Short Communication

Effects of Alcohol and Menstrual Cycle on Insulin-like Growth Factor-I and Insulin-like Growth Factor Binding Protein-3

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Abstract

Alcohol ingestion and insulin-like growth factor-I (IGF-I) have been associated with increased breast cancer risk, the latter primarily in premenopausal women. We investigated whether alcohol ingestion altered IGF-I or its major binding protein (BP), IGFBP-3, in a controlled feeding study in premenopausal women. We also determined whether IGF-I or IGFBP-3 was affected by menstrual cycle phase. Serum was collected from 31 individuals who were randomly assigned to consume either 0 or 30 g (two drinks) of alcohol daily for three menstrual cycles and who then crossed over to the other alcohol level for three cycles. All calories were provided and weight was maintained during the study. For both alcohol levels, serum was collected during the final cycle at early follicular, periovulatory, and luteal phases. Relative to the follicular phase, IGF-I levels increased by 3.3% and 7.6% in the periovulatory and luteal phases, respectively (P for trend = 0.004). Although alcohol ingestion did not affect this increase, it significantly reduced IGF-I concentrations at all phases (9.5%; $P < 0.001$), whereas IGFBP-3 was unaffected by either menstrual phase or alcohol. This is the first controlled diet study to show that alcohol decreases serum IGF-I in premenopausal women and that IGF-I significantly increases over the course of the menstrual cycle whether or not alcohol is present. (Cancer Epidemiol Biomarkers Prev 2004;13(12):2264–7)

Introduction

Insulin-like growth factor-I (IGF-I) is an endocrine hormone produced primarily in the liver that also exerts autocrine and paracrine effects in numerous tissues (1). It is critical for long bone growth during puberty, and it causes cell proliferation and inhibits apoptosis in both normal and neoplastic cells (2). Over 90% of circulating IGF-I is bound to IGFBP-3, one of a family of six major IGF binding proteins, and is therefore unavailable to bind to and activate the IGF receptor.

In vitro exposure of breast epithelial cells to IGF-I results in their proliferation and survival (1, 2). In support of these findings, recent epidemiologic evidence indicates that increased serum concentrations of IGF-I and/or decreased levels of IGFBP-3 are associated with increased risk of several types of cancer, including prostate (3), colon (4-7), lung (8, 9), and breast (10-13). For breast cancer, the associations have been stronger in premenopausal than in postmenopausal women.

Because of its association with breast and other cancers, there has been much interest in dietary factors associated with changes in the IGF axis. One factor, alcohol, has been studied in several cross-sectional studies; however, these studies have produced mixed results. For example, in a study of 1,037 premenopausal and postmenopausal women, Holmes et al. (14) showed that high levels of alcohol intake were unrelated to IGF-I but were significantly associated with increased IGFBP-3 concentrations. The latter finding contrasted with a previous study of women ages 30 to 84 in which no association between alcohol and IGFBP-3 was seen (15). Neither study stratified by menopausal status, but in a sample of only premenopausal women, Jernstrom et al. (16) found a negative relationship between alcohol intake and IGFBP-3. None of these three studies found any significant association between alcohol intake and serum IGF-I, although there was a nonsignificant, negative association found by Jernstrom et al. (16). No adjustment was made for phase of menstrual cycle in the studies (14-16).

Numerous studies have shown an association between alcohol intake and breast cancer risk. A recent meta-analysis concluded that breast cancer risk increases 7.1%

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for every 10 g of alcohol consumed per day, regardless of menopausal status (17), but the causal mechanisms have not been clearly elucidated. Because both alcohol and the IGF pathway are associated with breast cancer risk, the association of alcohol with breast cancer could be mediated in part via the IGF pathway. Because the association between IGF-I and breast cancer risk has been strongest in premenopausal women, we sought to investigate the relationship between alcohol consumption and IGF-I and IGFBP-3 levels in premenopausal individuals under controlled dietary conditions. Our study permitted a more definitive determination of this relationship because all food was provided and weight was maintained during the study; thus, serum hormone changes could be attributed more clearly to alcohol intake alone. By focusing on premenopausal women and timing specimens during the menstrual cycle, we were also able to determine whether serum IGF-I and/or IGFBP-3 concentrations were affected by cycle phase.

Materials and Methods

Participants and Study Design. A detailed description of the study participants and experimental design has been reported previously (18). Briefly, 37 healthy, normally menstruating, nonsmoking women ages 21 to 40 years completed the entire study. Three were eliminated from analyses due to menstrual irregularities, and samples were no longer available from three additional individuals. The study was approved by the institutional review boards of Georgetown University School of Medicine and the National Cancer Institute.

Women were randomly assigned to consume either 0 or 30 g of alcohol daily for three menstrual cycles and were crossed over to the other alcohol level for an additional three cycles. All food and beverages were provided by the study and prepared at the Beltsville Human Nutrition Research Center (Beltsville, MD). On weekdays, morning and evening meals were eaten in the Center's dining facility and a carryout lunch was provided. Weekend meals were packaged for home consumption. Women were encouraged to report any deviation from the study diet. Alcohol (95% ethanol) was served in fruit juice, and calories from alcohol were replaced by calories from a cola drink during the nonalcohol consumption period. Women were weighed daily, and calories were adjusted to maintain constant weight. During the third (final) menstrual cycle of each diet period, fasting blood specimens were drawn daily from each individual on days 5 to 7, 12 to 15, and 21 to 23 of the menstrual cycle, corresponding to the follicular, periovulatory, and midluteal phases, respectively. For each phase, equal volumes of the daily blood draws from each day of that phase were combined, resulting in three pools: follicular, periovulatory, and midluteal. These were then stored at −80°C.

Laboratory Methods. IGF-I and IGFBP-3 concentrations were measured in duplicate from two aliquots of each pooled sample by ELISA (both from Diagnostic Systems Laboratories, Inc., Webster, TX) and all values were averaged to generate a final concentration. Blinded controls were assayed on each plate, and coefficients of variation from these samples were 6.4% for IGF-I (n = 10) and 10.3% for IGFBP-3 (n = 10).

Data Analysis. For statistical analysis, IGF-I and IGFBP-3 concentrations were transformed to the natural log scale to evaluate treatment effects as relative changes and to have error terms approximately normally distributed. Linear mixed models (19) with a single random intercept reflecting a subject effect were used to estimate and test for changes in IGF-I and IGFBP-3 at 30 g relative to 0 g of alcohol per day. Fixed effects included menstrual cycle (two indicator variables for the three phases), alcohol group, and treatment order. Conditional t and F tests (19) were used to test for treatment effect, changes within menstrual phase, period effect, and order effect. A significant order effect would suggest the presence of a carryover effect, which could result in biased inferences. Modification of the alcohol effect by age, body mass index, and height was examined by testing a cross-product term for alcohol treatment by the variable of interest, but no effect modification was seen (data not shown). All reported Ps are based on two-sided tests.

Results

Means ± SD for age, height, and body mass index for the 31 women who completed the study were 30.2 ± 4.8 years, 163.4 ± 6.7 cm, and 24.7 ± 4.52 kg/m^2, respectively. Geometric means of plasma IGF-I and IGFBP-3 in the follicular phase of cycle and percentage change at other phases of the cycle when women were consuming no alcohol are reported in Table 1. Under these conditions, compared with levels in the follicular phase, IGF-I concentrations increased in the periovulatory phase by 3.3% and in the luteal phase by 7.6% (P for trend = 0.004). We further tested for the interaction between alcohol group and menstrual phase (data not shown), and the results were not statistically significant, indicating that IGF-I increased through the menstrual cycle regardless of alcohol status. IGFBP-3 did not change over the menstrual cycle, whether or not alcohol was ingested.

There was an overall 9.5% reduction in serum IGF-I concentration during the entire menstrual cycle when alcohol was consumed (P < 0.001; Table 2). Decreases in IGF-I were present at all phases of the cycle and ranged from 7.9% to 11.2%, but the decreases were not significantly different by menstrual phase (P = 0.38). There was a 4.8% reduction in serum IGF-I concentrations from

Table 1. Effect of menstrual cycle on IGF-I and IGFBP-3

Menstrual cycle phase	IGF-I (ng/mL)	IGFBP-3 (μg/mL)
Follicular, mean (95% confidence interval)	192.2 (173.0-213.4)	18.3 (17.3-19.3)
Periovulatory, %Δ from follicular (95% confidence interval)	3.3 (−1.0 to 7.9)	−0.8 (−3.6 to 2.0)
Midluteal, %Δ from follicular (95% confidence interval)	7.6 (1.8-13.6)	0.8 (−2.5 to 4.2)

NOTE: Geometric means and percentage change (Δ) in serum concentrations in periovulatory or midluteal phases compared with follicular phase while on control diet (no alcohol).

Table 2. Effect of alcohol, by menstrual phase on IGF-I

Menstrual cycle phase	IGF-I	
	No alcohol, geometric mean (95% CI)	Alcohol, %Δ from no alcohol (95% CI)*
Combined phases	199.8 (181.7-219.7)	−9.5 (−12.5 to −6.4)
Follicular	192.2 (173.0-213.4)	−7.9 (−13.5 to −1.9)
Periovulatory	198.7 (180.3-218.9)	−9.3 (−14.2 to −4.2)
Midluteal	206.7 (187.8-227.5)	−11.2 (−17.0 to −4.9)

NOTE: Geometric mean and percentage change (Δ) in IGF-I concentrations on 30 g of alcohol/d versus no alcohol by menstrual cycle phase.
*Estimates are from linear mixed models.

the first to the second period ($P = 0.004$). However, there was no evidence of a carryover effect because there was no effect of order on mean serum levels ($P = 0.99$ for order effect using the linear mixed model).

Discussion

In a controlled diet study in premenopausal women where alcohol intake was the only variable dietary factor, we sought to investigate whether alcohol, which has been associated with increased breast cancer risk, had any effect on serum IGF-I or its major binding protein, IGFBP-3, both of which have also been implicated in breast cancer risk. Our results indicate that alcohol decreases serum IGF-I levels; therefore, the increased breast cancer risk associated with alcohol intake must be through an IGF-independent mechanism. In addition, we found that, whether women consumed alcohol or not, IGF-I increased over the course of the menstrual cycle.

This study is the first controlled feeding study to report differences in IGF-I throughout the menstrual cycle. The significant increase in IGF-I seen in the later compared with the earlier part of the cycle could indicate that IGF-I is being regulated by other ovarian hormones (e.g., estradiol or progesterone) that also fluctuate during the menstrual cycle and/or that there is an important role for IGF-I in human ovarian function. There have been several other noncontrolled feeding studies that have investigated whether IGF-I levels vary during the menstrual cycle, but results from these studies have been inconsistent, perhaps due to dietary or design differences (reviewed in ref. 20). Nonetheless, three studies have reported a 10% to 15% increase in blood IGF-I concentrations in the luteal compared with the follicular phase, which is similar to the change seen in our controlled feeding study (16, 21, 22). Also similar to our findings, studies have failed to detect cyclic variation in IGFBP-3 in premenopausal women (16, 20). The effects observed are particularly intriguing given the recent interest in the relationship between increased IGF-I and premenopausal breast cancer (10, 13, 23-25). Only one previous IGF-I-breast cancer study reported matching by menstrual cycle phase. Although data on menstrual cycle phase are not always available in epidemiologic studies, our findings strongly suggest timing of blood draw during the cycle could affect overall associations between IGF-I and breast cancer and that controlling for phase of blood draw is therefore necessary.

This study was also the first controlled feeding study to show that when weight and all other dietary factors are kept constant, alcohol intake decreases serum concentrations of IGF-I. Our results indicated that although IGF-I levels increased during the menstrual cycle, 30 g of alcohol daily suppressed IGF-I levels similarly at all phases by ~9.5%. We recently reported similar findings from another controlled study[3] involving postmenopausal women in which 30 g of alcohol daily significantly reduced serum IGF-I and increased IGFBP-3 concentrations. Although the current study showed no effect of alcohol on IGFBP-3, the effect of alcohol intake on IGF-I seems to be similar regardless of menopausal status. This finding indicates that, in addition to assessments of phase of cycle at blood draw, studies investigating the association between IGF-I and premenopausal breast cancer should assess and adjust for alcohol intake.

We initially hypothesized that the effect of alcohol on breast cancer risk may in part be mediated by its effects on IGF-I. However, our findings do not support this hypothesis if, as suggested by numerous studies, increased IGF-I contributes to increased breast cancer risk. IGF-I levels are, throughout life, primarily under the control of growth hormone (26). There is some evidence from studies of acute alcohol intake (27, 28) that alcohol has an impact on the growth hormone-IGF-I axis by inhibiting the growth hormone surge that typically occurs during the sleep part of the sleep-wake cycle. For example, Ekman et al. (28) studied four women and two men, ages 21 to 23 years, who ingested 0.5 or 1.0 g of alcohol/kg of body weight at approximately 7:00 to 8:00 p.m. Blood was then drawn at 1- to 2-hour intervals during the night. At 1:00 a.m., the lower dose of alcohol led to a significant decrease in the growth hormone surge compared with control, and the higher dose led to almost complete inhibition. The lower dose of alcohol used in this study corresponds very closely with the 30 g/d dose used in our study. Therefore, it is possible that, because IGF-I production is under the control of growth hormone, the decrease we observed in serum IGF-I concentrations after 3 months of alcohol intake was due to the inhibition by alcohol of the normal nocturnal growth hormone surge. Alternatively, the effect of alcohol on IGF-I could be due to toxic effects on hepatocytes that produce IGF-I or to decreased IGF mRNA production and/or increased protein turnover. In support of a non–growth hormone–mediated effect on IGF-I, Rojdmark et al. (29) found that, in eight subjects who consumed 0.45 g of alcohol/kg body weight at 8:00, 9:30, and 11:00 a.m., serum IGF-I decreased 7 hours after the first drink without decreasing growth hormone levels. This finding indicates that IGF-I levels can decrease in response to alcohol ingestion without affecting a nighttime growth hormone surge. Because the IGF-I decrease was observed after 7 hours, the effects were probably not due to immediate effects on RNA production but could be due to cell death or a reduction in overall protein production. Any of these mechanisms

[3] J.A. Lavigne et al. Effects of alcohol on insulin-like growth factor-I and insulin-like growth factor binding protein-3 in postmenopausal women, submitted for publication.

may be relevant to the finding of decreased IGF-I in our study, although our subjects did consume alcohol at night before bedtime. Although we have only fasted blood samples from early morning, it would be interesting in the future to determine whether there are any discernable effects on growth hormone levels associated with our protocol of alcohol intake.

The major strengths of this study were its highly controlled nature and its crossover design. These factors allowed us to identify the effects of alterations in a single dietary component, alcohol, on IGF-I and IGFBP-3 concentrations. A disadvantage of the crossover design is the potential for a carryover effect, which could lead to bias results. However, we found no evidence for a carryover effect for either IGF-I or IGFBP-3. Although a recent meta-analysis indicates that alcohol is associated with an increase in breast cancer risk (17), our results indicate that alcohol decreases the concentration of circulating IGF-I despite the fact that IGF-I has also been positively associated with breast cancer risk. These combined studies show that the effects of alcohol on breast cancer risk are clearly complex and are very likely modified by complicated issues such as dose and frequency of exposure, which are difficult to assess by dietary questionnaire. Our study showed that although alcohol and IGF-I are independently associated with breast cancer risk, there is a clear effect of alcohol in reducing IGF-I. Future epidemiologic studies will be needed to understand more clearly how alcohol affects breast cancer risk and how it may alter the balance between increased risk at some level or frequency of intake and decreased risk, perhaps by reducing IGF-I, at another level or frequency of intake. Future epidemiologic studies will also need to consider menstrual cycle phase when evaluating the IGF-I-breast cancer relationship in premenopausal women.

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