	20.2	19.6	
0.86-0.90	21.1	18.9	18.1	
> 0.90	23.1	17.1	15.5	<0.000
Age at menarche (years)				
≤ 11	16.3	14.6	14.7	
12	26.8	27.5	26.9	
13	29.2	30.5	29.9	
≥ 14	27.7	27.4	28.5	<0.000
Age at menopause (years)				
≤ 44	25.1	23.4	23.6	
45-49	26.0	26.2	26.5	
50-54	38.1	39.5	39.8	
≥ 55	10.8	10.9	10.1	0.009

Parity				
Nulliparous	9.0	8.3	9.9	
1-2	32.3	31.2	32.6	
3-4	39.4	40.5	39.4	
>4	19.3	20.0	18.1	0.001
Age at first live birth (years)				
≤19	21.4	18.2	18.3	
20-24	44.8	47.0	45.8	
25-29	18.8	20.5	21.1	
≥30	6.0	5.9	4.9	
nulliparous	9.0	8.4	9.9	<0.000
Oral contraceptive use				
Never	83.8	79.5	76.2	
Ever	16.2	20.5	23.8	<0.000
Noncontraceptive estrogen use				
Never	63.8	60.7	58.5	
Ever	36.2	39.3	41.5	<0.000
Colon/rectal polyps				
No	93.0	93.1	92.9	
Yes	7.0	6.9	7.1	0.93
Physical activity				
Low	49.9	44.1	44.0	
Moderate	26.1	29.7	29.3	
Vigorous	24.0	26.2	26.7	<0.000
Cigarette smoking				
Never	74.5	65.5	43.2	
Ex-smoker	14.7	21.2	29.4	
Current	10.8	13.3	27.4	<0.000

* p-value for the Pearson chi-square of a general association.

TABLE 5. Relative risk of colon cancer among postmenopausal women by average daily alcohol intake within strata of potential effect modifiers, Iowa Women's Health Study, 1986-1990, (n=237 cases).

	Relative risk*			
Risk Factor	Abstain	<4 g/day	≥4 g/day	p-value§
Education				
< High school	1.0	0.82	1.28	
High school	1.46	0.83	0.98	
> High school	0.97	1.09	0.79	0.30
Body mass index (kg/m^2)				
< 24.3	1.0	0.86	0.99	
24.3-28.39	1.80^	1.62	1.15	
≥ 28.4	1.69^	1.05	1.70	0.59
Waist-to-hip ratio				
≤ 0.795	1.0	0.92	0.73	
0.795-0.869	0.91	0.47^	0.72	
> 0.869	1.20	1.11	1.07	0.64
Age at menarche (years)				
≤ 11	1.0	0.49	0.76	
12	0.74	0.65	0.58	
13	0.67	0.55	0.63	
≥ 14	0.87	0.80	0.59	0.90
Age at menopause (years)				
≤ 44	1.0	1.17	0.95	
45-49	1.05	0.90	0.69	
50-54	1.15	0.55	0.87	
≥ 55	0.56	0.47	0.38	0.58
Parity				
Nulliparous	1.0	2.05	0.65	
1-2	1.69	1.38	0.90	
3-4	1.83	1.34	2.05	
> 4	1.92	1.08	1.14	0.22

Age at first live birth (years)				
≤ 19	1.0	0.84	0.73	
20-24	0.94	0.71	0.89	
≥ 25	0.92	0.60	0.82	0.88
Oral contraceptive use				
Never	1.0	0.68	0.87	
Ever	0.93	1.10	0.44	0.12
Noncontraceptive estrogen use				
Never	1.0	0.66	0.78	
Ever	0.82	0.78	0.69	0.61
Colon/rectal polyps				
No	1.0	0.80	0.79	
Yes	1.41	1.02	0.82	0.90
Physical Activity				
Low	1.0	1.03	0.97	
Moderate	1.12	0.66	0.62	
High	1.03	0.68	0.75	0.52
Cigarette Smoking				
Never	1.0	0.58^	0.57	
Ex-smoker	0.79	1.00	0.67	
Current	0.81	1.01	1.08	0.15

* Relative risk computed using CPH regression to control for age.

§ p-value for the -2 log likelihood test for interaction

^ significantly different from 1.0 ($p<0.05$)

TABLE 6. Relative risk of rectal cancer among postmenopausal women by average daily alcohol intake within strata of potential effect modifiers, Iowa Women's Health Study, 1986-1990, (n=75 cases).

	Relative risk*			
Risk Factor	Abstain	<4 g/day	≥4 g/day	p-value§
Education				
< High school	1.0	0.58	2.43	
High school	1.80	1.26	2.41	
> High school	1.84	2.79^	1.55	0.30
Body mass index (kg/m^2)				
< 24.3	1.0	1.12	1.16	
24.3-28.39	1.27	1.60	1.69	
≥ 28.4	1.21	1.01	1.65	0.98
Waist-to-hip ratio				
≤ 0.795	1.0	1.19	1.43	
0.795-0.869	0.92	1.34	0.68	
> 0.869	1.20	0.85	1.02	0.61
Age at menarche (years)				
≤ 11	1.0	1.82	0.83	
12	0.58	0.54	1.70	
13	1.18	0.99	0.78	
≥ 14	0.48	0.54	0.59	0.37
Age at menopause (years)				
≤ 44	1.0	0.55	0.63	
45-49	0.77	1.24	1.12	
≥ 50	1.06	1.14	1.16	0.80
Parity				
Nulliparous	1.0	0.69	2.00	
1-2	0.93	0.94	0.63	
3-4	1.00	1.59	1.30	
> 4	0.92	0.34	1.69	0.56

Age at first live birth (years)				
≤ 19	1.0	2.70	1.82	
20-24	2.0	1.40	1.40	
≥ 25	2.1	1.60	2.24	0.46
Oral contraceptive use				
Never	1.0	1.33	0.40	
Ever	1.29	1.43	1.18	0.29
Noncontraceptive estrogen use				
Never	1.0	1.46	0.94	
Ever	1.12	0.91	0.84	0.79
Colon/rectal polyps				
No	1.0	1.37	1.08	
Yes	1.38	4.24^	3.52^	0.18
Physical Activity				
Low	1.0	0.84	0.79	
Moderate	1.23	0.60	0.47	
High	0.57	1.58	2.57^	0.016
Cigarette Smoking				
Never	1.0	1.01	0.79	
Ex-smoker	1.16	1.18	1.18	
Current	0.24	0.49	1.58	0.28

* Relative risk computed using CPH regression to control for age.

^ significantly different from 1.0 ($p<0.05$)

TABLE 7. Age-adjusted relative risk of site-specific colon cancer incidence among 38,006 postmenopausal women, Iowa Women's Health Study, 1986-1990.

	Proximal (n=90)				Distal (n=141)			
Alcohol Intake (g/day)	No. of cases	Total person-years	Age-adjusted RR†	95% CI‡	No. of cases	Total person-years	Age-adjusted RR†	95% CI‡
0	51	101,939	1.0		94	102,029	1.0	
< 4.0	21	41,047	1.06	0.63-1.76	24	41,049	0.64	0.41-0.99
≥ 4.0	18	37,248	1.02	0.59-1.74	23	37,279	0.69	0.44-1.09
p for trend			0.94				0.04	

† Age-adjusted relative risk (RR) of proximal or distal colon cancer by 5-year groupings using the Mantel-Haenszel method

‡ CI, Confidence Interval

§ Based on Mantel's extension test

TABLE 8. Relative risk of proximal colon, distal colon and rectal cancer associated with specific types of alcoholic beverages among 38,006 postmenopausal women, Iowa Women's Health Study, 1986-1990.

	Proximal Colon (n=90*)				Distal Colon (n=141*)				Rectal (n=75*)			
Type of beverage	No. of cases	Total P-Y#	RR†	95% CI‡	No. of cases	Total P-Y#	RR†	95% CI‡	No. of cases	Total P-Y#	RR†	95% CI‡
Abstainer	51	101,946	1.0		94	102,036	1.0		41	102,385	1.0	
Beer	14	31,169	1.02	0.55-1.87	21	31,186	1.04	0.63-1.70	16	31,254	1.39	0.76-2.53
Wine	22	51,421	0.87	0.52-1.48	27	51,435	0.62	0.40-0.98	19	51,555	0.74	0.42-1.30
Liquor	19	44,629	0.89	0.51-1.55	28	44,663	0.91	0.58-1.43	22	44,746	1.37	0.79-2.39

* number of cases may add up to more than the total because cases were not assigned to one type of alcoholic beverage exclusively.

P-Y, Person-years

§ Total alcohol abstainers

† relative risk (RR) of proximal, or distal colon cancer or rectal cancer adjusted for age and indicator variables for each type of alcoholic beverage using Cox proportional hazard regression.

‡ CI, Confidence Interval

CHAPTER III

ALCOHOL CONSUMPTION AND POSTMENOPAUSAL ENDOMETRIAL CANCER: THE IOWA WOMEN'S HEALTH STUDY

INTRODUCTION

Epidemiologic studies have found a consistent link between alcohol consumption and many types of cancer; the strongest association is with aerodigestive cancers (1). Although weak, there is also a consistent association between alcohol and breast cancer; the risk of breast cancer among moderate consumers of alcohol is about fifty percent higher than abstainers (2). Because breast cancer and endometrial cancer share many of the same risk factors and at least one plausible mechanism for the association of alcohol with cancer involves hormone metabolism, it is reasonable to ask whether alcohol also may play a role in the development of endometrial cancer.

To date, there has been no report examining this association from a cohort study, but at least three case-control studies have been reported. In an analysis that compared cancer cases to "other cancer" controls, using data from the Third National Cancer Survey (3), a nonsignificant, inverse association between alcohol and uterine corpus cancer was shown. Similarly, Webster et al. (4) reported a significant inverse association using data from the Cancer and Steroid Hormone Study. In contrast to these two studies, the results of a third case-control study showed a significant, dose-related positive association (5) after adjusting for other potential endometrial cancer risk factors. Differences in selection criteria for study populations and other methodologic considerations may explain in part the conflicting results. For example, although the authors were careful to note that the control group used in the Third National Cancer Survey analysis excluded anyone with cancer of the mouth, larynx, esophagus, lung or bladder in order to avoid a possible exposure bias leading to false, inverse associations, breast cancer cases were not excluded and composed the majority of the comparison group (3). In addition, case-control studies also are subject to both potential recall bias and the possibility that the disease itself results in changes in

behavior among cases and among controls who are also patients.

Accordingly, further research of this association, using prospective data, is warranted. The present study was undertaken to examine the association between alcohol consumption and the incidence of endometrial cancer over five-years of follow-up in the Iowa Women's Health Study (IWHS), a large cohort study that collected information on usual alcohol intake and other variables. Moreover, recent evidence from this cohort study (6) (Appendix A) and from the Nurse's Health Study (7) suggests that there is a significant interaction between alcohol and noncontraceptive estrogen use on the risk of breast cancer; therefore, the possibility that other predisposing characteristics such as history of estrogen use or body mass index may modify the association between alcohol and endometrial cancer also was considered.

METHODS

The Iowa Women's Health Study cohort

The IWHS is a longitudinal cohort study designed to examine the association between several host, dietary, and lifestyle factors and the incidence of cancer in postmenopausal women who were age 55-69 at baseline. Study participants were selected randomly from Iowa's 1985 Department of Transportation's drivers license list, (which contained approximately 94 percent of all age-eligible women living in Iowa (8)). In January 1986, 99,826 randomly selected women were mailed a questionnaire and a letter describing the purpose of the study. From the original sample, 41,837 eligible questionnaires were returned (a response rate of 42%).

There were 24,848 women considered in the at-risk cohort. Excluded were women who, at baseline, reported a) a prevalent cancer other than skin cancer (n=3,831); b) a prior hysterectomy (n=12,632), or c) that they had menstruated within the past year (n=526).

Measurement of alcohol intake and other risk factors

Information on cancer risk factors was ascertained by a 16-page self-completed questionnaire. Items included reproductive and menstrual history, use of oral contraceptives and noncontraceptive estrogens, history of cancer in a female relative, and personal history of cancer. Participants also were asked to report their current height and weight, as well as weight at specified ages. To assess body fat distribution, a paper tape measure was enclosed along with detailed instructions for circumference measurements of the waist, hips, upper arm and lower leg. Kushi et al. (9) have verified the high reliability and validity of these anthropometric data.

Usual alcohol consumption was assessed using the Harvard semi-quantitative food frequency questionnaire developed by Willett et al. (10). Participants recorded their average daily alcohol intake over the last year according to one of nine possible frequency responses: never or less than one drink per month, 1-3 per month, 1 per week, 2-4 per week, 5-6 per week, 1 per day, 2-3 per day, 4-5 per day and 6+ per day. Frequencies were recorded separately for red wine, white wine, beer, and liquor. Intake, in grams per day, was computed with the aid of the Harvard Nutrient Data Base: the frequency with which each beverage was consumed was multiplied by the ethanol content of the specific beverage, (10.8 grams of ethanol per 4 ounce glass of red or white wine, 13.2 grams per bottle or can of beer, and 15.1 grams per drink or shot of liquor). Average daily alcohol intake was calculated by summing the contribution from each type of alcoholic beverage.

Willett et al. (11) and Giovannucci et al. (12) have reported that the semi-quantitative food frequency questionnaire is highly valid and reproducible for assessing average daily alcohol consumption. Munger et al. (13) examined the accuracy and reproducibility of the questionnaire in this cohort. Pearson correlation coefficients of alcohol intake from the IWHS baseline food frequency questionnaire with a second and third questionnaires were 0.99 and 0.98, respectively. The correlation between average daily alcohol intake measured by the third questionnaire and the average of five 24-hour recalls data was 0.32 (13); this correlation is low possibly because five 24-hour periods is too few to characterize usual intake. Nevertheless, these data suggest that the food-frequency questionnaire is highly reliable.

Abstainers were defined as women whose reported usual daily alcohol intake was 0 grams. Of the baseline questionnaires returned, 3.8 percent of the women left intake information blank for all four alcohol beverages. For purposes of analysis, total daily alcohol consumption for these women was considered to be 0 grams per day. However, the analyses were repeated with alcohol intake considered missing for these women; the results were comparable.

Follow-up of cohort and identification of endometrial cancer cases

The cohort has been followed for 5 years to determine incident cancer cases. Two follow-up mail surveys for vital status and address-change have been conducted. The status of non-respondents to the follow-up surveys was determined by the National Change of Address service (to identify women who had moved out of Iowa), and by the National Death Index (to identify out-of-state deaths through 1989).

Incident endometrial cancers were identified using the Health Registry of Iowa, part of the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program. Identification involved cross-matching cases from 1986-1990 with IWHS participants using a combination of first and last names, maiden name, zip code, birthdate, and Social Security number. Endometrial cancer was identified as corpus uteri and isthmus uteri (ICD-O codes 182.0, 182.1). After five-years of follow-up, 167 endometrial cancer cases were reported in the at-risk cohort.

Statistical analysis

Person-years of follow-up for each individual were computed as the amount of time since completion of the baseline questionnaire to one of the following events: 1) endometrial cancer diagnosis, 2) death (if in Iowa), 3) a move out of Iowa (if known), 4) midpoint of interval between last contact date and either date of next follow-up or December 31, 1990 (if date of move was unknown), or 5) midpoint of interval between date of last contact and date of death (for non-Iowa deaths). For women without one of these events, follow-up was to December 31, 1990.

Women were classified *a priori* according to three levels of alcohol intake: 0 g per day (abstainers), and two others based on a median split of drinkers (< 4 and ≥ 4 g of ethanol per day). Cutpoints for quantiles of other risk factors were determined from the distribution of the total at- risk cohort.

Incidence rates were calculated by dividing the number of events by the person-years of follow-up. Relative risks (RRs) and their 95 percent confidence interval (CI) (14), were computed within categories of potential risk factors with adjustment for five-year age

categories: 55-59, 60-64, and 65-69, 70-74. Tests for linear trend based on Mantel's extension were computed (15). Consideration was given to the following variables as potential confounders: education (as a measure of socioeconomic status), body mass index (kg/m^2), body mass index at age 18, waist-to-hip ratio, age at menarche, age at menopause, parity, age at first live birth, family history of endometrial cancer, oral contraceptive use, noncontraceptive estrogen use, hypertension, sugar diabetes, physical activity, and cigarette smoking.

To further evaluate possible confounding, the proportion of women within categories of alcohol consumption (0, <4 and ≥ 4 g of ethanol per day) were compared across strata of other endometrial cancer risk factors and the Pearson chi-square for a general association between two factors in an R x C table examined.

Multivariate analysis was performed using Cox proportional hazards regression to control for age and other potential confounding factors. Models containing only indicator variables for alcohol were compared to models including each of the potential confounders; if the regression coefficient for any level of alcohol consumption changed by more than 10 percent in the larger model, confounding was considered to be present. Cox proportional hazard regression also was used to test for multiplicative effect modification by comparing the -2 log likelihood Chi-square estimates between a model containing the main effects and a model containing two-way interaction terms for alcohol and each risk factor separately. Analyses were performed using PROC PHREG of the SAS statistical package (16). The proportional hazards assumption was tested and confirmed.

The associations between endometrial cancer and specific types of alcoholic beverage were examined using Cox proportional hazards regression. A single dose-response model was

tested, incorporating potential confounders and indicator variables (one set for each type of alcoholic beverage: wine beer, liquor) to represent categories of consumption.

RESULTS

In this cohort, approximately 62 percent of the cases and 56 percent of the noncases reported drinking alcohol never or less than once per month over the last year. The average amount of alcohol consumed per day was similar between the cases and noncases (4.7 and 4.0 g per day, respectively, p=0.44, unpaired t-test)

Data in Table 1 show the age-adjusted RRs of endometrial cancer for women whose reported alcohol consumption was < 4 and ≥ 4 g per day compared to abstainers. These data were suggestive of a weak inverse association, but the confidence intervals included 1. For comparison with recent results of alcohol and breast cancer analyses (6,7), the age-adjusted RRs for four categories of alcohol consumption (<1.5, 1.5-4.9, 5-14.9 and ≥ 15 g of daily) were also computed; compared to abstainers, the RRs of endometrial cancer were 0.63, 0.85, 0.65 and 1.18, respectively. Again none of these estimates were significantly different from 1.0.

In order to evaluate possible confounding, the associations between the incidence of endometrial cancer and several potential risk factors were computed (Table 2). Current body mass index, body mass index at age 18, waist-to-hip ratio, age at menopause, noncontraceptive estrogen use, hypertension, and diabetes were significantly and positively associated with endometrial cancer. There was an inverse association between endometrial cancer and an older age at menarche and a greater number of live births (parity). There

were no significant associations between the incidence of endometrial cancer and education, age at first live birth, family history of endometrial cancer, oral contraceptive use, physical activity or cigarette smoking. Further evaluation of possible confounding (Table 3) revealed that women who consumed ≥ 4 g of alcohol daily had a higher education, lower current body mass index and lower waist-to-hip ratio, greater prevalence of both oral contraceptive use for more than 5 years and noncontraceptive estrogen use for more than 5 years, and greater prevalence of current cigarette smoking than those with lower alcohol consumption levels. The higher alcohol consumers were less likely to have had an early age at first live birth and or to have reported a history of hypertension or diabetes mellitus. Alcohol consumption did not appear to be different across strata of body mass index at age 18, age at menarche, age at menopause, parity, or family history of endometrial cancer.

In multivariate-analyses, using Cox proportional hazards regression to control for age, body mass index, age at menopause, parity and noncontraceptive estrogen use, the risk of endometrial cancer at each level of alcohol consumption was comparable to abstainers (Table 4). Inclusion of other control variables did not appreciably alter the RR estimates. We further stratified alcohol consumption into four levels of intake. Compared to abstainers the multivariate-adjusted RR and 95 percent CI of endometrial cancer for women whose average daily alcohol consumption was <1.5, 1.5-4.9, 5-14.9 and ≥ 15 g were 0.73 (95 percent CI 0.44-1.23), 0.83 (0.48-1.46), 0.79 (0.44-1.41) and 1.35 (0.78-2.33), respectively.

Consideration was given to the potential modifying effects of other risk factors on the association between alcohol and endometrial cancer. Using Cox proportional hazards regression to control for age, body mass index, noncontraceptive estrogen use, parity and age at menopause, two-way multiplicative interactions between alcohol and other risk

factors were tested (Table 5). Specifically, the investigation of an interaction between alcohol and body mass index showed that the relationship between alcohol and endometrial cancer was not different across categories of body mass index, not across cetagories of noncontraceptive estrogen use. Similarly, there was no suggestion of a multiplicative interaction between alcohol and the other endometrial cancer risk factors. The interaction between family history of endometrial cancer and alcohol consumption on the risk of endometrial cancer was not considered because there were only four cases with a positive family.

Analyses exploring the associations between endometrial cancer and specific types of alcoholic beverages (Table 6) indicated that there was no association with either beer or wine. However, there appeared to be a statistically nonsignificant increase in risk of endometrial cancer associated with the highest level of liquor consumption after adjusting for confounders and intake of other beverages. Compared to total alcohol abstainers, the RR of endometrial cancer for women who consumed ≥ 4 g of ethanol per day from spirits was 1.41 (95 percent CI 0.84-2.38).

DISCUSSION

In this cohort study of postmenopausal Iowa women, usual alcohol consumption was not associated with an increased risk of endometrial cancer. In analyses that examined the effect of specific types of alcoholic beverages on the risk of endometrial cancer there was no association with either beer or wine. There was suggestion of a 40 percent elevated risk

of endometrial cancer associated with the consumption of liquor, but this was not statistically significant, and therefore may be due to chance. Risk factors previously reported to be associated with endometrial cancer such as higher body mass index, late age at menopause, lower parity and noncontraceptive estrogen use (17,18) also were evident in this cohort.

These results do not support the findings of two case-control studies that have shown inverse associations between alcohol and endometrial cancer or one case-control study that reported a positive association (3,4,5). The Third National Cancer Survey (3) reported that compared to "other cancer" controls, the odds ratios for uterine corpus cancer for women whose total alcohol consumption was < 51 oz-years and ≥ 51 oz-years were 0.69 and 0.63, respectively (neither odds ratio was significant). Webster et al. (4) showed that the risk of endometrial cancer among women whose average weekly alcohol consumption was at least 150 g was 55 percent less than among abstainers. Conversely, in multivariate analysis LaVecchia, et al. (5) found that compared to abstainers, consumption of at least 4 drinks per day conferred more than a four-fold elevated risk of endometrial cancer.

Reasons for the different results among the three case-control studies are not clear. One possible explanation may be differences in study populations. As discussed earlier, the Third National Cancer Survey analyses may be biased towards an inverse association due to the method of control selection. In the study by LaVecchia et al. (5) , women with acute conditions related to any of the established or suspected endometrial cancer risk factors were excluded from the control group. The positive association found in this study may be falsely elevated only if alcohol consumption was also related to these acute conditions.

The null association between alcohol and endometrial cancer observed in this study was not

different across levels of other endometrial cancer risk factors, specifically body mass index and noncontraceptive estrogen use. In contrast, Webster et al. (4) reported that women who abstained from alcohol and had a high body mass index were at greatest risk of endometrial cancer when compared to either abstainers who were lean or women who consumed ≥ 50 g of ethanol per week and had a high body mass index. This suggests that alcohol may protect obese women from endometrial cancer, a condition normally associated with an increased risk of this cancer (19).

Recent findings from the IWHS (6) and at least one other cohort study (7) showed a significant interaction between alcohol and noncontraceptive estrogen use on the risk of breast cancer. However, no such interaction was found for endometrial cancer, suggesting a different mechanism in the hormonally-related etiologies of breast and endometrial cancer. However, these data should be interpreted cautiously since there were few endometrial cancer cases in each stratum of alcohol consumption, meaning statistical power for detecting an interaction was low.

The impetus to explore the association between alcohol and endometrial cancer was driven by the number of hormonally-associated risk factors that are common to both endometrial cancer and breast cancer, and by the consistent observation of an increased risk of breast cancer associated with alcohol consumption (2). There is no evidence to date that ethanol alone is carcinogenic (1). Because, human and animal studies have shown that elevated levels of circulating estrogens, particularly free estrogens, are positively correlated with tumor incidence (20), it is suspected that in the development of hormone-sensitive malignancies, such as breast cancer, ethanol may alter the metabolism of androgens and estrogens. However, any influence of ethanol on the concentration of steroid hormones in women appears to depend on the pattern of habitual consumption. Chronic, heavy alcohol

intake by women appears to lead to early menopause, lower postmenopausal gonadotropin levels and increased concentrations of the classic female steroid hormones (21). The few data that have considered low or moderate alcohol consumers suggest inconsistent effects of ethanol on sex steroid hormone levels. Cauley et al. (22) reported that both estrone and estradiol levels were lower with higher, self-reported alcohol consumption in normal, postmenopausal women.

However, the above results do not provide a ready explanation for the observed differences in the association between alcohol and the risk of the hormone-sensitive cancers, breast and endometrial. Recent findings from this cohort, exploring differences in the relationships between epidemiologic risk factors and estrogen and progesterone receptor status-specific breast cancers show that alcohol is associated only with estrogen negative-progesterone negative (hormone unresponsive) breast cancer (RR=1.74 for any drinkers versus abstainers) (23). Collectively, these observations suggest that the role of alcohol in the etiology of these cancers may not be through its influence on hormone levels as has previously been suggested.

Consideration must be given to the potential limitations and sources of bias in this study that may have lead to the observed null association. First, alcohol consumption was assessed by self-report using a food-frequency questionnaire. Although this has been shown to be an accurate and reliable method for measuring alcohol intake, one limitation is that heavy drinkers may underreport consumption. With the limited range of exposure among drinkers and a large percent of nondrinkers in the cohort, the possibility of detecting a dose-response is reduced. Further, virtually nothing is known about whether the duration of alcohol use may influence risk of endometrial cancer. In this cohort study, women were asked to report their usual intake over the last year; changes in alcohol

consumption throughout life may lead to misclassification. Finally, at baseline, IWHS participants reported whether they had ever had a hysterectomy; no attempt has been made to assess whether additional cohort members had a hysterectomy since baseline. It is unlikely, however, that women who drink alcohol were more likely have undergone a hysterectomy since baseline than nondrinkers.

Given the conflicting results from the three case-control studies and the lack of an association observed in this cohort study, it would appear that a causal relationship between alcohol and endometrial cancer is unlikely. However, to understand better the previous results, further epidemiologic studies using prospective data, focusing on specific types of alcoholic beverages, on higher consumers, and on high risk groups, are warranted to corroborate these observations.

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TABLE 1. Age-adjusted relative risk of endometrial cancer associated with alcohol consumption among postmenopausal women, Iowa Women's Health Study, 1986-1990, (n=167 cases).

Risk factor	No. of cases	Total person-years	Age-adjusted RR†	95% CI‡	p for trend§
Alcohol intake (g/day)					
0	104	65,903	1.00		
< 4.0	30	26,920	0.74	0.49-1.11	
≥ 4.0	33	25,234	0.88	0.60-1.31	0.37

† Age-adjusted relative risk (RR) by 5-year groupings using the Mantel-Haenszel method.

‡ CI,Confidence Interval

§ Based on Mantel's extended test

TABLE 2. Age-adjusted relative risk of endometrial cancer associated with potential risk factors among postmenopausal women, Iowa Women's Health Study, 1986-1990, (n=167 cases).

Risk factor	No. of cases*	Total person-years	Age-adjusted RR†	95% CI‡	p for trend§
Education					
< High school	25	21,757	1.00		
High school	70	49,273	1.33	0.84-2.10	
> High school	71	46,709	1.42	0.90-2.24	0.16
Body mass index (kg/m^2)					
≤ 22.73	23	23,770	1.00		
22.76-24.86	22	23,456	0.96	0.54-1.73	
24.87-27.22	22	23,647	0.95	0.53-1.70	
27.23-30.30	25	23,800	1.06	0.60-1.86	
> 30.30	75	23,384	3.26	2.04-5.19	0.0001
Body mass index at age 18 (kg/m^2)					
< 19.35	31	23,887	1.00		
19.35-21.449	29	23,138	1.00	0.60-1.66	
21.45-22.749	27	23,536	0.93	0.55-1.55	
22.75-24.599	31	23,523	1.06	0.64-1.75	
≥ 24.60	47	23,414	1.62	1.03-2.55	0.04
Waist-to-hip ratio					
≤ 0.76	21	24,335	1.00		
0.77-0.80	20	21,163	0.98	0.53-1.81	
0.81-0.85	40	27,018	1.55	0.91-2.64	
0.86-0.90	36	21,284	1.71	0.99-2.93	
> 0.90	48	23,749	2.05	1.22-3.44	0.0008
Age at menarche (years)					
≤ 11	29	16,845	1.00		
12	54	31,252	0.96	0.61-1.51	
13	52	35,289	0.81	0.52-1.28	
≥ 14	30	33,303	0.50	0.30-0.83	0.002

Age at menopause (years)					
≤ 44	18	12,878	1.00		
45-49	24	29,742	0.63	0.34-1.15	
50-54	77	55,672	1.05	0.63-1.76	
≥ 55	43	16,225	1.83	1.05-3.18	0.0008
Parity					
Nulliparous	31	11,398	1.00		
1-2	59	37,286	0.59	0.38-0.90	
3-4	59	45,907	0.53	0.34-0.81	
> 4	17	22,708	0.31	0.17-0.56	0.0001
Age at first live birth (years)					
≤ 19	32	20,541	1.00		
20-24	61	52,543	0.72	0.47-1.11	
25-29	28	24,530	0.62	0.37-1.04	
≥ 30	13	7,666	0.92	0.48-1.78	0.35
Nulliparous	31	11,398	1.51	0.91-2.48	
Family history of endometrial cancer					
No	154	109,182	1.00		
Yes	4	5,037	0.55	0.20-1.48	
Oral contraceptive use					
Never	144	95,804	1.00		
< 5 years	15	15,460	0.79	0.46,1.36	
≥ 5 years	8	6,346	1.17	0.57-2.43	0.91
Noncontraceptive estrogen use					
Never	99	86,683	1.00		
< 5 years	38	26,148	1.22	0.84-1.77	
≥ 5 years	28	4,263	5.24	3.43-7.98	0.001
Hypertension					
No	86	74,921	1.00		
Yes	77	40,506	1.54	1.13-2.10	
Sugar Diabetes					
No	149	110,460	1.00		
Yes	17	6,817	1.76	1.06-2.90	

Physical Activity					
Low	87	54,403	1.00		
Moderate	37	32,072	0.70	0.48,1.03	
High	40	29,277	0.83	0.57-1.20	0.21
Cigarette Smoking					
Never	115	76,336	1.00		
Ex-smoker	30	22,241	0.93	0.62-1.39	
Current	17	17,705	0.68	0.41-1.14	0.179

* number of cases may add up to less than 167 due to missing data

† Age-adjusted relative risk (RR) by 5-year groupings using the Mantel-Haenszel method.

‡ CI,Confidence Interval

§ Based on Mantel's extended test

TABLE 3. Relationship between alcohol consumption and potential endometrial cancer risk factors among 24,848 postmenopausal women, Iowa Women's Health Study, 1986-1990, (n=167 cases).

	% With Risk Factor			
Risk factor	Abstain	<4 g/day	≥4 g/day	p-value*
< High school	21.6	15.7	13.2	
High school	42.5	42.0	39.5	
> High school	35.9	42.3	47.3	<0.000
Body mass index (kg/m^2)				
≤ 22.73	17.7	19.9	27.2	
22.76-24.86	17.6	21.4	24.1	
24.87-27.22	18.9	21.4	21.1	
27.23-30.30	21.6	20.3	16.0	
> 30.30	24.2	17.0	11.6	<0.000
Body mass index at age 18 (kg/m^2)				
< 19.35	20.1	20.5	20.9	
19.35-21.449	18.8	20.1	21.7	
21.45-22.749	19.2	20.5	21.6	
22.75-24.599	20.2	20.4	18.8	
≥ 24.60	21.8	18.6	16.9	<0.000
Waist-to-hip ratio				
≤ 0.76	18.4	23.4	23.3	
0.77-0.80	16.5	18.7	21.0	
0.81-0.85	22.8	23.5	22.8	
0.86-0.90	19.0	17.2	17.1	
> 0.90	23.3	17.2	15.8	<0.000
Age at menarche (years)				
≤ 11	15.3	13.5	13.5	
12	26.5	27.3	26.6	
13	29.7	31.6	30.4	
≥ 14	28.5	27.6	29.5	<0.001

Age at menopause (years)				
≤44	11.7	10.7	10.8	
45-49	25.9	25.5	26.8	
50-54	47.9	49.4	49.1	
≥55	14.5	14.4	13.3	0.038
Parity				
Nulliparous	9.8	9.3	10.4	
1-2	32.0	30.7	32.5	
3-4	38.7	40.0	39.1	
≥5	19.5	20.0	18.0	0.016
Age at first live birth (years)				
≤19	18.8	16.3	16.0	
20-24	44.6	45.8	44.9	
25-29	20.0	21.9	22.8	
≥30	6.7	6.7	5.9	
nulliparous	9.9	9.3	10.4	<0.000
Family history of endometrial cancer				
No	95.3	96.2	95.9	
Yes	4.7	3.8	4.1	0.011
Oral contraceptive use				
Never	84.2	79.4	76.3	
< 5 years	11.3	14.8	16.2	
≥5 years	4.5	5.8	7.5	<0.000
Noncontraceptive estrogen use				
Never	75.6	73.1	70.5	
< 5 years	21.1	22.9	25.0	
≥5 years	3.3	4.0	4.5	<0.000
Physical activity				
Low	49.5	44.4	43.9	
Moderate	26.0	29.7	29.6	
Vigorous	24.5	25.9	26.5	<0.000
Cigarette smoking				
Never	74.5	64.7	42.1	
ex-smoker	14.6	21.2	29.2	
Current	10.9	14.1	28.7	<0.000

Hypertension				
No	62.0	68.2	68.0	
Yes	38.0	31.8	32.0	<0.000
Sugar Diabetes				
No	91.6	96.6	97.5	
Yes	8.4	3.4	2.5	<0.000

* p-value for Pearson chi-square for a general association between the risk factor and alcohol

TABLE 4. Relative risk endometrial cancer associated with alcohol intake among postmenopausal women using Cox proportional hazards regression, Iowa Women's Health Study, 1986-1990, (n=167 cases).

Alcohol intake (g/day)	No. of cases*	Total person-years	RR †	95% CI ‡
0	101	62,803	1.00	
< 4.0	27	25,946	0.70	0.48-1.12
≥ 4.0	32	24,220	1.04	0.69-1.56

* number of cases may add up to less than 167 due to missing data

† Relative risk (RR) of endometrial cancer adjusted for age, body mass index, parity, age at menopause, and noncontraceptive estrogen use using Cox proportional hazards regression.

‡ CI,Confidence Interval

TABLE 5. Relative risk of endometrial cancer among postmenopausal women by average daily alcohol intake within strata of potential risk factors, Iowa Women's Health Study, 1986-1990, (n=167 cases).

	Relative risk*			
Risk Factor	Abstain	<4 g/day	≥4 g/day	p-value§
Education				
< High school	1.0	0.69	2.05	
High school	1.42	0.91	1.40	
> High school	1.53	0.94	1.28	0.58
Body mass index (kg/m^2)				
≤ 22.89	1.0	0.48	1.66	
22.90-25.04	1.13	1.12	1.41	
25.05-27.45	1.27	0.91	1.47	
27.46-30.70	1.36	0.75	1.69	
> 30.70	5.06^	3.90^	2.47	0.64
Body mass index at age 18 (kg/m^2)				
≤ 19.35	1.0	0.48	1.40	
19.35-21.449	1.27	0.47	0.97	
21.45-22.749	0.61	1.33	1.10	
22.75-24.599	1.18	0.66	0.36	
≥ 24.60	1.03	0.78	1.31	0.13
Waist-to-hip ratio				
≤ 0.76	1.0	0.44	1.65	
0.77-0.80	1.03	0.96	1.47	
0.81-0.85	1.45	1.14	1.88	
0.86-0.90	1.78	1.16	1.52	
> 0.90	1.52	1.36	0.84	0.76
Age at menarche (years)				
≤ 11	1.0	0.43	0.83	
12	0.96	0.85	0.89	
13	1.03	0.63	0.95	
≥ 14	0.51	0.46	0.90	0.78

Age at menopause (years)				
≤ 44	1.0	1.64	1.05	
45-49	0.78	0.46	0.69	
50-54	1.56	0.89	1.56	
≥ 55	2.16^	1.89	2.71^	0.79
Parity				
Nulliparous	1.0	1.62	1.92	
1-2	0.81	0.42	0.78	
3-4	0.74	0.46	0.64	
> 4	0.38	0.31	0.27	0.49
Age at first live birth (years)				
≤ 19	1.0	0.68	0.76	
20-24	0.71	0.60	0.76	
25-29	0.80	0.09^	0.34	
≥ 30	0.62	0.27	1.50	0.16
Family history of endometrial cancer				
No	1.0	0.77	0.99	
Yes	0.19	-	0.62	0.51
Oral contraceptive use				
Never	1.0	0.83	1.05	
< 5 years	1.04	2.14	1.12	
≥ 5 years	1.38	0.57	1.03	0.58
Noncontraceptive estrogen use				
Never	1.0	0.80	0.84	
< 5 years	1.25	0.91	1.69	
≥ 5 years	6.06^	3.34^	7.28^	0.79
Hypertension				
No	1.0	0.76	1.03	
Yes	1.28	0.77	1.27	0.87
Sugar Diabetes				
No	1.0	0.73	1.05	
Yes	1.25	1.27	1.28	0.91

Physical Activity				
Low	1.0	0.90	1.11	
Moderate	0.74	1.23	1.20	
High	1.32	0.55	0.65	0.25
Cigarette Smoking				
Never	1.0	0.83	1.13	
Ex-smoker	1.04	0.36	0.80	
Current	0.79	0.71	0.93	0.71

* Relative risk (RR) of endometrial cancer adjusted for age, body mass index, parity, age at menopause, and noncontraceptive estrogen use using Cox proportional hazards regression.
§ p-value for the -2 log likelihood test for interaction
^ significantly different from 1.0 ($p<0.05$)

TABLE 6. Relative risk of endometrial cancer associated with specific types of alcoholic beverages among postmenopausal women using Cox proportional hazards regression, Iowa Women's Health Study, 1986-1990, (n=167 cases).

Type of beverage	No. of cases	Total person-years	RR †	95% CI ‡
Beer				
abstainer*	101	65,908	1.00	
< 4.0 g per day	16	13,220	0.94	0.54-1.66
≥ 4.0 g per day	6	7,818	0.71	0.31-1.62
p for trend			0.41	
Wine				
abstainer*	101	65,908	1.00	
< 4.0 g per day	34	27,109	0.86	0.56-1.33
≥ 4.0 g per day	8	7,086	0.79	0.35-1.75
p for trend			0.55	
Liquor				
abstainer*	101	65,908	1.00	
< 4.0 g per day	19	17,392	0.89	0.53-1.51
≥ 4.0 g per day	20	12,284	1.41	0.84-2.38
p for trend			0.19	

* total alcohol abstainers

† Relative risk (RR) of endometrial cancer adjusted for age, body mass index, parity, age at menopause, and noncontraceptive estrogen use and indicator variables for each type of alcoholic beverage using Cox proportional hazards regression.

‡ CI,Confidence Interval

CHAPTER IV

BIOLOGIC MARKERS OF ALCOHOL INTAKE: A LITERATURE REVIEW

INTRODUCTION

As much as 80 percent of all cancer cases may be related to environmental risk factors (1). The associations between cancer and some of these risk factors, such as tobacco use and sun exposure, are well established; however, the relationship between other environmental exposures, such as diet, and cancer have been more difficult to assess. Although it has been estimated that from 10-70 percent of cancer cases are attributable to diet (1), the evidence to support associations between specific dietary components and disease has been inconsistent. Assessing intake of both micro- and macro-nutrients is dependent on self-report, with attendant misclassification, possibly biasing the estimates of associations toward the null and may contribute to the inconsistencies among studies. Of the dietary factors examined in epidemiologic studies, alcohol consumption may be reliably and validly measured in some populations (2,3) and poorly estimated in others (4). Objective measurements of alcohol consumption, using biologic markers of exposure, may help to reduce some of the problems associated with self-report data.

BACKGROUND AND SIGNIFICANCE

General Consideration of Biomarkers

Biochemical or molecular epidemiology has been described as "the incorporation of biologic markers (biomarkers) into analytic epidemiologic research" (5). Biomarkers are generally used in epidemiologic studies: a) "for improving validity and reducing bias" (misclassification); b) to "enhance our understanding of disease pathogenesis and allow early detection of disease"; c) to "assist in providing more homogeneous classifications of disease"; d) to "study individual susceptibility"; e) to measure compliance to interventions

trials; and f) to "improve our understanding of the mechanisms of disease occurrence" (5).

Perera and Weinstein (6) have suggested that types of biomarkers may be broadly classified into categories of internal dose, biologically effective dose, biologic response, and susceptibility. However, it is important to consider that these categories are not independent; if a person is exposed to factor X, then factors associated with susceptibility may affect the internal dose, the biologically effective dose and/or the biologic response.

The use of biomarkers in epidemiologic studies is not without limitations (5). The problems of inter- and intraindividual variation, as well as, variation between groups may affect the validity of some biomarkers. Most importantly, experimental, both *in vivo* and *in vitro*, studies, often, have preceded the use of biomarkers in humans. Therefore, the feasibility and applicability of markers must be considered before implementing them in large epidemiologic studies.

Determining the consistency among studies, and across different groups (sex, race) will ensure biomarker quality. The methodologic issues in biochemical epidemiology are not different from those in studies where self-report data are used-appropriateness and reliability of measurement, variability, and possible confounding. All must be carefully examined before inferring causality between exposure and/or susceptibility and disease.

Methods of Assessing Alcohol Consumption By Self Report

In epidemiologic studies, alcohol consumption is measured most commonly in individuals by self-report or by collaterals (e.g. spouse, relative, or friend). In validation studies of self-report data, the "gold standard" against which these data are compared also require self-report. Thus, validation in this sense, is just a measure of comparison between two

imprecise estimates.

One self-report method is the Quantity-Frequency-Variability Index (Q-F-V) that was developed for the national survey of American drinking practices (7). This index classifies individuals into five categories of intake: abstainers, infrequent drinkers, light drinkers, moderate drinkers and heavy drinkers. Using data from a pilot study (8), the original authors reported that the Q-F-V index has a high degree of validity. However, the individuals included in the pilot study all had a history of alcoholism; therefore, it is unclear how applicable this method is for the general population.

A food-frequency questionnaire (FFQ) developed by Willett et al. (9) queries individuals on their consumption over the last year for several different dietary components including beer, red wine, white wine and spirits; from these data, the average number of grams of alcohol consumed per day is computed. Giovannucci et al. (2) have reported this food-frequency questionnaire is a highly "valid" and reliable method of measuring alcohol intake. The IWHS FFQ (the questionnaire is identical to the original Willett FFQ) alcohol data showed reasonable reliability when compared to five daily dietary recalls (3). One limitation to the food-frequency questionnaire is that is does not distinguish lifetime abstainers from past-drinkers.

Other methods of determining alcohol consumption include dietary recall and daily diaries. Prospective diaries require the subject to report his/her daily alcohol consumption over the course of some period of time (e.g. one week , one month). This method can be intrusive and may actually affect the individuals alcohol intake (10). In addition, daily diaries necessitate accurate recording of intake and a high degree of compliance, factors that may differ among certain subpopulations based on their degree of motivation and, probably,

education.

Most, if not all, of these methods of self-report alcohol consumption are limited by subjectivity, the issue of social desirability, and possible seasonal variation. Further, respondents may differ systematically according to their disease status.

Finally, some research have attempted to distinguish lifetime abstention from past consumption using self-report. Results of one study defined 15 percent of the participants as abstainers (did not drink alcohol with the last year). After further evaluation, of those initially described as abstainers, 58 percent were actually lifetime abstainers, 34 percent were current abstainers with no history of alcohol abuse and 9 percent had a history of alcohol abuse (11). Defining lifetime abstainers from current abstainers may have important implications for accurately describing associations between alcohol consumption and the occurrence of disease.

Physiologic Methods of Assessing Alcohol Consumption and Abuse

Laboratory measures of alcohol intake potentially are more objective and therefore more accurate than self-report data. However, as with any biologic marker of exposure, there may be a great deal of variability affecting the measurement. Physiologic levels of alcohol can be affected my gender, amount consumed, whether or not food is eaten during the same occasion that an alcoholic beverage is consumed, and disease status of the individual (12). In addition, the quality of the laboratory assay (sensitivity and specificity) will also affect the measured estimates.

There are four type of biologic tissues commonly used to measure alcohol: breath (13), blood (14), or urine (15) and sweat (16). With the exception of the sweat patch, most of

the methods using these tissues only detect recent exposure (up to 10-24 hours) and do not differentiate between acute, occasional, and chronic regular alcohol consumption. Blood, breath and urine assays have merit for evaluating compliance to alcohol-treatment programs and the degree of alcohol intoxication in motor vehicle drivers. The sweat-patch collects alcohol excreted from the skin and can be worn continuously, thus providing a means of assessing alcohol consumption over a 7-day period (16). However, studies evaluating the validity of this method have shown that the sweat-patch lacks sensitivity and cannot discriminate between known amounts of alcohol consumed (17).

Recently, there has been a great deal of interest in a new biomarker of alcohol intake measured in urine, 5-hydroxytryptophol (5HTOL), normally a minor metabolite of serotonin (18). After alcohol consumption there is a change in seratonin catabolism such that 5-hydroxyindoleacetic acid (5HIAA), also a metabolite of serotonin, decreases and 5HTOL increases. However, the ratio of 5HTOL to 5HIAA can be used as a marker of only recent alcohol exposure (14-22 hours).

Several laboratory assays have been developed to determine chronic alcohol exposure (19). These include assays to measure elevated serum levels of the two liver enzymes gamma-glutamyltransferase (20), aspartate transaminase (21), increased concentrations of plasma high-density lipoprotein cholesterol (22) and increased mean corpuscle volume (22). These assays are generally indicative of alcoholism and/or associated liver disease and may be useful for follow-up after alcohol treatment; however, they are not useful for estimating alcohol use in the general population. Finally, carbohydrate deficient-transferrin is another serum marker that is measurable after consumption of 50-60 grams of ethanol per day for at least one week (23); the half-life of this marker is 15 days. The appearance of this marker occurs independent of liver damage unlike many of the other

serum markers.

Developing a Biomarker of Alcohol Exposure

Criteria for establishing a biochemical marker of alcohol consumption include: a) formation of stable products with relatively long half-lives (days to weeks); b) dose-dependent product formation; c) the presence of measurable physiologic concentrations; d) the absence of artifactual formation; and e) easily obtainable tissue. For population studies, there are additional needs such as a rapid and inexpensive assay. The acetaldehyde adducts of hemoglobin represent one potential marker. Acetaldehyde is the primary oxidative metabolite of ethanol and readily reacts with hemoglobin *in vitro* to form both stable and unstable modifications (24, 25).

Indirect evidence suggesting the formation of acetaldehyde-modified hemoglobin *in vivo* was determined by comparing the amount of "fast" hemoglobin between heavy drinkers and abstainers (Appendix B). The amount of "fast" hemoglobin is a measure of all hemoglobin changes causing an increased rate of migration on cation-exchange columns. Site-specific acetaldehyde-hemoglobin modification in heavy drinkers were confirmed using mass spectrometric analysis (26). Peterson et al. (27), have also reported indirect evidence of alcohol-associated modifications from blood samples of alcoholics and from normal volunteers after consuming a known amount of alcohol using a fluorogenic high performance liquid chromatography assay. Increased levels of acetaldehyde-modified hemoglobin in the blood of healthy volunteers who consumed a single dose of alcohol were detected using an immunologic assays (28). In addition, antibodies against acetaldehyde-hemoglobin adducts have been detected in the blood of alcoholics (29).

Although the above studies clearly support the presence of acetaldehyde-hemoglobin

adducts *in vivo*, the formation and structure of these adducts have not been characterized in a way that enhances the development of a biomarker of alcohol consumption. Acetaldehyde reacts with the N-terminal valine residues of both the alpha- and beta-chains of hemoglobin (24) and with other sites on the hemoglobin molecule including lysine residues (30). The formation of acetaldehyde-hemoglobin adducts, however, is affected by acetaldehyde concentrations (25, 31, 32) and other reaction conditions (30) which may differ significantly between *in vivo* and *in vitro* reactions. In addition, metabolic activity of red blood cells may be essential for the formation of certain adducts (33). Detection, isolation, and characterization of acetaldehyde-hemoglobin adducts formed *in vivo* is difficult because they are present in very low amounts. The generation of acetaldehyde-hemoglobin adducts by the exposure of red blood cells to [^{14}C]acetaldehyde *in vitro* (at concentrations mimicking *in vivo* conditions) is one method for obtaining reasonable amounts of these adducts in order to characterize their formation. The development of an *in vitro* model system, mimicking *in vivo* conditions, in order to characterize further the reaction between acetaldehyde and hemoglobin is described in Chapter 5 of this thesis.

The overall purpose of this research is to develop a biomarker of alcohol intake that can be readily measured in order to facilitate, in the future, the better quantitation of alcohol in studies examining associations between alcohol and site-specific cancers.

Determining the biologic half-life of acetaldehyde-hemoglobin modifications and describing the relationship of known doses of alcohol consumed to the amount of modified hemoglobin is necessary before biologic markers can be used in large epidemiolgic studies. The strategies involved in a series of alcohol feeding studies with different dosing schedules and the initial recruitment of a pool of participants is decribed in Appendix C.

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CHAPTER V

THE FORMATION OF STABLE ACETALDEHYDE-HEMOGLOBIN ADDUCTS IN A RED BLOOD CELL MODEL

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The Formation of Stable Acetaldehyde-Hemoglobin Adducts in a Red Blood Cell Model

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GAPSTUR, S. M., E. G. DEMASTER, J. D. POTTER, J. D. BELCHER AND M. D. GROSS. *The formation of stable acetaldehyde-hemoglobin adducts in a red blood cell model.* ALCOHOL 9(6) 563–569, 1992.—The formation of stable hemoglobin adducts was examined (in the absence of an added reducing agent) in metabolizing red blood cells (RBCs) exposed to micromolar concentrations of acetaldehyde for up to 48 hours in vitro. The rapid disappearance of acetaldehyde due to oxidation by RBC aldehyde dehydrogenase was prevented by pretreating the cells with the inhibitor cyanamide. The RBCs remained viable for 48 hours (37°C) as determined by cell hemolysis and glycolytic activity. [^{14}C]acetaldehyde-modified hemoglobin was assessed in untreated and in cyanamide-pretreated cells. In untreated cells, after 3 hours of exposure to 50 and 200 nmol/ml of [^{14}C]acetaldehyde, the molar ratios of acetaldehyde to hemoglobin were 0.00069 and 0.0038, respectively; [^{14}C]acetaldehyde concentrations decreased to less than 4% of the initial levels within 3 hours. In cyanamide-pretreated RBCs, the molar ratios of acetaldehyde bound to hemoglobin ranged from 0.0013 after 3 hours of exposure to 20 nmol/ml [^{14}C]acetaldehyde up to 0.039 after 48 hours of exposure to 200 nmol/ml [^{14}C]acetaldehyde. Following tryptic digestion of [^{14}C]acetaldehyde-hemoglobin and separation of peptides by high-performance liquid chromatography, significant incorporation of [^{14}C]acetaldehyde was observed in nine peptides. Modifications of the labeled peptides remain to be characterized.

Acetaldehyde Acetaldehyde-modified hemoglobin Cyanamide Ethanol Hemoglobin
Red blood cells Acetaldehyde-hemoglobin adducts

ACETALDEHYDE, the primary oxidative metabolite of ethanol, readily reacts with hemoglobin in vitro, to form both stable and unstable modifications (21,24). Similarly, acetaldehyde-hemoglobin modifications occur in vivo (7). Elevated levels of acetaldehyde-modified hemoglobin have been detected in alcoholics by fluorigenic high-performance liquid chromatography (19) and, in normal volunteers after acute ethanol consumption, by immunologic assays (17). In addition, antibodies to acetaldehyde-modified proteins have been detected in the blood of alcoholics (16).

The formation of acetaldehyde-hemoglobin adducts is affected by acetaldehyde concentration (12,15,24) and other reaction conditions (28), factors which may differ significantly between in vivo and in vitro reactions. In addition, the metabolic activity of red blood cells may be essential for the formation of certain adducts (9,11,23). Thus, adducts formed in vivo may differ from those formed in vitro (30).

Acetaldehyde-hemoglobin adducts have potential as biochemical markers for alcohol consumption, however, the kinetics, sites, and structure of adducts formed in vivo have not been determined to date. Information on these aspects of acetaldehyde-hemoglobin adduct formation may greatly enhance the development of a biomarker for alcohol consumption. Detection, isolation, and characterization of acetaldehyde-hemoglobin adducts formed in vivo is difficult because they are present in very low amounts. The generation of acetaldehyde-hemoglobin adducts by the exposure of red blood cells to [^{14}C]acetaldehyde in vitro (at levels mimicking in vivo conditions) is an alternative approach for obtaining measurable amounts of these adducts.

The aim of this study was to develop a model system for examining acetaldehyde-hemoglobin adducts formed in vitro using intact red blood cells. Three important aspects of such a system required consideration. First, the long-term viability of red blood cells in vitro is essential (9) for exploring site-specific modifications. Second, inhibition of red blood cell aldehyde dehydrogenase (20) is necessary to sustain measurable, physiologic concentrations of acetaldehyde over long incubation periods. Third, the presence of highly reactive contaminants in commercially available preparations of

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radioactive acetaldehyde may cause misleading results (10). These three aspects were examined in developing the red blood cell model. Accordingly, the model system was used to: (1) examine the amount of acetaldehyde-modified hemoglobin as a function of incubation time and acetaldehyde concentration; (2) compare the amount of stable acetaldehyde-modified hemoglobin in cyanamide-pretreated and untreated red blood cells; and (3) determine the presence of peptide-specific acetaldehyde modifications of hemoglobin.

METHODS

Enzymes and Chemicals

[1-^{14}C]ethanol (50 mCi/mmol) was purchased from New England Nuclear Co. (Boston, MA). Glucose-oxidase, bovine liver catalase, Drabkin's reagent, Trinder reagents, thiodiglycol, and cyanamide were purchased from Sigma Chemical Co. (St. Louis, MO). Gentamicin was purchased from Gibco Labs (Grand Island, NY). *N*-tosyl-L-phenylalanyl chloromethyl ketone-trypsin (TPCK-trypsin) was purchased from Worthington Biochemical Corp. (Freehold, NJ). Liquid scintillation cocktail, Ecoscint A, was purchased from National Diagnostics (Manville, NJ). All other chemicals were of the highest purity and purchased from standard suppliers.

Generation of [^{14}C]Acetaldehyde From [^{14}C]Ethanol

A two-compartment closed system was used consisting of a 25-ml Erlenmeyer flask equipped with an upper, center well suspended from a rubber stopper (Kontes Scientific, Vineland, NJ). Sodium phosphate buffer, 20 mM, pH 7.4 (1 ml), was added to the bottom of the flask. An acetaldehyde-generating system, containing 0.1 M [^{14}C]ethanol (20–40 mCi/mmol), 0.3 M glucose, 0.1 mg/ml glucose-oxidase, 1 mg/ml catalase, and 37.5 mM sodium phosphate, pH 7.0, in a total volume of 0.2 ml, was placed in the upper center well. The reaction vessel was sealed and incubated in a shaking water bath (37°C) for 4 hours. The generated [^{14}C]acetaldehyde and a fraction of the [^{14}C]ethanol diffused from the center well into the phosphate buffer below. The reaction was stopped by removing the upper well from the reaction flask. An aliquot of the [^{14}C]acetaldehyde preparation in phosphate buffer was analyzed for acetaldehyde and ethanol content by headspace gas chromatography (2). Specifically, 20 μl of the preparation was expelled onto an azide-treated paper disk (Whatman No. 1 filter paper impregnated with 1 M sodium azide) contained within a glass septum vial. The samples were heated for 10 minutes at 55°C in the heating block of a Perkin-Elmer model Sigma 2000 gas chromatograph, equipped with an HS-100 headspace autosampler and a flame ionization detector. The chromatography conditions were as follows: column, 2 mm (i.d.) × 2 m glass packed with Tenax-GC, 80–100 mesh (Alltech, Deerfield, IL); column temperature, 85°C; carrier gas, nitrogen; carrier gas flow rate, 40 ml/min.

[^{14}C]acetaldehyde preparations were analyzed for the presence of contaminants by gas chromatography–mass spectrometry. The gas chromatograph–mass spectrometer was a LKB 9000 equipped with a Tenax, 80–100 mesh, 6 ft. × ⅛ in. steel column. The analysis was done under isothermal conditions at 140°C with helium as the carrier gas at a flow rate of 25 ml/min. Samples were prepared as described above and two ml of headspace were applied with a splitless injector. The mass spectrometer was operated at 70 eV, scans were taken at 1.2-second intervals, and data analyzed on a Teknibit 1050 data system. Sample blanks were analyzed and background subtracted from each unknown sample spectra.

Isolation of Red Blood Cells

Blood was drawn by venipuncture into EDTA-containing 15-ml tubes and immediately placed on ice. The blood was collected as needed from a single subject who had abstained from alcohol for a minimum of 5 days prior to the collection. The blood was centrifuged at 2500 rpm (4°C) for 15 minutes and the plasma was removed. Red blood cells were washed three times with ice-cold, sterile saline.

Preparation of Cyanamide-Treated Red Blood Cells

The washed red blood cells in each venipuncture tube were resuspended to a final volume of 15 ml with ice-cold sterile Tris buffer (150 mM), pH 7.4, containing 1.0 mM cyanamide, 12 mM glucose, 10 μg/ml glucose-oxidase, 50 μg/ml gentamicin, 5.0 mM adenine, 5.0 mM inosine, and 64 mM NaCl (preincubation buffer). The red blood cell suspensions were incubated at 37°C for 1 hour in a shaking water bath, followed by centrifugation at 2500 rpm (4°C) for 15 minutes and the supernatants were removed. The packed cells were washed twice with ice-cold, sterile red blood cell incubation buffer (RBC media) to remove unreacted cyanamide and glucose-oxidase. Finally, the washed, packed red blood cells were resuspended in RBC media to a volume of 15 ml. The RBC media (26) contained 12 mM glucose, 50 μg/ml gentamicin, 5.0 mM adenine, 5.0 mM inosine, 64 mM NaCl, and 150 mM Tris (final pH 7.4). Adenine and inosine were added to the cyanamide-pretreated cells to help maintain red blood cell viability over the 48-hour incubation period.

The cyanamide inhibition of red blood cell aldehyde dehydrogenase was confirmed by addition of unlabeled acetaldehyde (200 nmol) to 1 ml of: (a) RBC media; (b) untreated washed red blood cells; and (c) cyanamide-treated red blood cells. After incubation at 37°C for 0, 3, 24, and 48 hours, the samples were centrifuged at 2500 rpm (4°C) for 15 minutes and the concentrations of acetaldehyde in the supernatants were determined by headspace gas chromatography as described above.

Reaction of [^{14}C]Acetaldehyde With Metabolizing Red Blood Cells

Cyanamide-treated and untreated red blood cells were incubated with 50 and 200 nmol/ml of [^{14}C]acetaldehyde for 3 hours (37°C) in sterile RBC media. A 40% suspension of red blood cells (0.9 ml), was added to sterile 12 × 75 mm capped polystyrene Falcon tubes followed by the addition of generated [^{14}C]acetaldehyde and sterile RBC media to yield a final volume of 1.0 ml. The initial acetaldehyde/hemoglobin molar ratio was 0.10. After incubation, the samples were centrifuges at 2500 rpm (4°C) for 15 minutes and the supernatants were removed. Red blood cells were washed seven to nine times with 3-ml aliquots of cold, sterile saline to remove the unreacted [^{14}C]acetaldehyde. The removal of the unreacted [^{14}C]-acetaldehyde was monitored by counting the radioactivity present in the supernatant. When radioactivity, counted in successive wash supernatants, remained less than 100 disintegrations per minute (DPM) per 50 μl, washing was discontinued. The packed cells were resuspended with 0.75 ml of sterile, cold saline. Total hemoglobin was measured using Drabkin's reagent (3).

The association of [^{14}C]acetaldehyde with intact red blood

cells was determined by measuring the radioactivity in a 0.025-ml aliquot of the red blood cell suspension added to 5 ml of Ecoscint A and counted on a Beckman Model LS 3801 scintillation counter. The amount of [^{14}C]acetaldehyde bound to hemoglobin was computed from the specific activity of the [^{14}C]acetaldehyde, the amount of bound [^{14}C]acetaldehyde, and the hemoglobin concentration. Bound [^{14}C]acetaldehyde was expressed as moles of acetaldehyde per mole of hemoglobin. Because there is evidence that acetaldehyde binds to erythrocyte membrane proteins (6), the amount of bound [^{14}C]acetaldehyde was also determined in a red blood cell hemolysate. To prepare the hemolysate, the red blood cell suspensions were centrifuged at 2500 rpm (4°C) for 15 minutes and the supernatant removed. To hemolyze the cells, 0.75 ml of H_2O was added and the samples vortexed for 1 minute. The samples were transferred to 1.5-ml microfuge tubes, toluene (0.4 ml) was added, and the samples were continuously mixed for 20 minutes at 4°C. To remove the cell membranes, the samples were centrifuged at 10,000*g* for 20 minutes in a Beckman model E microfuge. The hemolysates were transferred to new microfuge tubes and, after first removing a portion for measuring total hemoglobin and radioactivity, immediately frozen in a dry ice/acetone bath, and stored at −70°C. The amount of [^{14}C]acetaldehyde bound to hemoglobin was calculated and expressed as described above for intact red blood cells.

The effect of incubation time and acetaldehyde concentration on the amount of [^{14}C]acetaldehyde bound to hemoglobin was investigated by incubating the cell suspensions at 37°C for 3, 24, or 48 hours in the presence of 20, 50, or 200 nmol/ml [^{14}C]acetaldehyde. At the end of each incubation period, the suspensions were processed as above.

Assessment of Red Blood Cell Viability

Cell viability was monitored over the 48-hour period in duplicate sets of samples incubated with unlabeled acetaldehyde and ethanol by: (a) degree of hemolysis; (b) glucose utilization; and (c) change of media pH. The degree of hemolysis was determined spectrophotometrically (415 nm) by comparing the absorbance in the supernatant with the absorbance after hemolysis of the remaining intact red blood cells with 0.6 ml of H_2O. Glucose utilization was determined by the Trinder reaction (25): briefly, after each acetaldehyde incubation, the supernatant was removed and a 10% red blood cell suspension was prepared in an isotonic glucose incubation media (26) containing 12 mM glucose, 50 mM Tris-base, 25 mM Na_2PO_4, and 83 mM NaCl, pH 7.8, and incubated for 1 hour (37°C). After centrifugation (2500 rpm for 15 minutes), the glucose concentration was measured in the supernatant. Glucose utilization was computed per gram of hemoglobin.

Analysis of Hemoglobin Peptides by HPLC

Reverse-phase HPLC was used to separate and identify stable [^{14}C]acetaldehyde-hemoglobin peptides in tryptic digests: briefly, tryptic digests were prepared (1) by diluting 1 mg of hemoglobin from each incubation condition to 0.165 ml with distilled water in 0.3-ml autosampler vials, followed by the addition of 0.055 ml of 0.5 M NH_4HCO_3 buffer (pH 8.2) containing 0.5 mM $CaCl_2$, 0.00275 ml 1% thiodiglycol (vol/vol in ethanol), and 0.055 ml of 1 mg/ml TPCK-trypsin (in 0.1 mM HCl). The vial was sealed under nitrogen and the digestion allowed to continue for 30 minutes at 4°C. The reaction was stopped by addition of 0.015 ml trifluoroacetic acid (10%). The samples were immediately frozen in a dry ice/acetone bath and stored at −70°C until analyzed by HPLC.

The HPLC system included a Beckman System Gold (Fullerton, CA) consisting of a 507 autosampler, an Altex manual injector, a 168 diode array detector module, a 116 programmable solvent module, and a Vydac (Herpsian, CA) reverse-phase C_{18} column (0.46 cm × 25 cm) with a C_2 guard column (Chromtech, Apple Valley, MN). The digested samples were injected and the peptides were separated (22) with a linear gradient of solvent A (0.1% trifluoroacetic acid in water) and B (0.1% trifluoroacetic acid in acetonitrile) using a flow rate of 1 ml/min. The gradient was from 0–45.8% solvent B in 100 minutes, followed by a second gradient of 45.8–66.0% solvent B in 10 minutes. One-minute fractions were collected using a fraction collector equipped with an ice-cold water bath. Liquid scintillation cocktail (5 ml) was added to each 1-ml collected fraction.

RESULTS

Others have reported the presence of condensation products of acetaldehyde, such as aldol and crotonaldehyde, in commercial preparations of [^{14}C]acetaldehyde. Like acetaldehyde, these substances can also react with hemoglobin and form [^{14}C]labeled adducts (10). To avoid this potential problem, [^{14}C]acetaldehyde was produced enzymatically from [^{14}C]ethanol, as needed, using the two compartment closed system described in Methods. Newly formed [^{14}C]acetaldehyde and a fraction of the [^{14}C]ethanol diffused into the second compartment producing a solution containing an average concentration of 3.9 mM [^{14}C]acetaldehyde and 13.2 mM [^{14}C]-ethanol in phosphate buffer. Various amounts of the [^{14}C]-acetaldehyde-ethanol preparations were analyzed by gas chromatography to determine the number of components present. Two components accounting for at least 99.9% of all volatile components were detected. These two components were analyzed by gas chromatography–mass spectrometry. The mass spectra are shown in Fig. 1. Component 2 was ethanol (EtOH) (Fig. 1A). The mass spectra of our sample (EtOH) was consistent with published mass spectra (8), and the mass spectra of a standard analyzed on our system. Mass spectra published for acetaldehyde (8) and from the analysis of a standard acetaldehyde preparation were consistent with the mass spectra of component 1 (Fig. 1B). These results indicate ethanol and acetaldehyde were the only volatile components present in the generated acetaldehyde-ethanol preparations.

In order to expose red blood cells to acetaldehyde during long incubation periods, red blood cell aldehyde dehydrogenase activity was inhibited by pretreating cells with cyanamide as described in Methods. The stability of [^{14}C]acetaldehyde added to cell-free media, red blood cells, and cyanamide-pretreated red blood cells over time is shown in Fig. 2. There was essentially no change in the concentration of acetaldehyde in the cell-free media over 48 hours. The acetaldehyde concentration in the supernatant of the untreated red blood cells decreased to 3.3% of the original concentratin within 3 hours and remained depressed throughout the 48-hour incubation period. In contrast, the acetaldehyde concentration in the supernatant of the cyanamide-pretreated red blood cells remained unchanged at 3 hours, and thereafter gradually increased with time. This suggests that the ethanol present in the acetaldehyde preparation was slowly oxidized to acetaldehyde during the long incubation period. Although the initial concentration of acetaldehyde added to each preparation was 200 nmol/ml, the free acetaldehyde concentrations were significantly reduced due to the known reversible interactions of

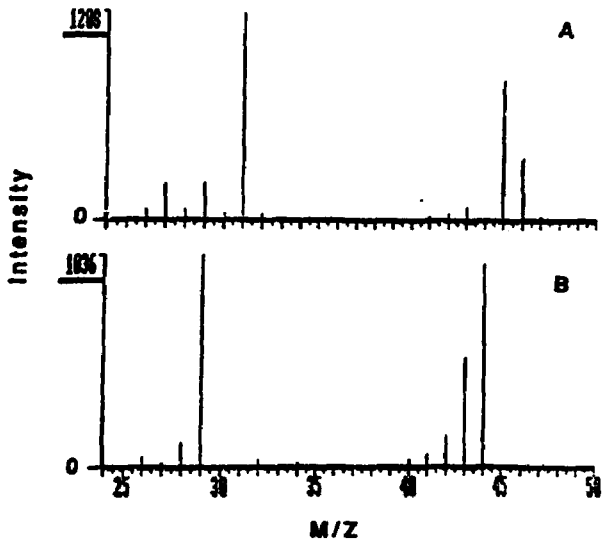

FIG. 1. Mass Spectra of the [^{14}C]acetaldehyde-ethanol preparations: (A) component two [^{14}C]ethanol, and (B) component one [^{14}C]acetaldehyde. The mass/charge (M/Z) ratio is given on the *x*-axis and signal intensity is given on the *y*-axis.

Tris with aldehydes (13). Standard curves for acetaldehyde prepared in isotonic saline and the Tris-RBC media over a range of 0–200 nmol/ml were linear and showed that the Tris decreased the free acetaldehyde levels by 60% (data not shown). Therefore, the expected concentration of free acetaldehyde is 40% of the initial value, as shown by the measured concentration at time zero in Fig. 2.

There were no differences in the amount of [^{14}C]acetaldehyde/mg hemoglobin measured in intact red blood cells compared to hemolyzed red blood cells after removal of the membranes (data not shown).

In hemolyzed samples, after removal of the membranes, the molar ratio of [^{14}C]acetaldehyde bound to hemoglobin in control and cyanamide-pretreated red blood cells was determined following a 3-hour incubation in the presence of 50 or 200 nmol/ml [^{14}C]acetaldehyde (Fig. 3). For these levels of acetaldehyde, the amount of [^{14}C]acetaldehyde bound to hemoglobin in cyanamide-pretreated red blood cells was three- to four-fold greater than that bound to hemoglobin in the untreated red blood cells. This difference in adduct formation most likely reflects the effect of cyanamide treatment on the free acetaldehyde concentration as shown in Fig. 2.

The molar ratio of [^{14}C]acetaldehyde bound to hemoglobin in intact red blood cells was also examined as a function of both free acetaldehyde concentration and incubation time (Fig. 4). Cyanamide-pretreated red blood cells were incubated for 3, 24, and 48 hours with three different amounts of [^{14}C]-acetaldehyde. For each incubation time, the molar ratio of [^{14}C]acetaldehyde bound to hemoglobin increased linearly as a function of free [^{14}C]acetaldehyde measured in the reaction media. The amount of [^{14}C]acetaldehyde bound to hemoglobin also increased with time.

Duplicate samples incubated with various concentrations of unlabeled acetaldehyde were monitored to determine cell viability over the 48 hours of incubation (Table 1). The degree of hemolysis remained relatively low throughout the time period with only slight increases observed at 24 and 48 hours. Red blood cells showed glycolytic activity under all of the incubation conditions. The higher glycolytic activity of those cells incubated for 24 and 48 hours may be the result of increased cell hemolysis. That is, hemoglobin released during hemolysis is free to bind to glucose which may result in a falsely elevated glycolytic activity. The pH of the incubation media decreased from pH 7.3 at zero time to pH 6.86 at 48 hours, indicating lactic acid production from glucose metabolism.

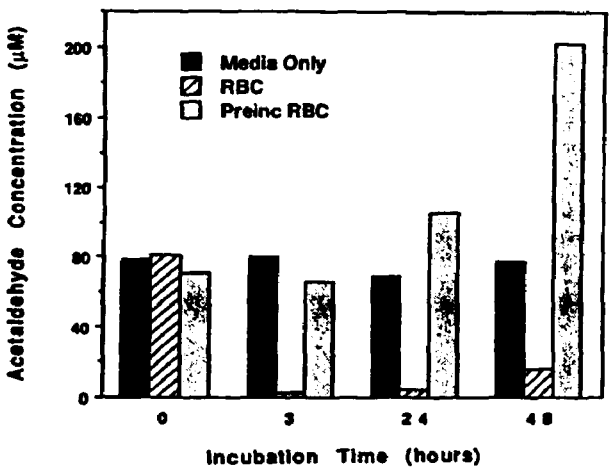

FIG. 2. Time course for free acetaldehyde concentrations in incubations containing untreated red blood cells (RBC), cyanamide-pretreated red blood cells (Preinc RBC) and media only. Experimental values shown represent means of duplicate incubations.

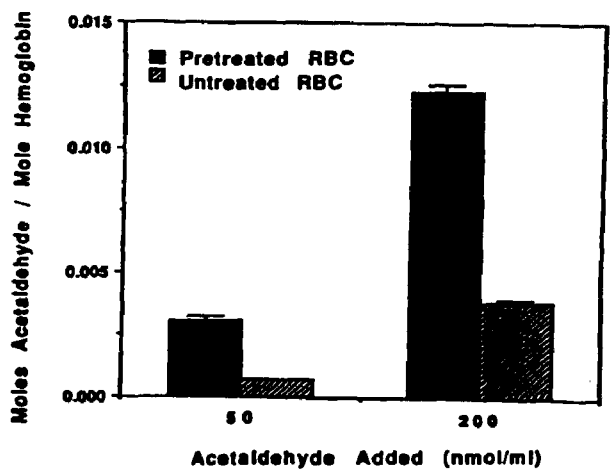

FIG. 3. Effect of cyanamide pretreatment on acetaldehyde-hemoglobin adduct formation. Intact red blood cells were incubated with 50 or 200 nmol/ml [^{14}C]acetaldehyde for 3 hours. The results are the mean values (± SEM, n = 3) after removal of the RBC membranes.

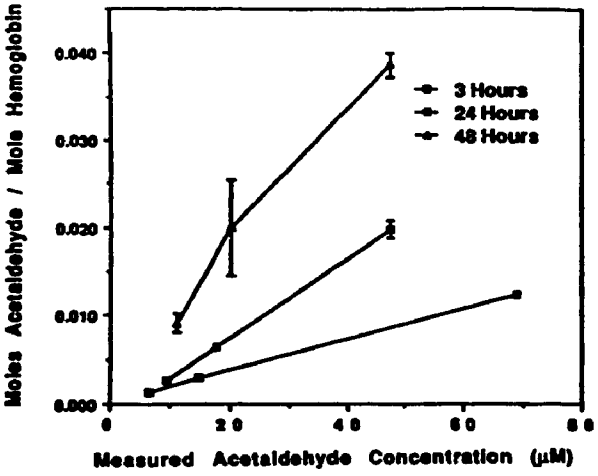

FIG. 4. Effect of various [^{14}C]acetaldehyde concentrations and incubation times on acetalhyde-hemoglobin adduct formation in cyanamide-pretreated, red blood cells. The results represent the mean ± SEM (n = 3) after removal of the membranes.

Using reverse-phase HPLC to separate the tryptic digests of [^{14}C]acetaldehyde-modified hemoglobin isolated from cyanamide-pretreated red blood cells incubated for 48 hours with 200 nmol/ml [^{14}C]acetaldehyde, nine peptides were identified which contained a minimum of 10 DPM (disintegrations per minute) over background (Fig. 5). The total number of counts in each peak ranged from 15,133 DPM in peak 1 to 23 DPM in peak 8. Characterization of each peak by mass-spectrometry and amino acid analysis is necessary in order to determine accurately the number of different binding sites; peaks 1 and 2 coeluted with peptides which were modified at

TABLE 1

RED BLOOD CELL VIABILITY IN CYANAMIDE-PRETREATED RED BLOOD CELLS

Incubation Condition		Measures of Cell Viability		
Time (h)	Acetaldehyde Concentration (nmol/ml)	pH	Glucose utilization (μmol glucose/g Hb*/h)	Hemolysis (%)
0	20	7.29	12.2	1.5
	50	7.30	12.0	1.1
	200	7.29	12.8	0.5
3	20	7.16	16.8	1.8
	50	7.23	15.1	2.7
	200	7.25	11.2	1.8
24	20	6.98	22.0	7.5
	50	6.93	20.4	3.5
	200	6.92	11.2	2.5
48	20	6.86	57.0	5.5
	50	6.88	49.8	3.5
	200	6.87	50.3	2.8

*Hb: hemoglobin.

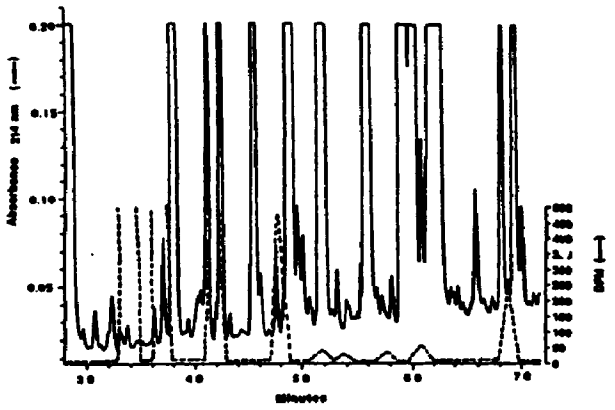

FIG. 5. Separation of the tryptic digests of [^{14}C]acetaldehyde-modified hemoglobin by reverse-phase HPLC. The peaks are numbered left to right. Peaks 1, 2, and 3 contained 15,133, 7032, and 889 disintegrations per minute (DPM), respectively.

the N-termini valine of both the α and β chains of hemoglobin already identified in our previous experiments (7).

DISCUSSION

Criteria for establishing an objective marker of usual alcohol consumption include the following: (a) exposure to alcohol results in the formation of stable products with relatively long half-lives; (b) product formation is dose-dependent; (c) physiologic concentrations can be quantified; and (d) additional products do not form as a result of artifactual processes.

An early step in the development of a marker is the design of an in vitro model that closely mimics in vivo conditions. We have described an in vitro model system using human intact red blood cells. The model was designed to expose these cells to amounts of acetaldehyde under conditions which approach those associated with chronic alcohol consumption. This model allows for maintenance of cell viability over long incubation periods, inhibition of aldehyde dehydrogenase in red blood cells in order to sustain elevated concentrations of acetaldehyde, and the use of contaminant-free acetaldehyde.

Cell hemolysis and glycolytic activity were used as the primary indicators for monitoring red blood cell viability over the lengthy incubation period. Over 90% of the cells remained intact after 48 hours (Table 1). Glycolytic activity was not only maintained but actually appeared to increase with time. This apparent increase in glucose utilization is probably due to binding of glucose to released hemoglobin following hemolysis; however, this possibility was not established experimentally. Although there was some indication of decreased cell viability, the overall condition of the red blood cells appeared satisfactory for use in this in vitro model.

Ethanol-derived acetaldehyde spills over from the liver into the systemic circulation. During chronic consumption of ethanol, a low concentration of acetaldehyde in the range of 1 to 20 μM (18) may persist over prolonged periods of time. To reproduce these conditions in our in vitro system, it is necessary to pretreat the red blood cells with cyanamide, an inhibitor of aldehyde dehydrogenase (4,14). Inhibition of red blood cell aldehyde dehydrogenase by cyanamide-pretreatment

blocked the rapid loss of acetaldehyde from the system (Fig. 2). Elevated levels of free acetaldehyde could be maintained for 48 hours in the presence of the cyanamide-pretreated red blood cells. At the longer incubation times, the concentration of free acetaldehyde actually increased above the starting concentrations. Since the [^{14}C]acetaldehyde preparations also contained ethanol, this increase must reflect oxidation of ethanol to acetaldehyde and may be due to the persistent artifactual formation of acetaldehyde known to occur in red blood cells (5).

The amount of stable acetaldehyde-hemoglobin adducts has been shown to increase proportionally to increasing acetaldehyde concentrations when incubated with purified hemoglobin preparations in vitro (12,15,24). The amount of these acetaldehyde-modifications increased with successive exposures (24) and increasing incubation time (15). In addition, the formation of stable acetaldehyde-adducts on bovine serum albumin is time dependent (29). Incubation of intact red blood cells with acetaldehyde concentrations ranging from 0-5 mM resulted in 6-83% of altered hemoglobin as determined by cation exchange chromatography (27). Using our in vitro red blood cell system, the amount of acetaldehyde bound to hemoglobin also increased proportionally to the concentration of acetaldehyde (Fig. 3). However, the concentrations of free acetaldehyde used in these experiments were much lower than those used in other studies, thus more closely mimicking the concentrations present in vivo after chronic alcohol exposure. A time-dependent relationship also was observed.

Acetaldehyde may form stable adducts at several sites when incubated with purified hemoglobin. Modifications have been identified on at least two sites (21), the *N*-terminal valine residues of both the α and β chains of hemoglobin. HPLC analysis of the labeled hemoglobin generated in our experiments, under conditions which exposed intact red blood cells to the highest acetaldehyde concentration for the greatest amount of time, showed nine modified peptides. However, reactivity of different sites on the protein may be time and dose-dependent. That is, differences in the reactivity of acetaldehyde (21) and glucose (23) with the *N*-termini of hemoglobin have been observed in other studies. Therefore, the exact relationship between acetaldehyde dose or incubation time and site-specific modifications found in this study will require characterization of each unique site of modification. At present, we are characterizing acetaldehyde-labeled peptides to determine the number of unique sites. However, reaction products of ethanol metabolites and hemoglobin formed in vitro may differ from those formed in vivo both quantitatively, and in relation to site-specificity. Nonetheless, confirmation of the modification sites on isolated hemoglobin is an essential step in the verification of in vivo adduct formation.

A red blood cell model system may be useful for examining acetaldehyde-hemoglobin adducts formed under a variety of metabolic conditions. For example, stable acetaldehyde-protein adducts formed on lysine residues were enhanced by ascorbic acid, a physiologic reducing agent which is present in vivo (28). Using the red blood cell model system described above, the effect of ascorbic acid at physiologic levels on stable acetaldehyde-hemoglobin adduct formation may be explored. In red blood cells, acetaldehyde can be metabolized to 5-deoxy-D-xylulose-1-phosphate (DXP) (9), which may form stable adducts with hemoglobin. The reaction of DXP as well as other metabolic products of ethanol and acetaldehyde with hemoglobin may also be examined using a red blood cell model. In addition, examination of the reaction products between alcohol metabolites and hemoglobin from a red blood cell model may provide information about the structure, stability, and dose-dependency of the adducts formed. This information may facilitate the development of objective measures for quantifying alcohol consumption including, particularly, the identification of appropriate targets for immunologic methods.

The results of this study provide evidence that stable acetaldehyde-hemoglobin adducts are formed in metabolizing red blood cells exposed to concentrations of acetaldehyde ranging from 6-70 μM. Inhibition of red blood cell aldehyde dehydrogenase substantially increased adduct formation. The amount of hemoglobin adduct formed in metabolizing red blood cells is dependent on the free acetaldehyde concentration and on incubation time. In addition, tryptic digests of [^{14}C]acetaldehyde-hemoglobin peptides can be separated by reverse-phase high-performance liquid chromatography for further characterization and comparison of the modifications formed under varying incubation conditions.

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CHAPTER VI

SUMMARY

The influence of usual alcohol consumption on the risk of colon, rectal and endometrial cancer was explored in The Iowa Women's Health Study, a large cohort study of postmenopausal women. An inverse association between usual alcohol consumption and colon cancer, probably limited to the distal colon was observed. There was a nonsignificant increased risk of rectal cancer with increasing alcohol consumption. After exploring potential effect modification by other risk factors, there appeared to be a significantly higher risk of rectal cancer among women who were vigorously active and drank alcohol compared to less active women and alcohol abstainers. Further, alcohol was not associated with the incidence of endometrial cancer in this prospective study, nor was there any evidence of effect modification by other endometrial cancer risk factors.

In the absence of experimental data, criteria used to judge causality for exposure-disease associations, such as those between alcohol and each site-specific cancer considered in this thesis, include consistency, strength, dose-response, specificity, temporality and coherence. After considering all of these criteria, the net effect of alcohol consumption on risk of colon and rectal cancer indicates there may be a weak association that is not specific to any type of alcoholic beverage. Because of the inconsistencies among the few studies published to date, there is no clear evidence to support a causal association between alcohol and endometrial cancer.

Observational epidemiologic research of noninfectious diseases, such as cancer, has been criticized for reporting the presence of weak, cause-effect relations in the absence of experimental methods. This is particularly true for studies published in prominent journals that receive publicity by the mass media; clearly, the public's reaction to these reports is the major concern. Therefore, the question arises, are traditional epidemiologic methods adequate for assessing the strength of exposure-chronic disease associations? There has

been considerable support for incorporating biochemical and molecular biological methods into epidemiologic research. Cooperation between these two disciplines not only will enhance the understanding of biologic processes involved in the development of cancer and other chronic diseases but also will allow epidemiologists to evaluate exposure more objectively.

In this thesis, I have presented results for a first step in the development of an objective biological marker for alcohol consumption. The in vitro formation of acetaldehyde-modified hemoglobin in viable red blood cells was dependent on both dose of acetaldehyde and length of incubation time. Also, using HPLC, preliminary results indicated these modification were stable and may be specific to certain amino acid residues on the hemoglobin molecule.

Although a biologic marker for measuring alcohol consumption will provide epidemiologists with an objective tool for assessing usual intake, its shortfall may be in determining past alcohol exposure. Thus, better questionnaires that query individuals about their lifetime history of alcohol consumption are also needed. Incorporating these two methods will allow epidemiologists to gather more accurate, alcohol exposure information.

The detrimental role of heavy alcohol consumption and abuse on public health is well recognized because of its contribution to the occurrence of cirrhosis of the liver, stroke, accidents, homicides and suicides. Conversely, it is believed low alcohol intake may have a beneficial influence on coronary heart disease, the primary cause of mortality among adults in the United States. Regarding carcinogenicity, there are consistent data from animal experimental studies and human epidemiologic studies showing alcohol is one of

the primary risk factors for cancer of the oral cavity, pharynx, larynx, esophagus, and the liver. In addition, epidemiologic data are suggestive of a weak, positive association between alcohol consumption and breast cancer, the most commonly occurring cancer among women.

In conclusion, from a public health perspective, evidence indicates drinking moderate amounts of alcohol is not detrimental. In terms of individual decision making, consideration must be given to personal risk factor profiles; for example, a women who is at low risk of coronary heart disease but high risk for colon or rectal cancer may consider limiting her intake of alcoholic beverages. More research on the association between alcohol and site-specific cancers focusing high-risk groups and better measurement methods for both recent and past consumption is needed to better understand whether alcohol is indeed causally related to cancers where consistent, weak associations have been observed.

APPENDIX A

INCREASED RISK OF BREAST CANCER WITH ALCOHOL CONSUMPTION IN POSTMENOPAUSAL WOMEN

Increased Risk of Breast Cancer with Alcohol Consumption in Postmenopausal Women.

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Abbreviations: RR, relative risk; CI, confidence interval; df, degrees of freedom.

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Running Head: Alcohol consumption and breast cancer

ABSTRACT

The association between breast cancer incidence and alcohol consumption among postmenopausal women was examined in the Iowa Women's Health Study. In January 1986, a cohort of 41,837 postmenopausal women, aged 55-69, completed a questionnaire that included alcohol intake and other information. Through December 1989, 493 incident breast cancer cases were identified. Age-adjusted relative risks (RR) of consumption of < 1.5, 1.5-4.9, 5.0-14.9 and ≥ 15.0 g of alcohol per day compared to abstention were 1.08, 1.10, 1.08 and 1.28 respectively (p for trend = 0.11). After controlling for age, body mass index, age at first live birth, age at menarche, and family history of breast cancer, the RR were 1.18, 1.20, 1.25 and 1.46 (p for trend = 0.04). Multivariate modeling, using Cox proportional hazards regression, revealed a significant multiplicative interaction between alcohol intake and noncontraceptive estrogen use. The RR of breast cancer associated with average daily alcohol consumption of 5.0-14.9 and ≥ 15.0 g were 1.88 (95% confidence interval (CI) 1.30-2.72) and 1.83 (95% CI 1.18-2.85), respectively, among ever-users of estrogen; no association between alcohol and breast cancer was observed among never-users of estrogen.

Am J Epidemiol

Key Words: alcohol drinking, breast neoplasms, cohort studies, epidemiology, estrogen replacement therapy

INTRODUCTION

Breast cancer is the most common cancer among women in the United States, and is a major public health concern. It is estimated that one in nine women will develop breast cancer in their lifetime (1). Thus, identifying those risk factors amenable to modification would be useful for implementing primary prevention strategies. In 1977, Williams and Horm (2) reported results from the Third National Cancer Survey, a population-based cross-sectional study of incident cancers, suggesting for the first time that alcohol consumption may be related to breast cancer. This report lead to an extensive examination of the alcohol-breast cancer relationship.

In a recent review, Hiatt (3) summarized most of the studies that examined the association between breast cancer and alcohol consumption. He noted that 11 of 17 case-control studies (2, 4-20), five of the six cohort studies (21-26) and one meta-analyses (27) have shown a positive association. Three of four case-control studies (28-31) and one meta-analysis (32) published between 1989 and 1991 also reported a positive association. In general, the relative risks (RR) and odds ratios for developing breast cancer ranged from 1.4 to 2.0 for women who consumed a moderate amount of alcohol compared to abstainers (3). The meta-analysis conducted by Longnecker et al. (27) suggested a dose-response relationship in both case-control and cohort studies with RR of 1.4 and 1.7, respectively, for women who consume on average $\geq$ 24 g of alcohol daily compared to abstainers. Thus, although the excess risk tends to be low, results across studies are consistent and appear to show a dose-response. Furthermore, since over 18 percent of women report consumption of at least 1 drink per day (33), the population attributable risk of breast cancer is at least as high as 14 percent (3,25,27).

Several studies have addressed the influence of menopausal status on the association between breast cancer and alcohol intake. A positive association was noted for postmenopausal women in 2 (24,25) of the 4 cohort studies that stratified on menopausal status (23-26) with RR up to 4.2 (25) for consumption of 6 or more drinks per day; however, data from the Framingham cohort (26) were suggestive of an inverse relationship between alcohol and breast cancer for postmenopausal women. Odds ratios for five (4, 11, 12, 14, 30) of eight case-control studies showing a positive association were consistent across strata of menopausal status. However, two case-control studies (13, 29) found a positive association for premenopausal women, and no association for postmenopausal women; results from a population based case-control study in Australia (16) were suggestive of a dose-response relationship between alcohol and breast cancer among premenopausal women and, more weakly, also among postmenopausal women.

The Iowa Women's Health Study is a large cohort study of postmenopausal women. The association between alcohol and breast cancer incidence in postmenopausal women can be effectively examined in the Iowa Women's Health Study cohort, including exploration of potential modifying effects of other breast cancer risk factors.

MATERIALS AND METHODS

The Iowa Women's Health Study cohort

The Iowa Women's Health Study is a longitudinal cohort study designed to examine the association between several risk factors and the incidence of cancer in postmenopausal women, age 55-69 at baseline. Study participants were selected randomly from Iowa's 1985 Department of Transportation's drivers license list, (which contained approximately

94 percent of all age-eligible women living in Iowa (34)). In January 1986, 99,826 selected women were mailed a questionnaire and a letter describing the purpose of the study. From the original sample, 1,796 women were ineligible for participation (wrong age or gender); 41,837 eligible questionnaires were returned.

The population at-risk of developing breast cancer excluded women who at baseline reported 1) a prevalent cancer other than skin cancer, 2) menstruating within the past year, or 3) a prior mastectomy. The "at risk" cohort for the final analysis was 37,105.

Measurement of alcohol intake and other risk factors

Information on the major breast cancer risk factors was ascertained by the questionnaire. Self-reported items included birth date, race, education, reproductive and menstrual history, use of oral contraceptives and noncontraceptive estrogens, family history of breast cancer, and personal history of cancer. Participants also were asked to report their current height and weight, as well as weight history. To assess body fat distribution, we enclosed a paper tape measure along with detailed instructions for circumference measurements of the waist, hips, upper arm and lower leg (35).

Usual dietary and alcohol consumption were assessed using the Harvard semi-quantitative food frequency questionnaire developed by Willett et al. (36). Participants recorded their average daily alcohol intake over the last year according to one of nine possible frequency responses: never or less than one drink per month, 1-3 per month, 1 per week, 2-4 per week, 5-6 per week, 1 per day, 2-3 per day, 4-5 per day and ≥6 per day. Frequencies were recorded separately for red wine, white wine, beer, and liquor. Alcohol intake, expressed in grams per day was computed with the aid of the Harvard Nutrient Data Base: the frequency with which each beverage was consumed was multiplied by the ethanol

content of the specific beverage, (10.8 g of ethanol per 4 ounce glass of red or white wine, 13.2 g per bottle or can of beer, and 15.1 g per drink or shot of liquor). Average daily alcohol intake was computed by summing the contribution from each type of alcoholic beverage. Abstainers were those women whose reported usual daily alcohol intake was 0 g.

Of the baseline questionnaires returned, 3.8 percent of the women had intake information blank for all four alcohol beverages. Total daily alcohol consumption for these women was assumed, for analysis, to be 0 g per day. Analyses were repeated in which alcohol intake was considered missing and the results were not significantly different.

Follow-up of cohort and identification of breast cancer cases

The cohort was followed for 4 years to determine incident breast cancer cases. Two follow-up mail surveys for vital status and address change were conducted in October, 1987 and August, 1989. The status of non-respondents to the follow-up surveys was determined by the National Change of Address service to identify women who had moved out of Iowa, and by the National Death Index to identify out-of-state deaths. The vital status for all but 197 women (0.5 percent) has been determined.

Incident breast cancers were identified using the Health Registry of Iowa, part of the National Cancer Institute's Surveillance, Epidemiology and End Results program; identification involved cross-matching cases from 1986-1989 with IWHS participants using a combination of first and last names, maiden name, zip code, birthdate, and Social Security number. Of the 493 incident breast cancer cases identified, 48 were carcinomas in situ. Analysis included all 493 cases; excluding those diagnosed with carcinomas in situ did not change the results.

Data analysis

Person-years of follow-up for each individual were computed as the amount of time since completion of the baseline questionnaire to one of the following events: 1) breast cancer diagnosis, 2) death (if in Iowa) 3) a move out of Iowa (if known), 4) midpoint of interval between last contact date and either date of next follow-up or December 31, 1991 (end of follow-up) if date of move was unknown, or 5) midpoint of interval between date of last contact and date of death (for non-Iowa deaths).

Women were classified according to five levels of alcohol intake: 0 g per day (abstainers), < 1.5 g per day, 1.5-4.9 g per day, 5.0-14.9 g per day, and ≥ 15 g per day. Alcohol intake was set to missing for 1 non-case whose reported daily intake was 299 g, more than twice as much as the next highest reported intake and equivalent to a usual intake of approximately 20-30 drinks per day. Cutpoints for quintiles of Quetelet body mass index and waist-to-hip ratio were determined from the distributions in the total at-risk cohort.

Incidence rates were calculated by dividing the number of events by the person-years of follow-up. RR and 95 percent confidence intervals (CI) (37), were computed within categories of several potential breast cancer risk factors with adjustment for five-year age categories: 55-59, 60-64, and 65-69. Tests for linear trend based on Mantel's-extension were computed to examine dose-response (38).

Possible confounding was examined by comparing the proportion of women within two categories of alcohol consumption (split at the median) across strata of other breast cancer risk factors. To determine whether these variables modified the association between alcohol and breast cancer, age-adjusted RR for the alcohol-breast cancer association were

compared across strata of the covariates.

Cox proportional hazards regression was used to analyze the association between breast cancer and alcohol consumption while simultaneously controlling for age, and other potentially confounding factors as well as to test for potential multiplicative effect modification. Analysis was performed using PROC PHREG of the SAS statistical package (39). The proportional hazards assumption was tested and confirmed.

The associations between breast cancer and specific types of alcoholic beverage were examined using Cox proportional hazards regression and indicator variables for beer, wine, and liquor. A single dose-response model was tested incorporating potential confounders and three sets of two indicator variables (one set for each type of alcoholic beverage: wine, beer, liquor) to represent three categories of alcohol consumption: 0, < 4.0 and ≥ 4.0 g per day.

RESULTS

Over the four years of follow-up, the at risk cohort of 37,105 women contributed 140,704 person-years. A total of 493 cases of breast cancer were identified. Approximately 57 percent of the non-cases and 54 percent of the cases reported drinking no alcohol.

Table 1 shows the age-adjusted RR of breast cancer for several potential risk factors including alcohol intake. Increasing body mass index, waist-to-hip ratio, older age at first live birth, and a history of breast cancer in a first degree relative (mother, sister, daughter) were all significantly and positively associated with breast cancer. There was an inverse

association of breast cancer with body mass index at age 18, and age at menarche. In the age-adjusted analysis, any alcohol intake was associated with an elevated but nonsignificant risk of breast cancer (RR = 1.14, 95 percent CI 0.95-1.35) and there was suggestion of a weak dose-response relationship. Education, age at menopause, parity, use of oral contraceptives, and use of noncontraceptive estrogens were not associated with age-adjusted breast cancer incidence.

The proportion of women who consumed alcohol was examined across strata of other breast cancer risk factors within three categories (nondrinkers versus drinkers split at the median) of alcohol intake: 0, < 4.0 and ≥ 4.0 g per day (table 2). Women who consumed ≥ 4 g of alcohol daily had higher education levels, lower body mass indices and waist-to-hip ratios, and a higher prevalence of oral contraceptive and noncontraceptive estrogen use. Cox proportional hazards regression was performed to control for the potential confounding effects of age, body mass index, age at menarche, age at first birth and family history of breast cancer. Table 3 shows the multivariate relative risks of breast cancer for each of the five levels of alcohol consumption that are shown as age-adjusted estimates in Table 1. Inclusion of the other risk factors in the model did not materially change the results.

To determine whether the association between alcohol and breast cancer differed across levels of other risk factors, age-adjusted relative risks for three levels of alcohol intake were calculated within strata of the non-alcohol risk factors (table 4). Across most strata, the alcohol-breast cancer relationship appeared consistent. However, the association between alcohol and breast cancer risk for ≥ 4 g versus 0 g per day was greatest for women in the lowest quintile of body mass index (RR = 2.26, 95 percent CI 1.33-3.82). Women who reported a later age at menarche had a higher risk of breast cancer with ≥ 4 g

of alcohol per day than women reporting a younger age at menarche. The RR for women who consumed > 0 but < 4 g of alcohol per day and who had a family history of breast cancer was 1.81 (95 percent CI 1.09-3.02) compared to a RR of 1.01 (95 percent CI 0.79-1.30) for those without a family history. Noncontraceptive estrogen use also appeared to modify the alcohol-breast cancer relationship.

There was no evidence of a statistically significant, multiplicative interaction between alcohol and body mass index or age at menarche when tested in multivariate analysis including terms for age, body mass index, age at menarche, age at first birth and family history of breast cancer. Although the multiplicative interaction between family history of breast cancer and alcohol consumption also was not statistically significant (χ^2= 6.77, df = 4, p = 0.15) in multivariate analysis, family history of breast cancer did appear to influence the relationship between alcohol and breast cancer incidence. The RR and 95 percent CI of breast cancer for women who were family history positive and whose average daily alcohol consumption was < 1.5, 1.5-4.9, 5-14.9 and ≥ 15.0 g per day were 2.59 (95 percent CI 1.45-4.64), 2.23 (95 percent CI 1.34-3.66), 1.18 (95 percent CI 0.52-2.66), and 2.56 (95 percent CI 1.32-5.00), respectively. The interaction between alcohol and noncontraceptive use was significant (χ^2= 16.48, df = 4, p < 0.005). The RR associated with alcohol intake was elevated and showed a significant dose-response among women who ever used noncontraceptive estrogens (table 5).

The association between breast cancer incidence and each type of alcoholic beverage consumed was also explored. The increased risk of breast cancer was associated primarily with beer consumption. After adjusting for potential confounders and intake of all other alcoholic beverages, the RR and 95 percent CI for women who drank < 4 or ≥ 4 versus 0

g of alcohol per day from beer were 1.01 (95 percent CI 0.74-1.39) and 1.54 (95 percent CI 1.10-2.17), respectively, (p for trend = 0.017). The relative risk associated with < 4 versus 0 g of alcohol per day from wine was 1.21 (95 percent CI 0.96-1.52). No association was observed between breast cancer and liquor intake. Because of low power, the interaction between beer and noncontraceptive estrogen use was not tested.

DISCUSSION

The relationship between breast cancer and most measured risk factors in this study were comparable to those reported previously for postmenopausal women (40). After adjustment for these characteristics, there was a significant, dose-response association between alcohol and breast cancer incidence. This association appeared to be limited to women who reported ever using noncontraceptive estrogens. Our results indicate an 80-90 percent higher incidence among women who have ever used estrogen and whose average daily alcohol intake was 5 or more g compared to never-users of estrogen who abstained from alcohol. There was no increased risk of breast cancer for women who consumed alcohol but had never used estrogens.

Willett et al. (24) reported results from the Nurses Health Study, in which alcohol consumption was measured using the same questionnaire as used in this study. They found that the risk of breast cancer was 1.5-fold higher for women who consumed at least 5 g of alcohol per day compared to abstainers, but possible interactions with estrogen use were not reported. However, subsequent analysis by the same investigators suggested that alcohol consumers who were currently using estrogens had an age-adjusted RR of developing breast cancer of 1.56 (95 percent CI 1.2-2.0); abstainers currently taking

estrogens were at no increased risk of developing breast cancer (41).

The risk of breast cancer associated with specific types of alcohol has been examined in several epidemiologic studies (4, 10-12, 14, 16, 17, 24-26, 28, 30). No single alcoholic beverage appears to be consistently implicated; however, data from this study and at least two other studies (14, 24) that controlled for the use of other types of alcoholic beverages in multivariate analysis showed beer consumption to be an independent risk factor for breast cancer. The other two studies (14, 24) also noted an independent association with liquor consumption, whereas, our data were suggestive of a weak association with wine consumption.

Although the Iowa Women's Health Study cohort design has the advantage of assessing exposure prior to the diagnosis of breast cancer, the follow-up was short and will be extended. RR estimates may be attenuated by exposure misclassification, although the reliability of this questionnaire is high. Willett et al. have reported that this semi-quantitative food frequency questionnaire is highly valid and reproducible for assessing average daily alcohol consumption (24, 42). Munger et al. (43) have examined the accuracy and reproducibility of the questionnaire in this cohort. The correlation between the alcohol intake from the baseline food frequency questionnaire and a second and third questionnaires were 0.99 and 0.98, respectively. The correlation between average daily alcohol intake measured by the third questionnaire and the average from five 24 hour recalls in 44 subjects was 0.32; this correlation may be low possibly because five 24 hour periods is too few to characterize usual intake. Nevertheless, these data suggest that the food-frequency questionnaire is a highly reliable and reasonably accurate method of assessing alcohol consumption in this sample.

It is improbable that women who developed breast cancer during follow-up were more likely to have overestimated their average daily alcohol intake at baseline than women who did not develop breast cancer. Our estimates would have been underestimated if breast cancer cases were more likely to leave alcohol intake information blank, thus being classified as abstainers in this analysis. However, there was no difference in the proportion of cases and noncases who left the alcohol questions blank. There is some evidence to suggest that an early age at first exposure to alcohol may be a greater risk factor for breast cancer than drinking later in life (14, 18, 25, 29), but we do not have data to test this association. A differential loss to follow-up across the various levels of alcohol consumption could also lead to bias. However, the follow-up rate of this cohort was virtually complete.

Several mechanisms have been proposed to explain how alcohol may be etiologically involved in the development of breast cancer. Alcohol may have a direct effect on cell membrane permeability to carcinogenic substances (44). In addition, indirect effects such as stimulation of the anterior pituitary to secrete prolactin, which enhances mitotic activity in rat mammary tissue (45, 46) have been suggested. Alcohol may have an inhibitory effect on the metabolism of nitrosamines by the liver. Nitrosamines can induce carcinogenesis in laboratory animals (47), thus, with impaired liver function due to alcohol intake, there is potential for these compounds to remain in the body longer. Alternatively, there may be other constituents of alcoholic beverages, and not the alcohol itself, that play a role in the etiology of breast cancer. None of these hypothesized mechanisms clearly explains the association between alcohol and breast cancer.

This is at least the second epidemiologic cohort study (41) to note an interaction between noncontraceptive estrogen use and alcohol consumption. Further, although the interaction

between alcohol and obesity is not statistically significant in this study, these results are similar to those of at least two cohort studies (23, 24), suggesting that leaner women who drink alcohol were at higher risk of breast cancer than heavier women who consumed the same amount of alcohol. Finally, data from this study suggest (for the first time) that the relationship between alcohol and breast cancer may be restricted primarily to women who have a positive family history of breast cancer; this is inconsistent with the results of at least two other cohort studies (23,24). Inasmuch as laboratory, animal, and epidemiologic studies have shown not only that sex hormones can influence the risk of breast cancer but also that some risk factors that influence hormone metabolism may be limited to those who have a positive family history, future investigations should focus on the possible joint effects of obesity, noncontraceptive estrogen use, family history and alcohol consumption on the risk of this malignancy.

In summary, the results of this study are consistent with most other cohort studies that have examined the relation between breast cancer and alcohol consumption; that is, there appears to be an elevated risk among postmenopausal women who consume at least a moderate amount of alcohol. This association was limited in this study to women who reported ever using noncontraceptive estrogens. Several issues still need to be addressed: the uncertainty of a biologically plausible mechanism by which alcohol may be causally related to breast cancer; the consistency but weakness of the relationship; the development of methods to measure alcohol consumption objectively and to characterize both recent and past alcohol exposure in individuals; and the influence of other variables on the alcohol/breast cancer association.

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TABLE 1. Age-adjusted relative risks for potential breast cancer risk factors among postmenopausal women, Iowa Women's Health Study, 1986-1989, (n=493 cases).

Risk factor	No. of cases*	Total person-years	Age-adjusted RR†	95% CI‡	p for trend§
Education					
< High school	88	27,415	1.00		
High school	205	58,701	1.12	0.87-1.44	
> High school	200	54,193	1.20	0.93-1.54	0.19
Body mass index (kg/m²)					
≤ 22.89	76	27,773	1.00		
22.90-25.04	90	28,842	1.14	0.84-1.54	
25.05-27.45	88	27,860	1.15	0.84-1.56	
27.46-30.70	112	28,133	1.44	1.08-1.93	
> 30.70	127	28,096	1.65	1.24-2.19	0.0001
Waist-to-hip ratio (inch/inch)‖					
≤ 0.76	78	27,959	1.00		
0.77-0.80	82	24,973	1.16	0.85-1.58	
0.81-0.85	105	32,170	1.13	0.84-1.52	
0.86-0.90	96	25,931	1.25	0.92-1.69	
> 0.90	128	29,058	1.50	1.13-2.00	0.005
Body mass index at age 18 (kg/m²)					
< 19.35	70	18,169	1.00		
19.35-21.449	157	38,630	1.08	0.81-1.43	
21.45-22.749	104	28,482	0.96	0.71-1.30	
22.75-24.599	94	28,624	0.86	0.63-1.17	
≥ 24.60	68	26,775	0.68	0.48-0.95	0.003
Age at menarche (years)					
≤ 11	95	21,532	1.00		
12	137	37,698	0.80	0.61-1.04	
13	134	41,171	0.72	0.56-0.94	
≥ 14	121	38,664	0.69	0.52-0.90	0.006
Age at menopause (years)					
≤ 44	111	32,919	1.00		
45-49	117	35,608	0.98	0.75-1.27	
50-54	191	52,902	1.07	0.85-1.35	
≥ 55	58	14,437	1.13	0.83-1.56	0.36
Parity					
Nulliparous	48	12,514	1.00		
1-2	164	44,917	0.95	0.69-1.32	
3-4	209	55,430	1.03	0.75-1.42	
> 4	70	26,958	0.71	0.49-1.03	0.11

Age at first live birth (years)					
≤ 19	76	27,741	1.00		
20-24	206	63,395	1.16	0.89-1.51	
25-29	103	27,380	1.28	0.94-1.75	
≥ 30	52	7,986	2.29	1.58-3.32	0.0001
Nulliparous	48	12,514	1.36	0.94-1.97	
Family history of breast cancer					
No	386	119,752	1.00		
Yes	83	16,490	1.53	1.21-1.94	0.0004
Oral contraceptive use					
Never	404	114,166	1.00		
Ever	89	26,249	1.09	0.86-1.38	0.47
Noncontraceptive estrogen use					
Never	295	86,580	1.00		
Ever	198	53,515	1.07	0.90-1.28	0.48
Alcohol intake (g/day)					
0	267	79,679	1.00		
< 1.5	49	13,611	1.08	0.80-1.47	
1.5-4.9	78	21,382	1.10	0.86-1.42	
5.0-14.9	56	15,770	1.08	0.81-1.44	
≥ 15.0	43	10,260	1.28	0.93-1.76	0.11

* number of cases may add up to less than 493 due to missing data
† Age-adjusted relative risk (RR) by 5-year groupings using the Mantel-Haenszel method.
‡ CI,Confidence Interval
§ Based on Mantel's extended test
ll 1 inch = 2.54 cm

TABLE 2. Relationship between alcohol consumption and potential breast cancer risk factors among postmenopausal women, Iowa Women's Health Study, 1986-1989, (n=493 cases).

	% With Risk Factor		
Risk factor	Abstain	<4 g/day	≥4 g/day
Education			
> High school	34.8	41.3	46.3
Body mass index (kg/m^2)			
≤ 22.89	17.2	19.7	27.2
Waist-to-hip ratio (inch/inch)*			
≤ 0.76	17.5	22.4	23.5
Body mass index at age18 (kg/m^2)			
≤ 19.35	13.0	12.7	12.9
Age at menarche (years)			
≤ 11	16.1	14.5	14.5
Age at menopause (years)			
≤ 44	24.0	22.7	22.9
Parity			
1-2	32.1	31.2	32.4
Age at first live birth (years)			
≤ 19	21.0	18.0	18.1
Family history of breast cancer			
Yes	11.7	11.7	12.1
Oral contraceptive use			
Ever	16.1	20.4	23.7
Noncontraceptive estrogen Use			
Ever	36.4	39.4	41.4

*1 inch = 2.54 cm

TABLE 3. Relative risk of breast cancer associated with alcohol intake among postmenopausal women using Cox Proportional hazard regression, Iowa Women's Health Study, 1986-1989, (n=493 cases).

Alcohol intake (g/day)	No. of cases*	Total person-years	RR †	95% CI ‡	p for trend
0	242	74,872	1.00		
< 1.5	47	12,951	1.18	0.86-1.61	
1.5-4.9	75	20,419	1.20	0.93-1.56	
5.0-14.9	54	15,038	1.25	0.93-1.68	
≥ 15.0	41	9,794	1.46	1.04-2.04	0.04

* number of cases may add up to less than 493 due to missing data

† Relative risk (RR) of breast cancer adjusted for age, body mass index, age at menarche, age at first live birth, and family history of breast cancer using Cox proportional hazard regression.

‡ CI,Confidence Interval

TABLE 4. Age-adjusted relative risk of breast cancer among postmenopausal women by average daily alcohol intake within strata of potential risk factors, Iowa Women's Health Study, 1986-1989, (n=493 cases).

	Relative risk*		
Risk Factor	Abstain	<4 g/day	≥4 g/day
Education			
< High school	1.0	1.18	1.26
High school	1.0	1.00	1.07
> High school	1.0	1.15	1.17
Body mass index (kg/m^2)			
≤ 22.89	1.0	1.90	2.26
22.90-25.04	1.0	0.95	1.25
25.05-27.45	1.0	0.81	1.05
27.46-30.70	1.0	1.60	1.20
> 30.70	1.0	0.91	0.86
Waist-to-hip ratio (inch/inch)†			
≤ 0.76	1.0	1.17	1.07
0.77-0.80	1.0	1.47	1.58
0.81-0.85	1.0	1.08	1.48
0.86-0.90	1.0	0.94	1.22
> 0.90	1.0	1.10	0.74
Body mass index at age 18 (kg/m^2)			
≤ 19.35	1.0	1.31	0.82
19.35-21.449	1.0	0.87	1.31
21.45-22.749	1.0	1.13	1.13
22.75-24.599	1.0	1.14	1.10
≥ 24.60	1.0	1.27	1.02
Age at menarche (years)			
≥ 11	1.0	0.76	1.19
12	1.0	1.11	0.89
13	1.0	1.06	1.10
≥ 14	1.0	1.58	1.58
Age at menopause (years)			
≤ 44	1.0	1.02	1.16
45-49	1.0	1.36	1.62
50-54	1.0	0.86	1.04
≥ 55	1.0	1.84	0.69

Parity			
Nulliparous	1.0	1.01	1.01
1-2	1.0	0.82	1.04
3-4	1.0	1.23	1.45
>4	1.0	1.44	0.71
Age at first live birth (years)			
≤19	1.0	0.65	0.59
20-24	1.0	1.27	1.29
25-29	1.0	1.18	1.37
≥30	1.0	1.20	1.43
Nulliparous	1.0	1.01	1.01
Family history of breast cancer			
No	1.0	1.01	1.15
Yes	1.0	1.81	1.57
Oral contraceptive use			
Never	1.0	1.12	1.15
Ever	1.0	1.04	1.14
Noncontraceptive estrogen use			
Never	1.0	1.10	0.83
Ever	1.0	1.12	1.66

* Age-adjusted relative risk by 5-year groupings using the Mantel-Haenszel method.
† 1 inch = 2.54 cm.

TABLE 5. Interaction between alcohol consumption and noncontraceptive estrogen use* on risk of breast cancer in postmenopausal women, Iowa Women's Health Study, 1986-1989, (n=493 cases†).

	Noncontraceptive estrogen use							
	Never				Ever			
Alcohol intake (g per day)	No. of cases	Total person-years	RR‡	95% CI§	No. of cases	Total person-years	RR‡	95% CI§
0	162	47,308	1.0		80	27,564	0.88	0.67-1.15
< 1.5	32	7,895	1.25	0.86-1.83	15	5,056	0.93	0.55-1.58
1.5-4.9	46	12,238	1.16	0.84-1.62	29	8,181	1.13	0.76-1.68
5.0-14.9	19	8,908	0.71	0.44-1.15	35	6,130	1.88	1.30-2.72
≥ 15.0	18	5,605	1.07	0.65-1.74	23	4,189	1.83	1.18-2.85
p for trend			0.46				0.0001	

* -2 log likelihood test for interaction (χ^2 = 16.48, df=4, p<0.005)

† number of cases may add up to less than 493 due to missing data

‡ Relative risk (RR) of breast cancer, adjusted for age, body mass index, age at menarche, age at first live birth, and family history of breast cancer using Cox proportional hazards regression.

§ CI, Confidence Interval

APPENDIX B

CROSS-SECTIONAL COMPARISON OF ABSTAINERS AND HEAVY DRINKERS

Samples of several different tissue types were obtained from heavy drinkers of alcohol and alcohol abstainers in order to examine differences in fatty acid ethyl estter profiles and hemoglobin adducts. Tissue types included red blood cells, white blood cells, plasma, adipose, buccal celss and saliva.

Heavy drinking individuals were recruited from either the VA Medical Center or the Hennepin County Detoxification Center. Only those heavy drinkers who consumed no alcohol-containing beverages for at least 24 hours before sampling were invited to participate. Abstainers were those people who drank no alcohol-containing beverage in the last five years. The population of abstainers included individuals employed by the University of Minnesota, long-term abstinent alcoholics and members of a Mosque.

The purpose of the study was explained to all participants and written consent obtained prior to sampling (see consent form). Data regarding demographic information as well as smoking and alcohol consumption histories were collected. However, during th eearly stages of sampling detailed questionnaires were not administered. Those individuals whose identification codes were AB01-AB09 or HD01-HD21 were asked only for information regarding diabetic status, current smoking and drinking status, age, and time since last alcohol drink. All other participants (AB10-AB25 and HD22-HD35) were administered a more detailed questionnaire (see questionnaire). The information obtained was explanded to include birthdate, weight, height and smoking histories, in addition to a more detailed drinking history.

Following completion of the questionnaire, blood and adipose samples were drawn by a physician; saliva and buccal cell samples were collected by a trained technician. All samples were kept on ice until processing. Briefly, the samples were returned to the lab

where the whole blood was centrifuged to separate the plasma, red blood cells and white blood cells. The plasma was immediately transferred to storage vials and the red cells washed x3 with sterile saline and resuspended in saline-EDTA before transferring to the vials. The red blood cell remnants were lysed and removed from the white blood cells before washing with sterile saline. The white blood cells were resuspended with a Tris-EDTA buffer and transferred to storage vials. The saliva was removed from the dental rolls and 1 ml aliquots or less were transferred into storage vials. The buccal cells were also centrifuged and the supernatant discarded. One milliliter of sterile saline was added to the Buccal Cell pellet and the suspension was transferred to a storage vial. All samples were evacuated and stored under N_2 at -70°C. In addition, both glycosylated and fast hemoglobin were quantitated on most blood samples.

The demographic and blood glucose data were examined comparing heavy drinkers to abstainers (Table 1). The demographic data show that 31 (89%) of the 35 heavy drinkers, and 14 (56%) of the 25 abstainers, were male. Heavy drinkers were significantly older than abstainers but the two groups did not differ in height or weight. Of the 60 people who participated in the study, only 1 heavy drinker was a diabetic. There was no difference in the mean percent glycosylated hemoglobin between heavy drinkers and abstainers however; there was a significant difference in the percent fast hemoglobin. This is consistent with the findings of other studies.

Table 2 compares the smoking characteristics of heavy drinkers and abstainers. Among heavy drinkers, 28 (85%) are currently smoking cigarettes and 3 (9%) have never smoked, whereas among abstainers 6 (25%) are current smokers and 10 (42%) have never smoked. The number of packyears was computed by multiplying the total number of years smoked by the average number of packs of cigarettes smoked during this time.

There was no difference in the average number of cigarettes smoked per day or the number of packyears between heavy drinkers and abstainers who had ever smoked. Among current smokers, the average (minimum and maximum) number of cigarettes smoked per day for heavy drinkers and abstainers were 22 (3-60) and 16 (12-22) respectively. In addition, heavy drinkers who are currently smoking tended to have been smoking longer and the total number of packyears was larger compared to currently smoking abstainers.

Alcohol drinking characteristics were examined among heavy drinkers and abstainers (Table 3). The population of abstainers was composed of 13 people who had consumed alcohol in the past (exdrinkers)and 12 who had not. The average time since the last alcohol drink was 8.4 years among exdrinkers, whereas for heavy drinkers the last drink was consumed an average of 3.1 days prior to sampling. Because the rate at which alcohol is absorbed is markedly effected by food intake, the heavy drinkers were asked to report on their eating habits while consuming alcohol. Of the 9 heavy drinkers questioned, 44% reported that less than half to none of the occasions where alcohol was consumed was this accompanied by a meal. In addition, of the 9 heavy drinkers who were asked if they had abstained from alcohol during the last two years, 8 responded positively. Among those 8, the length of abstention ranged from 2-24 months (mean=5.9). Four heavy drinkers reported consuming alcohol 1-3 days per week during the two weeks prior to sampling, whereas 5 heavy drinkers reported drinking alcohol more than 3 days per week.

The detailed questionnaire included separate questions regarding beer, wine and liquor intake. The ethanol content in a 12 oz. can/bottle of beer, 4 oz. glass of wine, 1.5 oz shot of liquor was assumed to be 13.2, 10.8 and 15.1 grams, respectively, and the grams of

ethanol consumed was computed for each beverage type. Average daily intake over the last six months as well as intake on each occasion that alcohol was consumed was estimated from the reported number of days per week alcohol was drunk and the number of drinks per day. The total average daily intake of EtOH was 183.3 grams (SD=162) (Table 4, computed by summing the average daily intake of beer, wine and liquor). When examining average daily intake versus intake on each occasion, the ratio of the mean average daily intake to the mean intake on each occasion is 0.39, 0.46 and 0.43 for beer, wine and liquor, respectively. In addition, using the median of the average daily intake to determine cutpoints for stratifying grams of ethanol per occasion, there is a much greater proportion of drinkers in the higher strata for all three drinks. These results suggest that alcohol consumption is characteristically binge drinking in these heavy drinkers.

Table 1. Demographic characterisitics and blood glucose measurements of heavy drinkers and abstainers.

	Heavy Drinkers (n=35)	Abstainers (n=25)
Sex (n):		
male	31	14
female	4	11
Avg. Age (yrs)	45	35[a]
Avg. Weight (kg)	73	71
Avg. Height (cm)	173	174
Diabetes (n):		
Yes	1	0
No	33	23
Avg. Glycosylated Hemoglobin (%)	5.93 (n=27)	5.56 (n=15)
Avg. Fast Hemoglobin (%)	7.01 (n=23)	6.08[a] (n=15)

[a] significantly different from heavy drinkers ($p<0.01$)

Table 2. Smoking characteristics of heavy drinkers and abstainers

	Heavy Drinkers (n=35) *	Abstainers (n=25)
Smoking status (n):		
Never	3	10
Ex	2	6
Current	28	6
Ever smokers:		
Avg. # cig. /day	21	20
Avg. # yrs. smoked	27.4	19.0
Avg.packyears	23.7	19.1
Current smokers		
Avg. # cig. /day	22	16
Avg. # yrs. smoked	27.0	21.5
Avg. packyears	28.9	17.1

* total n may not equal total sample size because of missing data

Table 3. Drinking history of heavy drinkers and abstainers

	Heavy Drinkers (n=35)	Abstainers (n=25)
Drinking status (n):		
Never	-	12
Ex	-	13
Current	35	-
Time since		
Last drink:	3.1 days	8.4 years
Eat while drinking (n):		
Always	1	
Mostly	2	
Half the time	2	
Less than half	4	
Abstain in last 5 years (n):		
Yes	8	
No	1	
Avg. length of		
Abstention (months)	5.9	
Avg. days per week consuming alcohol in last 2 weeks (n):		
<1 day /week	0	
1-3 days /week	4	
4-5 days /week	3	
6-7 days /week	2	

Table 4. Number of heavy drinkers at various alcohol levels over last 6 Months and average daily ethanol intake and intake per drinking occasion among 14 heavy drinkers.

Alcohol type (gr EtOH/day)	No. of Drinkers per day	Avg. Intake per Day (gr EtOH)	No. of Drinkers per occasion	Avg. Intake per Occasion (gr EtOH)
Beer:		51.8		132.9
>0-30	7		2	
>30	7		12	
Wine:		56.6		122.7
>0-30	6		0	
>30	5		11	
Liquor:		101		234
>0-76	6		4	
>76	6		8	

* split on median daily intake for each alcohol type
a) Mean Daily Intake During Last 6 Months: 183 gr EtOH /day (SD=162).

ALCOHOL MARKER STUDY
QUESTIONAIRE

Study Subject Name_____

Study Subject Number_____

Social Security # _____

Address _____

_____

_____

Study Subject Number_____

ALCOHOL MARKER STUDY:

The following questions are to be asked of each study participant. The questionaire is to be completed by a member of the research staff. Please follow appropriate skips. An attempt should be made to get as much information that is relevent to the questions asked as possible.

1. Today's Date (month,day,year)_____

2. Sex (circle one) **M** **F**

3. Date of Birth_____
(mon,day,year)

4. Height (ft,in)_____. 5. Weight (lbs)_____

6. Have you ever been told by a physician that you have diabetes or high blood sugar?

YES
NO
Don't Know

****

7. Have you ever smoked cigarettesa, that is at least a total of 100 cigarettes or more over your lifetime? **YES**
NO --->Q14

8. How old were you when you first began smoking?
_____ yrs old

9. Do you currently smoke cigarettes? **YES**
NO --->Q11

10. How many cigarettes per day do you currently smoke?

_____ (packs or cigarettes) ---> **Q12**

11. How long ago did you quit smoking? _____ (yrs,months)

12. Thinking back over your lifetime, how many total years have you smoked?_____ (years)

13. During this time period, what is the average number of cigarettes per day that you smoked?_____(packs, cig)

****

NOW I WOULD LIKE TO ASK YOU SOME QUESTIONS ABOUT YOUR CONSUMPTION OF ALCOHOLIC BEVERAGES.

14. Have you ever drunk alcoholic beverages? YES
NO ----->End

15. At what age did you start drinking alcohol regularly?_____

16. Have you had any alcohol drinks in the last five years?

YES ---->Q18
NO

17. How old were you when you stopped drinking alcohol?

_____(years old) -----> END

18. How long has it been since your last alcohol drink?

_____(days, months, years)

NOW I WOULD LIKE TO ASK YOU SOME QUESTION ABOUT HOW MUCH AND HOW OFTEN YOU DRINK SPECIFIC TYPES OF ALCOHOL.

19. Do you ever drink beer? YES
NO ---->Q22

20. Which of the following best describes how often you have consumed beer during the last six months?

a. less than one day per month
b. less then one day per week
c. 1-3 days per week
d. 4-5 days per week
e. 6-7 days per week

21. On the days that you drink beer, what is the average number of cans or bottles you drink?_____ cans/bottles

22. Do you ever drink wine? YES
NO ---->Q25

23. Which of the following best describes how often you have consumed wine or wine coolers during the last six months?

    a. less than one day per month
    b. less than one day per week
    c. 1-3 days per week
    d. 4-5 days per week
    e. 6-7 days per week

24. On the days that you drink wine, what is the average number of glasses of wine you drink? _____ glasses

25. Do you ever drink hard liquor? **YES**
    NO ---->Q29

26. Which of the following best describes how often you have consumed hard liquor during the last six months?

    a. less than one day per month
    b. less than one per week
    c. 1-3 days per week
    d. 4-5 days per week
    e. 6-7 days per week

27. On the days that you drink hard liquor, what is the average number of drinks do you drink?_____ drinks

28. When you drink hard liquor, what kind do you drink?

    _____(gin,vodka,whiskey,etc.)

29. Finally, which of the following best describes your total alcohol consumption over the last two weeks:

    a. less than one day per week
    b. 1-3 days per week
    c. 4-5 days per week
    d. 6-7 days per week

30. On those occasions when you drank in the last two weeks:

how much wine did you drink_____

how much beer did you drink_____

how much hard liquor did you drink_____

31. Of the times you drink, are all drunk with meals, are most drunk with meals, are about half drunk with meals or are none or almost none drunk with meals.

a. all drunk with meals
b. most are drunk with meals
c. about half are drunk with meals
d. non or almost none drunk with meals

32. Remembering back over the last two years have there been any time when you did not drink alcohol for a period longer than you normally abstain.

YES (if yes) WHEN AND HOW LONG_____
NO

32. Thinking about <u>how often</u> you drink alcoholic beverages, would you say that in the last six months you drink more often, less often or about the same as compared to the last five years?

a. more often
b. less often
c. about the same

33. Thinking about <u>how much</u> you drink on each occasion, would you say that in the last six months you drink more, less or about that same as compared to the last five years?

a. more
b. less
c. about the same

THANK the participant!!!

CONSENT FORM

We are asking for volunteers to participate in a study being conducted by researchers at the University of Minnesota to develop biological markers of alcohol consumption. For the study, we would like to obtain small samples of tissue. These include blood, saliva, buccal (cheek) cells and adipose (fat) tissue. The procedures used to obtain these tissues are unlikely to result in injury and involve the following:

1. You will be seated comfortably and a trained professional will draw 30 mls of blood or about 9 teaspoonfuls from your arm. The procedure is the same as is typically used by a physician to obtain blood in routine check-ups.

2. You will be asked to suck on a cotton swab for the collection of a saliva sample and then rinse your mouth for the collection of cheek cells.

3. For the collection of adipose (fat) tissue, a small amount of fat tissue will be collected by needle biopsy under the skin at the upper and outer part of the hip. The level of discomfort associated with this procedure is about that of a needle stick for drawing blood.

It is unlikely that these procedures will result in injury, however, a small bruise may result from the drawing of blood and obtaining adipose tissue.

In the event of injury, emergency medical care will be available. If follow-up treatment is required, this will be the responsibility of you and your third party health insurance payor.

You may volunteer to donate any of the requested tissues to this research project. The project is conducted by Drs. Belcher and Gross in the Division of Epidemiology at the University of Minnesota. You will receive $5.00 in compensation for the tissues contributed to the study.

Your decision whether or not to participate will not affect your future relations with the Division of Epidemiology or the University in any way. If you decide to participate, you are free to discontinue participation at any time without affecting such relationships. If you have any further questions about the research and/or research subject's rights or wish to report a research-related injury, please call John Belcher, 624-2183.

Data and information collected on individuals in this study will be treated as a confidential medical record, and will not be provided to anyone without your written request. The information collected will be used by the University of Minnesota for scientific purposes only and will not identify participants by name.

I have read the attached description of the Ethanol (Alcohol) Markers Study. I agree to participate in this study. I understand that the information collected on individuals in this study will be treated by the University of Minnesota as a confidential medical record and will not be provided to anyone without my written consent. I am free to discontinue my participation in the study at any time.

You will be offered a copy of this form to keep.

_____ _____

Signature Date

_____ _____

Signature of Witness Signature of Investigator

APPENDIX C

ALCOHOL FEEDING STUDY

SINGLE-DOSE ALCOHOL FEEDING STUDIES

Introduction

Healthy, non-smoking volunteers, aged 21-49, will be given a single dose of ethanol in orange juice after an evening meal. They will be seated in an evenly-lighted, temperature-controlled room. A trained professional will be present during the first 4 hours after alcohol ingestion. At indicated times, over the course of the experiment, blood samples will be drawn by venipuncture into EDTA containing tubes. Red and white blood cells will be separated from the plasma and all fractions will be stored under nitrogen atmosphere at
- 70°C. "Fast" and glycosylated hemoglobin will be determined on blood samples immediately after they are collected as well as blood alcohol levels.

Study Protocol:

DAY 1. After the subjects arrive, a baseline blood sample will be collected. A urine sample will also be collected from females to determine pregnancy status. The evening will be meal served. Approximately, 30 minutes following the meal the alcoholic beverage will be served. The subjects will be asked to consume the beverage over the course of an hour. Two more blood samples will be collected 1 and 3 hours after beginning consumption. The subjects will be sent home in a taxi cab.

DAY 2. The following morning subjects will return to the study site and asked to remain for 8 hours. A blood will be collected before breakfast is served, this will be 14 hours after the alcoholic beverage was consumed the evening before. At times 18 and 22 hours after consumption, two more samples will be drawn. Breakfast and lunch will be provided.

DAYS 3-7. Additional blood samples will be drawn at times 62, 110 and 158 hours either at the subjects home or at the study site.

SUBJECT RECRUITMENT AND PRELIMINARY STUDY

Recruitment of Subjects

For purposes of the single-dose alcohol feeding study, subjects have been recruited from the general population by advertising in the classified section of the local newspaper. Students and staff were specifically excluded except during the quarter breaks. Only nonsmokers, 21-49 years of age were eligible for participation. In an initial recruitment, 174 individuals responded to a single newspaper advertisement placed in three local newspapers. These 174 respondents were interviewed by telephone (Telephone Description) and asked to complete a more detailed questionnaire (Questionnaire for Alcohol Marker Study) which would be mailed to their home and to sign a consent form; 101 mail questionnaires were returned. Specific characteristics of these respondents are shown in Table 1.

First Screening of Respondents

Information collected from the mailed questionnaire included self-report alcohol intake, age, sex, smoking habits, history of specific diseases including psychiatric and alcohol or drug dependence, pregnancy status and use of medication. In addition, questions from a widely cited and validated alcohol abuse screening test (CAGE) were included in the questionnaire. Peopled with scores of 2 or more on the CAGE test (presumptive evidence

of drinking problem) will be ineligible for participation in the alcohol feeding studies as well as people who report alcohol dependence problems. In the future, these people will be considered as potential blood donors for experiments that do not administer alcohol. In addition, respondents who are pregnant, who are taking any medication where alcohol is contraindicated such as metronidazole, disulfiram, pargyline or other "Antabuse" type drugs, or who have any self-reported diseases-liver, heart, kidney, diabetes peptic ulcer, brain or nervous disorders, psychiatric problems, or drug dependence will be eliminated from any of our studies. Using these eligibility criteria, a pool of potential participants were selected for the feeding studies from the initial recruitment (Table 2).

Second Screening to Determine Eligibility

Four to five weeks before the each alcohol feeding study, selected participants were required to attend a brief orientation session. During this time, a short physical examination was conducted (Physical Examination). The nature of the effects of alcohol on a fetus were explained to women prior to asking about present and future pregnancy plans. A urine sample was collected and screened to determine pregnancy status (from fertile women) and for drug use (cocaine, barbiturates, opiates, amphetamines, cannabinoids, methadone and benzodiazapines). A small sample of blood (10 ml) was drawn by venipuncture to measure blood alcohol concentrations and hepatic gamma glutamyltransferase (a liver enzyme) in serum. If the results of the pregnancy test had been positive, or there was evidence of liver damage, or alcohol abuse or drug use, the subjects would have been informed and disqualified from further participation.

At this orientation session, a description of the study design and purpose as well as its likely benefits and hazards were explained. Subjects agreeing to participate were given a

beverage consumption diary and asked to record their daily alcohol use during the four weeks prior to the feeding study. They were specifically asked not to exceed their usual drinking habits during this time and to be as accurate as possible in keeping the diary. Subjects were required to abstain from any alcohol consumption for the two weeks prior to the experiment. A letter was sent to each subject reminding them when to begin abstaining. Although they were encouraged to maintain their abstinence, if they did consume any alcoholic beverage they were asked to record it in their diary.

Subjects who successfully completed the beverage diary were paid with $20.00. Further compensation for participation in the single-dose alcohol feeding studies involved an additional $100.00 for completing the entire study.

Preliminary Study

An initial study was conducted to determine the feasibility of the study protocol, to describe the disappearance curves of measured adducts for the purpose of optimizing sampling time, and to describe any gender differences in the marker. If no metabolites are identified, the procedure will be repeated using higher doses of ethanol compatible with usual, self-report alcohol consumption.

Briefly, twelve subjects (6 males, 6 females) were selected from the pool of potential participants. After the second screen, one female was deemed ineligible for participation (overweight, therefore the amount of alcohol administered would be excessive). Usual alcohol intake for these subjects was 5-10 drinks per week. An amount of alcohol equal to 0.6 grams per kilogram body weight was consumed by each participant and blood samples were collected as described above. Samples from the preliminary feeding study are

currently under analysis.

Table 1. Characteristics of mail questionnaire respondents

	Males (n=60)	Females (n=41)
Age (mean)	32.2	31.3
Race (%)		
White	90.0	97.6
Black	3.3	2.4
Other	6.7	--
Any medication (n)	6	13
Heart disease (n)	1	0
Drug dependence (n)	2	0
Smoking status (n)		
Past smoker	23	23
Current smoker	5	1
Never smoke	32	17
Pipe	1	0

Table 2. Number of mail questionnaire respondents excluded by exclusion criteria.

Criteria		N	
1.	Medication	19	
2.	Heart Disease	1	(also on medication)
3.	Drug Dependency	2	
4.	Current Smoker	6	
5.	2+CAGE	16	(1 also on medication)
			(2 also diagnosed drug dep.)
			(1 also a current smoker)
		39	people excluded
			(21 men and 18 women)

TELEPHONE DESCRIPTION

We are trying to measure the effects of ordinary drinking on body chemistry. The studies we are planning involve drinking a specified amount of alcohol varies by the experiment in which you participate. For each experiment you will drink alcohol and then have some blood samples drawn. These experiments will require you to be in the laboratory on two consecutive days, perhaps on the weekend, for several hours each day. We will then ask you to return a few more times during the week in order for us to draw a little more blood at each visit. In addition, we will also need a few cells from the inside of your cheeks and some saliva.

If you are selected to participate you will receive $50.00 per week. We would now like to ask you a few questions if you are interested.

(Instructions to telephone interviewer: The text in upper case is only for instructions to you and not to be asked to the participant.)

1) How old are you? _____

2) Gender MALE / FEMALE

3) (IF FEMALE) Are you pregnant? YES / NO

4) Do you currently smoke cigarettes? YES / NO

5) How many cans of beer, or glasses of wine, or glasses of liquor do you usually consume in a week?

_____ glasses

of _____ (TYPE)

6) Would you be willing to complete a questionnaire for this study? YES / NO

7) (IF YES) Could you tell me your name, address and telephone number and I will mail the questionnaire to you?

_____

_____

_____

_____

8) Could you tell me where you saw the advertisement? _____

Questionnaire for Alcohol Marker Study

We are trying to measure the effects of ordinary drinking on body chemistry. The studies we are planning involve drinking a specified amount of alcohol that varies by the experiment in which you participate. For each experiment you will drink alcohol and then have some blood, saliva and cheek cell samples drawn. These experiments will require you to be in the laboratory on two consecutive days, perhaps on the weekend, for several hours each day. We will then ask you to return a few more times during the week in order for us to draw a little more blood saliva and cheek cells at each visit.

In return for your participation you will receive $50.00 per week. There are several experiments with different time schedules. If you would like to participate please return this questionnaire in the business reply envelope. If you have any questions please call The Alcohol Marker Study Center at 625-6467.

For each of the following questions either circle the answer that is right for you or write your answers in the space provided.

Name _____

Address _____

_____

Telephone Number _____

1) How old are you? _____

2) What is your birthdate? _____
(mo, day, year)

3) Sex: MALE / FEMALE

4) Race or ethnic background: (circle one)

BLACK (not of Hispanic origin)
WHITE (not of Hispanic origin)
HISPANIC
ASIAN or PACIFIC ISLANDER
NATIVE AMERICAN INDIAN or ALASKAN NATIVE
OTHER (specify)_____

5) Are you in good health? YES / NO

6) Are you taking any medication? YES / NO

If YES, please list._____

_____

7) Have you ever had:

a. liver disease? YES / NO

b. heart disease? YES / NO

c. kidney disease? YES / NO

d. diabetes? YES / NO

e. brain or nervous disorder? YES / NO

f. psychiatric problems? YES / NO

g. alcohol or drug dependence problems? YES / NO

8) Have you ever smoked cigarettes? YES / NO

9) If **yes**, do you still smoke? YES / NO

10) If **yes**, How many cigarettes per day do you smoke?

_____ cigarettes/day

11) Do you smoke a pipe or cigars? YES / NO

12) Do you drink alcoholic beverages? YES / NO

13) **If you drink alcoholic beverages,** please complete the table below to describe your alcohol consumption habits in a usual week.

*If you consume less than 1 drink per week check the box below and do not complete the grid.

I consume less than 1 drink per week ___

For example, if you consume, on the average, a glass of wine on Wednesday, 2 beers on Friday and Saturday and no other form of alcohol at any other time during the week, place a 1 under NUMBER OF GLASSES OF WINE for Wednesday, and a 2 under NUMBER OF CANS/BOTTLES OF BEER for Friday and Saturday.

NOTE: one drink is equal to 1 1/2 oz of liquor (shot), 4 oz. glass of wine, or 12 oz. of beer (can/bottle).

DAY OF THE WEEK	NUMBER OF BOTTLES/CANS OF BEER	NUMBER OF GLASSES OF WINE	NUMBER OF DRINKS OF LIQUOR
MONDAY			
TUESDAY			
WEDNESDAY			
THURSDAY			
FRIDAY			
SATURDAY			
SUNDAY			

14) Have you ever felt the need to cut down on your drinking?

YES / NO

15) Have you ever felt annoyed by criticism of your drinking?

YES / NO

16) Have you had guilty feelings about drinking? YES / NO

17) Do you ever take a morning eye-opener? YES / NO

18) Are you able to spend part of 2 consecutive days at our laboratory?

YES / NO

19) If yes, what days are best, including weekends?

---

20) Can you come back to see us several times during the week for a short visit?

YES / NO

21) Can you make weekly visits over 1 month? YES / NO

22) Are you willing to not drink for 3 weeks before the start of one experiment and during the week of the experiment?

YES/ NO

I would like to be considered as a participant in the University of Minnesota Alcohol Studies. I give permission for one of your interviewers to call me if I am eligible.

Signature _____ Date _____

PHYSICAL EXAMINATION

Date _____ Study No. _____

Session No. _____

Subject Name _____ Subject ID _____

Sex _____

Height _____

Weight _____

Blood Pressure _____

Reflexes: knee _____ ankle _____ plantar _____

Chest Exam: _____

_____

Abdominal Exam: Liver _____ Spleen _____

other: _____

_____

Additional Physician Comments _____

_____

_____

_____ _____

Date Physician's Signature