Published in final edited form as:

Alcohol Clin Exp Res. 2008 November ; 32(11): 1899–1908. doi:10.1111/j.1530-0277.2008.00774.x.

Biphasic effects of moderate drinking on prolactin during lactation

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Abstract

Background—Contrary to popular lore that encourages women to drink alcohol as an aid to lactation, we previously showed that alcohol consumption disrupted lactational performance and the hormonal milieu of the lactating mother in the short term.

Methods—Thirteen lactating women participated in a 2×2 within-subject design, double-blind, 4-session study to test several hypotheses related to the effects of alcohol on prolactin (PRL) responses and milk yield over time. The two within-subject factors were beverage condition (control or 0.4g/kg dose of alcohol) and pumping condition (pumping occurred at fixed intervals once or twice during the 5.3-hr session). Plasma PRL, blood alcohol concentrations (BAC) and milk yield were measured.

Results—Alcohol consumption increased basal PRL levels ($P<0.0001$) and modified the PRL response to pumping ($P<0.0001$) but the directionality of the response depended on when pumping occurred along the BAC curve. Pumping enhanced PRL response when it occurred during the ascending BAC limb but blunted the response when it occurred during the descending limb, providing evidence that the effects were transient and of a biphasic nature. The slower alcohol was metabolized, the greater the relative PRL response to breast pumping ($P<0.05$). The dynamics of the PRL response between pumping sessions were also altered if women drank. If women pumped within the hour after drinking alcohol, the PRL response during the next pumping some 1.5 hours later, was delayed by a few minutes. Milk yield was significantly lower after drinking alcohol but such deficits were not significantly related to PRL or the speed at which alcohol was eliminated.

Conclusions—Effects of alcohol on suckling-induced PRL were biphasic in nature, but could not explain the deficits in lactational performance. Such findings provide further evidence that the dynamic changes in neuroendocrine state are integrally involved in alcohol's effects over time and underscore the complexity of lactation.

Keywords

alcohol; lactation; prolactin; women's health; biphasic effects; milk yield; moderate drinking

INTRODUCTION

The polypeptide hormone prolactin (PRL) is ubiquitous and multifunctional in vertebrates (Ben-Jonathan et al., 2008) but perhaps most well known for its role in the initiation of lactation. Once established, however, PRL is only one of an ensemble of hormones required for lactation and its role in regulating milk production in humans, both in the short- and long-term, is somewhat obscure (Czank et al. 2007). Nevertheless, in response to the

suckling stimulus of a nursing infant or breast pump, there is a transient increase in the synthesis and release of PRL (Cox et al. 1996; Mennella et al. 2005) by specialized cells in the anterior pituitary (e.g., lactotrophs) and other tissues including the breast (De Coopman, 1993). The pituitary control of PRL is mainly due to a suppressive (dopaminergic) hypothalamic process (Ben-Jonathan et al. 2008). Suckling, the most potent and best physiological stimulus for PRL release, does so, in part by increasing the release of opioids and other PRL-releasing factors which inhibit dopamine secretion into the portal circulation (Ben-Jonathan et al. 2008; Freeman et al. 2000; Goffin et al. 2002).

Given that many drugs of abuse (e.g., alcohol, tobacco, cocaine, opium) interfere with the opioidergic and mesolimbic dopaminergic systems (Kreek, 2007), it is not surprising that hyperprolactinemia is a common condition among addicts and that PRL levels are elevated after acute usage of these drugs (Ellingboe et al. 1980; De Rosa et al. 1981; Ginsburg et al. 1995; Koob and Kreek, 2007; Mendelson et al. 1987; Mennella et al. 2005; Moshtaghi-Kashanian et al. 2005; Patkar et al. 2004; Sarkola et al. 1999; Schuckit et al. 1987; Soyka et al. 1991; Wilkins et al. 1982). In the past, some argued that the alcohol-induced increases in PRL (Grossman, 1988), albeit in men and non-lactating women, lent scientific support for the age-old folklore that encourages lactating women to drink alcohol as a means to increase milk production (Mennella, 2002; Mennella, 2004; Pepino and Mennella, 2004). Only in recent years has such research focused on lactating women and the findings actually disputed the lore that the alcohol-induced increases in PRL enhanced lactational performance.

Although the PRL response was magnified when lactating women drank a mo of alcohol (Mennella et al. 2005), there was no enhancement of milk production during the single breast pumping session which occurred 35-51 minutes after consumption. Nor was there any evidence that the alcohol-induced elevations in PRL were stress-related or that there were temporal relationships between PRL or cortisol levels and the amount of milk produced by a mother during a given breastfeed (Mennella et al. 2005). On the contrary, women produced less milk (Mennella, 1998; Mennella and Beauchamp, 1991; 1993; Mennella et al., 2005), further disputing the argument that the alcohol-induced elevations in PRL enhanced lactational performance (Grossman, 1988) and the lore that alcohol is a galactagogue. The decreased milk yield and longer latency for milk ejection were not related to PRL, but rather they were significantly related to the alcohol-induced decreased oxytocin response in these women (Mennella et al. 2005).

The present study sought to replicate our findings that alcohol consumption increases suckling-induced PRL levels and decreases milk yield (Mennella et al. 2005) as well as determine alcohol's effects on basal PRL and the dynamic PRL response which occurs during lactation. To this end, the observation period following alcohol consumption was extended from 2.3 to 3.6 hours. This enabled us to evaluate the hormonal responses during two feeds and determine how one breastfeed affected the subsequent one and whether alcohol consumption modified this relationship. We obtained PRL levels in the absence of breast pumping to determine whether alcohol increases basal levels during the immediate hour after drinking in a manner similar to that previously observed in men and non-lactating women. We also determined the PRL response to breast pumping at different time points across the blood alcohol concentration (BAC) curve. Because biphasic responses of alcohol consumption have been observed for mood, motor activity, performance tasks, aggression, and a variety of other hormones and physiological responses (Calabrese and Baldwin, 2003; Cicero, 1981; He et al. 2007; Holdstock and deWit, 1998; King et al. 2002; Rosito et al. 1999), we hypothesized that alcohol affects PRL in a biphasic time-dependent manner, with ascending BAC levels being stimulatory and descending levels being inhibitory. Secondary objectives of the study were to determine whether the alcohol-induced changes in PRL were

related to milk yield and fat content as well as maternal perceptions of lactational performance since previous research revealed that PRL levels (albeit in the milk) are related to the lactating mother's perception of the fullness in her breasts (Cox et al. 1996).

MATERIALS AND METHODS

Subjects

Thirteen, healthy lactating women (53.8% multiparous) who were exclusively nursing 2- to 5-month-old infants and had not yet resumed menstruation were recruited. Women were excluded if they were pregnant, obese, smokers, anemic, alcohol dependent or lifetime alcohol abstainer, had previous gastric bypass surgery, or were on any medication including oral contraceptives. Two additional women began testing but were excluded because they participated in only one test session due to child care issues. All procedures were approved by the Office of Regulatory Affairs at the University of Pennsylvania and each subject gave informed written consent.

The women (6 Caucasian, 3 African American, and 4 Other/Mixed Ethnic Group) were 33.1 ± 1.4 years of age with a mean body mass index of 24.7 ± 1.1 kg/m². They reported that alcohol intake was low during pregnancy but significantly increased during lactation (0.3 ± 0.9 vs. 1.5 ± 0.3 standard drinks per month; $P < 0.008$). When they did drink alcohol during lactation, they drank 1.8 ± 0.3 standard drinks per occasion.

Procedures

A four-session, double-blind, 2×2 within-subject study was conducted. Each session occurred on a separate day and the test days were separated by one week. Each session lasted 5.3 hours (Table 1) so that subjects could be monitored for at least 3.6 hours after consuming the beverage. The sessions occurred at the same time of day since the composition of milk varies throughout the day (Prentice et al. 1981) and PRL has an endogenous circadian rhythm (Diaz et al. 1989). Using the methodologies established in our laboratory (Mennella et al. 2005) and developed for the study of neurally-mediated hormonal responses in humans (Teff et al. 1991), women were tested at the Clinical and Translational Research Center (CTRC) at the University of Pennsylvania on four separate testing days, each test day separated by one week. One of the 13 subjects completed only 3 of the 4 test sessions.

The women's infants were not present during testing and thus no breastfeeding occurred during the test sessions. For several reasons, a breast pump (Medela Symphony, Crystal Lake, IL) rather than the infant was used to study suckling-induced PRL response. First, the infants' suckling intensity changes when feeding mothers' milk that contains alcohol (Mennella, 1997; Mennella and Beauchamp, 1991). Second, the mere sound, sight or smell of the baby often stimulates milk letdown or leaking (Vuorenkoski et al. 1969). Third, exposure to alcohol in milk, albeit a minute fraction of that consumed by the mother, disrupts the infants' sleep-wake patterning (Mennella and Garcia-Gomez, 2001), a finding which contradicted popular beliefs that decades ago encouraged lactating mother to drink so that their infants will relax and become less colicky after drinking such milk (Lawrence, 1989).

Subjects arrived at the CTRC following an overnight fast and remained fasted during the entire testing procedures since PRL is potentiated by certain gastrointestinal hormones and high blood glucose levels (Widstrom et al. 1984). To ensure that the women had indeed fasted, capillary blood glucose was measured from a finger-prick sample (OneTouch®, LifeScan, CA) at the beginning of each test day. Only those whose blood glucose levels were lower than 99 mg/dl participated in the study. Women were asked to refrain from

drinking alcohol 3 days prior to each test day and a pregnancy test (First Response®, Church & Dwight Co., Inc, NJ) was administered each test day to confirm they weren't pregnant. Throughout the entire test sessions, subjects were not allowed to sleep, converse about babies or food, or watch television (which could expose them to images of babies and food) because these behaviors may affect the hormonal milieu of the mother.

To standardize conditions and minimize discomfort due to engorgement, all women emptied their breast with an electric breast pump (baseline pumping) at the beginning of each test day ($t = -100$ min; see Table 1). Shortly thereafter, an intravenous line was inserted into the antecubital vein of an arm. Because PRL increases during the first 30 minutes after a needle prick (Grayson et al. 1997), subjects acclimated in a private testing room for 45 minutes after the intravenous line was inserted. The CTRC nurses, who were blind to the experimental condition as were the subjects, took blood samples at fixed intervals before (-40, -25 and -10 minutes) and after drinking a beverage (25, 35, 37, 39, 41, 43, 45, 47, 49, 51, 65, 80, 95, 110, 125, 140, 142, 144, 146, 148, 150, 152, 154, 156, 170, 185, 200 and 215 min); time 0 refers to the time subjects drank the beverage. Each sample collection involved the removal of 1 ml of blood to clear the catheter tubing, followed by a 3 ml-collection into 2 Vacutainer tubes containing EDTA; one of these tubes contained Trayslol (1ml) and the other did not (2ml). Both tubes were kept on ice for no longer than 1 h and were centrifuged. The 2ml tube was then separated into 2 aliquots of plasma, and stored at -70°C for later assay of PRL and oxytocin (which will be reported separately). The second tube of plasma containing Trayslol was stored for future analyses.

As shown in Table 1, each subject was tested under two beverage conditions (ROH, CON) and two breast pumping conditions (Pump Once, Pump Twice). The order of testing was counterbalanced in a quasi-randomized manner such that pumping condition alternated every two test sessions and the beverage alternated every test sessions (ROH, CON, ROH, CON or CON, ROH, CON, ROH) so that 2 weeks elapsed between the two ROH conditions. During ROH - Pump Once and ROH-Pump Twice conditions, women drank a 0.4g/kg dose of alcohol in orange juice (15% v/v), a dosage equivalent to 1-2 drinks. This represents the amount lactating women typically consume during a drinking occasion (Mennella, 1998). During CON-Pump Once and CON-Pump Twice Conditions, they drank an equal volume of orange juice. The beverages were aliquoted into two equal volumes and each aliquot was consumed within consecutive 5-minute periods. For both types of beverages, 3 milliliters of alcohol were pipetted onto the surface of the cup to serve as a smell and taste mask (Holdstock and de Wit, 1998). The two breast pumping conditions differed in that during Pump Once Condition, women used an electric breast pump only once ($t=140$ -156 min) after drinking, whereas during Pump Twice Condition, they breast pumped twice, at 35-51 minutes and then again at 140-156 minutes post beverage consumption. The breast pump was turned on at $t=34$ min and $t=139$ min and pumping began within seconds after the blood sample was drawn at $t=35$ min and $t=140$ min timepoints. Because the strength of the applied vacuum from the breast pump can affect lactational performance (Kent et al. 2008), the same intensity setting on the breastpump (-250 mm Hg) was used for all subjects during each of the four test sessions.

During each breast pumping session, lactating women alternated the breast being stimulated every 2 minutes for 16 minutes. The total amount of milk pumped was measured (in milliliters) and milk samples were analyzed for fat content using the creatocrit technique (Lucas et al. 1978). After slight vortexing, the milk was immediately drawn into three microhematocrit tubes and sealed with Critoseal. The tubes were spun for 10 minutes in a microcentrifuge. The height of the cream layer was measured and then converted to fat by the following formula: $\text{fat (g/L)} = (\text{mean creatocrit \%} - 0.59) / 0.146$ (Lucas et al. 1978). Each mother also rated the degree of fullness of each breast at the beginning and during

pumping on a 4-point scale; larger numbers reflect greater perceptions of breast fullness. In addition, BAC (g/L) were determined by having subjects breathe into an Alco-Sensor IV (St Louis, MO) at fixed intervals throughout the test sessions (Inns et al. 1979; O'Connor et al. 1998). After the last data collection point at 215 minutes, lunch was provided. Subjects were not allowed to leave the CTRC until BAC levels registered zero.

PRL Assay

PRL levels in plasma samples were measured in duplicate by a direct, two-site immunoradiometric assay without extraction using materials supplied by ICN Diagnostics (Costa Mesa, CA). The antiserum used cross-reacts less than 0.01 % with HCG, TSH, LH, and FSH. According to the provider, the minimal detectable concentration is 0.19 $\mu\text{g/L}$. Intra-assay and inter-assay variations were 7.7%, and 11.3%, respectively. All assays were performed by the Diabetes Research Center at the University of Pennsylvania.

Calculation and Statistics

From the BAC data, we determined peak BAC, time-to-peak BAC, and alcohol disappearance rates (β_{60} ; g/L/hr). β_{60} were determined from the slopes of the linear least-squares regression lines calculated within the apparent linear portion of the descending limb of the BAC time curve for each subject (Mumenthaler et al. 1999). To exclude the upper distribution phase and lower first-order elimination phase of the linear portion of the curve, we used the first value taken 0.5 hours after peak BAC and all subsequent readings $\geq 0.20\text{g/L}$.

From the raw PRL data ($\mu\text{g/L}$) for each of the 4 test sessions, we determined peak PRL levels ($\mu\text{g/L}$) and area under the plasma PRL time curve (AUC; $\mu\text{g}\cdot\text{min/L}$) for two time periods (AUC_{35-140 min} and AUC_{140-215 min}). The first time period occurred at 35-140 min and the second occurred at 140-215 min, the time at which women started breast pumping in all four days of testing (140 min) until the end of the test session (215 min). AUC values were determined by using a software program (OriginLab® Corporation) based on the trapezoidal rule. PRL levels at 25 min were used as the baseline values to calculate the delta scores for the AUC calculations for 35-140 min time period and PRL levels at time 140 min were used as baseline values for the 140-215 min time period. Linear least-squares regression lines were calculated within the apparent linear portion of the ascending limb of the PRL rise for each period of breast pump stimulation and for each beverage condition to determine the slopes of the PRL rise. The correlation coefficients for these regression lines ranged from -0.75 to -1.00 (Mean+SD: -0.94+0.01); the slopes could not be determined for 1 of the 6 slopes for one woman and 3 of the 6 slopes for another.

As shown in Table 2, the study was designed to address four key hypotheses. We determined whether alcohol consumption by lactating women altered basal (Hypothesis 1) as well as suckling-induced PRL responses during the ascending (Hypothesis 2) and descending (Hypothesis 3) limbs of the BAC time curve. Slopes of the PRL rise were compared to determine whether alcohol's effect on PRL was additive or synergistic with breast pump stimulation. Next, we determined whether the dynamics of the PRL response between feeds by investigating whether one breast pump session ($t=35-51$ min) altered the PRL response to a subsequent session ($t=140-151$ min) and whether alcohol modulated these effects (Hypothesis 4). Separate repeated measures ANOVAs with Beverage Condition (Alcohol, Control) and/or Pumping Condition (Once, Twice) and time (when applicable) as the within-subjects factors were conducted. When significant, post-hoc Fisher Least Significant Difference analyses were performed.

Secondary analyses focused on the effects of alcohol consumption on measures of lactational performance such as milk yield, milk fat content, and maternal perceptions of breast fullness/engorgement. Here we conducted RM-ANOVA analyses on data obtained from the first pump session so that we could also factor in the effects of time since last pumping. Correlational analyses were conducted to determine whether there were relationships between PRL, classical alcohol pharmacokinetic parameters and measures of lactational performance. The critical value for significance of all analyses was set at $P < 0.05$. Data are presented as means $\pm$ SEM unless otherwise specified.

RESULTS

BAC Curve

Initial analyses characterized the BAC time curve for the alcohol dosage used in the present study. Figure 1 illustrates that BAC levels ascended during the first hour, reaching peak levels of 0.60 ± 0.03 g/L at around 42 minutes post consumption during the two sessions in which women drank alcohol. For the next 2.6 hours, BAC levels steadily descended reaching levels of 0.15 ± 0.02 g/L at the time the sessions ended. On average, the alcohol disappearance rate was 0.14 ± 0.01 g/L/hr. There were no significant differences in the BAC time curve during the two test session in which subjects drank alcohol.

PRL Responses to Breast Pumping

Initial analyses not only verified that the suckling stimulus used in the present study elicited a PRL response but characterized the response as well. Sixteen minutes of breast pump stimulation effectively increased plasma PRL levels ($F(30,330df)=10.44$; $P < 0.00001$). Breast pump-induced elevations in PRL levels were evident in all but one woman during the first pumping session and all but another during the second. During the control conditions, PRL levels increased from 54.5 ± 6.7 μ g/L before starting (e.g., baseline) to 68.0 ± 9.3 μ g/L at around 10 minutes after the start of breast pumping. PRL then peaked at 30 minutes (106.7 ± 15.9 μ g/L) and remained heightened until 75 minutes after the commencement of pumping ($t = 110$ min relative to beverage consumption) when levels were indistinguishable from baseline. This time course was characteristic of the PRL response during the one and only pump session in CON-Pump Once Condition ($t = 140$ -156 min) as well as during the first pump session in the CON-Pump Twice Condition ($t = 35$ -51 min).

PRL Responses: 4 Key Hypotheses

Overall plasma PRL levels were significantly affected by beverage ($F(30,330df)=5.43$; $p < 0.0001$) and pumping ($F(30,330df)=8.89$; $p < 0.0001$) conditions. Figure 2 summarizes the PRL findings as they relate to the four key hypotheses under study with each panel representing the primary comparisons made. First, alcohol consumption increased basal PRL (Hypothesis 1) ($F(30, 330df)=4.43$; $p < 0.0001$). When we examined individual timepoints during this period, basal PRL levels were significantly higher 25-65 minutes after drinking alcohol than the control beverage (all p 's < 0.05 ; see Figure 2A).

Second, alcohol consumption modified the PRL response to breast pumping but the directionality of the response depended on whether pumping occurred during ascending or descending BAC. When breast stimulation occurred during the ascending phase of the BAC curve (Hypothesis 2), alcohol significantly enhanced PRL response during and after breast stimulation (Figure 2B) and the slope of the PRL rise tended to be steeper ($F(1,11df)=3.92$; $P = 0.07$; Table 3) than that observed during the respective control condition. Consequently, PRL AUC_{35-140 min} was significantly larger when women drank alcohol than when they drank the control beverage ($F(1,12df)=9.56$; $P < 0.01$; Table 3). That alcohol potentiated the PRL response when suckling stimulation occurred during the ascending BAC limb was also

indicated by the significant correlation between alcohol disappearance rates and the relative differences in the systemic availability of PRL ($AUC_{35-140 \text{ min}}$) of the first pumping session in Pump Twice Condition. That is, the slower lactating women eliminated alcohol, the larger the alcohol-induced increase in PRL $AUC_{35-140 \text{ min}}$ relative to its control ($r(13df)=-0.59$; $P=0.03$).

In contrast, when breast stimulation occurred during the descending phase of the BAC curve, alcohol significantly blunted the PRL response (Hypothesis 3). Post-hoc analyses revealed that, regardless if women pumped for the first (Figure 2A) or second (Figure 2B) time after consuming alcohol, PRL levels were significantly lower at 165-215 minutes post consumption when compared to respective controls. There was also a main effect of beverage condition such that when pumping occurred during descending BAC, peak PRL levels were significantly lower ($F(1,11)=4.94$; $P<0.05$) and PRL $AUC_{140-215 \text{ min}}$ ($F(1,11df)=4.68$; $P=0.05$) was significantly smaller when women drank alcohol. However, there were no significant effect of beverage ($F(1,9df)=2.35$; $P=0.16$) or pumping ($F(1,9df)=3.00$; $P=0.12$) conditions or a significant interaction between these two conditions ($F(1,9df)=0.00$; $P=0.95$) for the slopes of the PRL rise (see Table 3).

The dynamics of the PRL response to breast pumping were also affected by how often women breast pumped and whether they drank (Hypothesis 4). Figure 2C illustrates that PRL levels were higher if women were breast pumping for the second time (and consequently less time had elapsed between pumping; CON Pump Twice) than if pumping for the first time (CON-Pump Once Condition). However, the magnitude of the PRL response to breast pumping, as determined by $AUC_{140-215 \text{ min}}$ and the slopes of the PRL rise were not significantly different between these two control pump sessions (Table 3). Nor were there differences in the time course of the PRL response. During both control conditions, post-hoc analyses revealed that PRL levels were significantly different from baseline beginning at 8 minutes after the commencement of pumping ($t=148 \text{ min}$). Alcohol significantly delayed this PRL response to breast stimulation by a few minutes when we focused on what happens at approximately 2 hours after drinking. As shown in Figure 2D, PRL levels did not become significantly different from baseline until 12 ($t=152 \text{ min}$; ROH-Pump Twice Condition) or 14 ($t=154 \text{ min}$; ROH-Pump Once Condition) minutes after the commencement of pumping (compare with Figure 2C).

Lactational Performance

To determine whether lactational performance was affected by the length of time since the women last breast pumped, we focused on the first pump session of each of the four test days. For two of the days (Pump Twice Condition) this first pump session occurred at 35-51 minutes post beverage consumption (or 120 minutes since baseline pumping) whereas it occurred at 140-156 minutes post beverage consumption (or 225 minutes since baseline pumping) during the other two sessions (Pump Once Condition). As expected, women felt more engorged (Pump Once vs. Pump Twice Condition: 1.4 ± 0.5 vs. 1.0 ± 0.7 ; $F(1,11df)=29.49$; $p<0.001$) and expressed more milk (90.0 ± 14.5 vs. $62.7\pm9.2 \text{ ml}$; $F(1,11df)=12.31$; $P=0.005$) but the milk tended to have a lower fat content (48.0 ± 2.8 vs. $60.3\pm3.5 \text{ g/L}$; $F(1,11df)=4.50$; $P=0.06$) when more time had passed since the last pumping.

There was a significant main effect of beverage condition on lactational performance ($F(1,11df)=8.74$; $P=0.01$). Although there were no significant differences between perceptions of breast fullness between the two beverage conditions ($F(1,11df)=2.65$; $P=0.13$), Figure 3 illustrates that women expressed significantly less milk during the sessions in which they drank the alcoholic beverage. Despite expressing less milk, there were no significant differences in the fat content of the milk expressed ($F(1,11)=0.40$;

$P=0.54$). Neither were there any significant and reliable correlations between PRL and the elimination of alcohol with any of the measures of lactational performance.

DISCUSSION

Plasma PRL increased transiently in response to suckling stimulation in lactating women whose infants were between the ages of 2 and 5 months. PRL levels rose steadily throughout 16 minutes of breast stimulation, peaking 30 minutes after the commencement of pumping and decreasing thereafter until levels returned to baseline values at approximately 75 minutes. Although this time course was characteristic of the PRL response for each session of pumping on the two control days, PRL levels at the start of breast pumping and for at least the next hour were higher when less time had elapsed between pumping sessions. Despite the higher PRL levels, the magnitude of the PRL response to breast pumping, as determined by AUC and the slopes of the PRL rise, was not different from that observed when a shorter time period had elapsed since women last pumped. This suggests that not only are PRL levels affected by the interval between breastfeeds/breast pumping sessions (Tay et al. 1996) but each woman may have some intrinsic level of PRL response to suckling that tracks throughout lactation (O'Brien et al. 2007).

Alcohol consumption modified PRL several ways. First, basal PRL levels increased within the hour after drinking. The magnitude and persistence of the alcohol-induced PRL response in lactating women observed herein was more robust than that previously observed in non-lactating women (Sarkola et al. 1999), further highlighting the dynamics of the system under study.

Second, alcohol consumption modified the PRL response to breast pumping but the directionality of the effects depended upon when breast pumping occurred along the BAC curve. That is, the same intensity and duration of breast pumping enhanced the PRL response when pumping occurred during the ascending limb of BAC curve but blunted the PRL response when it occurred during the descending limb, providing evidence that the effects of alcohol on suckling-induced PRL were transient and of a biphasic nature. A biphasic effect on basal PRL was not evident in the present study, although it has been shown to produce biphasic effects for other hormones (e.g., thyroid releasing hormone) which are known to regulate PRL release (Cicero, 1981). A longer observation period may be needed to capture the biphasic effects on basal levels but this would be difficult to do in lactating women since they would experience pain and discomfort due to breast engorgement if more time elapsed between breast pumping. Further evidence that the PRL response to suckling was modified by alcohol *per se* was the finding that the slower the woman metabolized alcohol, the greater the relative PRL response to breast pumping.

Third, alcohol consumption altered the dynamics of the PRL response between feeds. Specifically, if women breast pumped within an hour after drinking alcohol, the PRL response resulting from the subsequent pump session, which occurred some 1.5 hours later, was delayed by a few minutes. This suggests that alcohol alters the ability of PRL regulatory systems to subsequently respond to the same stimulus.

Uniquely among the pituitary hormones, PRL has a propensity for hypersecretion and is under tonic inhibition (Ben-Jonathan et al. 2008). In the short-term, alcohol causes hyperprolactinemia by affecting extra-pituitary tissues, such as breast tissue, which are capable of producing PRL and/or through a general depression of the central nervous system (Ben-Jonathan et al. 2008). Such depression would increase PRL levels because pituitary PRL is under a suppressive dopaminergic (hypothalamic) control (Ben-Jonathan and Hnasko, 2001; Freeman et al. 2000). Alcohol also stimulates PRL release by activating

inhibitors (e.g., endogenous opioid peptides) of the hypothalamic dopaminergic neurons (Ben-Jonathan and Hnasko, 2001). Although never studied in lactating women, beta-endorphins increase after alcohol consumption in men and non-lactating women (Gianoulakis et al. 1989; Sarkola et al. 1999).

At the present time, one can only speculate about the mechanisms underlying the biphasic nature of alcohol on suckling-induced PRL release. One explanation is that such effects are due to changes in the dopaminergic tone of the hypothalamus in a manner similar to how the tone changes throughout the day due to an endogenous circadian rhythm (Shieh et al. 1997). That is, there is a circadian rhythm on suckling-induced PRL secretion such that the same intensity of suckling elevates PRL more effectively in the evening, when basal PRL levels are higher, than during the day (Freeman et al. 2000; Stern and Reichlin, 1990). Because subjects in the present study were tested at the same time of day for each of the four sessions, we eliminated any circadian effects on PRL. Perhaps by increasing basal PRL levels immediately after consumption, alcohol rendered the system more sensitive to suckling stimulation during its ascending phase but desensitized or adapted the system during the descending phase of the BAC curve when PRL was on the decline (see also Kalant et al. 1978; Sarkola et al. 1999). That the slopes of the PRL rise tended to be steeper when breast stimulation occurred shortly after drinking alcohol suggests that during the ascending limb of the BAC curve, alcohol is interacting synergistically with the mechanisms underlying suckling-induced PRL release. Whether the mechanisms underlying alcohol's biphasic effects on PRL are similar to those described for how alcohol acutely impairs the normal entrainment process of the circadian clock (Prosser et al. 2008) remain unknown. Also unknown is whether the alcohol-induced changes in circulating levels of PRL are solely due to increased or decreased production rates or whether it also reflects changes in the metabolic clearance of the hormone (Cooper et al. 1979).

Previous research has repeatedly demonstrated that during the immediate hours after lactating women drank a moderate dose of alcohol (i.e., 1-2 standard drinks), their infants drank less milk at the breast (Mennella, 2001; Mennella and Beauchamp, 1991; Mennella and Beauchamp, 1993). If mothers refrained from any further drinking, infants then compensated and drank more from the breast 8 to 12 hours later (Mennella, 2001). Consistent with previous findings (Mennella, 1998; Mennella et al., 2005), the present study revealed that one of the reasons infants take in less at the breast is because their mothers produce less milk within the immediate hours after drinking. However, we found no relationships between milk yield and PRL during either the control or alcohol conditions, findings which are in agreement with a large body of research that concluded that PRL does not directly regulate milk synthesis in the short- or long-term once lactation is established (Chatterton et al. 2000; Cox et al. 1996; Czank et al. 2007; Howie et al. 1980). That there were no differences in the fat content, despite the lower milk yield, between the milk pumped after alcohol consumption and after drinking the control beverage suggests that women have difficulty expressing all the milk (particularly hindmilk) when they drink alcohol since the degree of breast emptying has been shown to be a major factor explaining the variance of fat content in the milk (Daly et al. 1993; Kent et al. 2008). We hypothesize that the changes in lactational performance observed after lactating women drink alcohol (Mennella, 1998; Mennella et al. 2005) were due to the effects on oxytocin. In contrast to PRL, our prior study revealed that oxytocin levels significantly decreased during the immediate hours following alcohol consumption and the diminished oxytocin responses were significantly related to the deficits in milk yield and delay in milk ejection (Mennella et al. 2005).

This leaves open the question as to whether alterations in PRL are affecting mothers in other ways besides lactational performance. Emerging evidence from animal model and some

human studies suggests that the PRL response triggered by breastfeeding affects maternal behaviors and moods, responsiveness to stress and coping skills, and immunity (Kelley et al., 2007; Mann and Bridges, 2001; Uvnas-Moberg et al., 1990). Whether the alcohol-induced disruptions in PRL alter the behavior and physiology of the mother in the short-term and, if so, the social and situational context in which such behaviors would be displayed (Fleming et al., 1997) are important areas for future research.

Breastfeeding was one of the five areas emphasized in the State of the Science Report by the National Institute on Alcohol Abuse and Alcoholism on the health risks and potential benefits of moderate drinking (Gunzerath et al. 2004). Although low to moderate drinking has been viewed by many cultures as an aid to lactation (Mennella, 2002; Pepino and Mennella, 2004), the present findings add to the growing body of scientific literature that refutes the folklore that alcohol is a galactagogue (Mennella, 1998; Mennella, 2004; Mennella and Beauchamp, 1991; Mennella and Beauchamp, 1993; Pepino and Mennella, 2004; Mennella et al., 2005; Chien et al., 2008). Drinking a moderate dose of alcohol modified the dynamic PRL response to breast pumping for several hours. The effects of alcohol on suckling-induced PRL were biphasic in nature, as has been demonstrated in many other physiological systems (Calabrese and Baldwin, 2003; Cicero, 1981), but could not explain the deficits in lactational performance. Such findings provide further evidence that the dynamic changes in neuroendocrine state are integrally involved in alcohol's acute effects over time and underscore the complexity of the under-investigated reproductive state of lactation.

Acknowledgments

This research was supported by NIH Grants R01AA09523 and NIDDK19525 and the Diabetes Research Center Grant RR00040. We would like to acknowledge the expert technical assistance of Janice Kennedy, Emily Perlman, Lauren Yourshaw, Allison Steinmeyer, Ms. A. Lorraine Norfleet, MHA, BSN, BS, RN and the nurses at the Clinical and Translational Research Center at the Hospital of the University of Pennsylvania, and Dr. Heather Collins at the Diabetes Research Center, University of Pennsylvania. Dr. Joseph Verbalis provided valuable discussions on the data and Drs. Karen Teff and Gary K. Beauchamp provided valuable comments on the manuscript.

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Figure 1. Mean ($\pm$ SEM) blood alcohol concentration (BAC) on the two test sessions in which women drank alcohol (Pump Once Condition; closed circles; Pump Twice Condition; closed triangles). To convert values for blood alcohol concentration to millimoles per liter multiply by 0.217.

Figure 2.

Mean ($\pm$ SEM) plasma PRL in 13 lactating women who were tested under two beverage conditions (ROH is represented by closed symbols and CON represented by open symbols) and two breast pumping conditions (Pump Once represented by circles; Pump Twice represented by triangles). Time zero represents the time the beverage was consumed and is represented on the figure by the arrow labeled BVG. The timing of breast pumping session(s) indicated by hatched bars. Each of the four panels (A-D) represents the primary analyses conducted to test the four hypotheses described in Table 2. *denotes values within each test session that were significantly different from their respective baseline values ($P < 0.05$). †denotes values that were significantly different from similar timepoints during the control (plasma levels; $P < 0.05$). Conversions: PRL in micrograms per liter $\times 43.5$ = PRL in picomoles per liter.

Figure 3.

Milk yield in milliliters (left axis; shaded bars) and fat content in g/L (right axis; closed circles) of milk expressed during the first pump sessions. The top panel represents the main effect of beverage condition. Lactating women drank either control beverage or alcoholic beverage. The bottom panel represents the main effect of pumping condition. The first pump session occurred 120 minutes since last pumping for the Pump Twice Condition and 225 minutes since last pumping for the Pump Once Condition. *signifies significantly different at $P < 0.05$. ‡signifies different at $P = 0.06$.

Table 1

Schedule of Events: Timing of breast pumping, insertion of the line, and drinking the beverage

Beverage Condition	Pumping Condition	Time Relative to Drinking the Beverage (in minutes)					
		-100 to -85	-85	0-10	35-51	140-156	240
ROH	Pump Once	Baseline Pumping	insert line	Drink ROH Beverage	No Pumping	Pumping	Study Ends
	Pump Twice	Baseline Pumping	insert line	Drink ROH Beverage	Pumping	Pumping	Study Ends
	Pump Once	Baseline Pumping	insert line	Drink CON Beverage	No Pumping	Pumping	Study Ends
	Pump Twice	Baseline Pumping	insert line	Drink CON Beverage	Pumping	Pumping	Study Ends

ROH, 0.4g/kg ethanol in orange juice

CON, control

Table 2**Key Hypotheses and Corresponding Data Analyses**

Hypotheses		Data Comparisons
1	Alcohol consumption alters basal PRL levels.	ROH-Pump Once vs. CON-Pump Once; t=25-140 min; Figure 2A
2	Alcohol consumption enhances suckling-induced PRL response when pumping occurs during ascending BAC levels.	ROH-Pump Twice vs. CON-Pump Twice; t=35-140 min; Figure 2B
3	Alcohol consumption blunts suckling-induced PRL response when pumping occurs during descending BAC levels.	ROH-Pump Once vs. CON-Pump Once; t=140-215 min; Figure 2A ROH-Pump Twice vs. CON-Pump Twice; t=140-215 min; Figure 2B
4	A. First pumping session influences PRL response to the second pumping session.	CON-Pump Once vs. CON-Pump Twice; t=140-215 min; Figure 2C
	B. Alcohol consumption modulates how the 1 st pumping session influences PRL response to the second pumping session	ROH-Pump Once vs. ROH-Pump Twice; t=140-215 min; Figure 2D) when compared with Figure 2C

PRL, prolactin

BAC, blood alcohol concentration

ROH, 0.4g/kg ethanol in orange juice

CON, control

Table 3
Effects of Beverage and Pumping Conditions on PRL responses to Breast Pumping

Beverage Condition	Control			Alcohol		
	Pump Once	Pump Twice	Pump Once and Pump Twice Combined	Pump Once	Pump Twice	Pump Once and Pump Twice Combined
PRL Responses:						
PRL AUC (µg·min/L) 35-140 min interval 140-215 min interval	-- 2937.4 ± 639.8	2653.5 ± 835.5 3176.7 ± 895.8	-- 3057.0 ± 993.9	-- 2056.2 ± 715.6	4153.2 ± 1224.3[*] 2237.7 ± 579.1	-- 2146.9 ± 875.4[†]
Slope of PRL rise 35-65 min interval 140-170 min interval	-- 2.5 ± 0.5	2.6 ± 0.7 2.9 ± 0.7	-- 2.7 ± 0.7	-- 1.7 ± 0.6	3.8 ± 1.0[‡] 2.2 ± 0.5	-- 2.0 ± 0.8
Peak PRL (µg/L) 35-140 min interval 140-215 min interval [§]	-- -98.5 ± 15.7	109.6 ± 15.5 132.1 ± 22.7	-- 115.3 ± 25.5	-- 85.4 ± 17.2	136.3 ± 24.9 107.8 ± 16.8	-- 96.5 ± 23.2[‡]

Significant effects are indicated by bold fonts.

* P<0.05 for the comparison with same Pumping Condition, Control Beverage.

[†] P≤0.05 for the comparison with Control Beverage (both pumping conditions combined).

[‡] P=0.07 for the comparison with same Pumping Condition, Control Beverage.

[§] P<0.05 for the comparison between Pump Once (beverages combined) and Pump Twice (beverages combined); data not shown.