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Acute Alcohol Consumption Elevates Serum Bilirubin, an Endogenous Antioxidant

Stephanie S. O'Malley^{1,2}, Ralitza Gueorguieva³, Ran Wu¹, and Peter I. Jatlow^{2,4}

Stephanie S. O'Malley: stephanie.omalley@yale.edu; Ralitza Gueorguieva: ralitza.gueorguieva@yale.edu; Ran Wu: ran.wu@yale.edu; Peter I. Jatlow: peter.jatlow@yale.edu

¹Department of Psychiatry, Yale School of Medicine, New Haven, CT, United States

²Yale Cancer Center, Yale School of Medicine, New Haven, CT, United States

³Department of Biostatistics, Yale Schools of Public Health and Medicine, New Haven, CT, United States

⁴Department of Laboratory Medicine, Yale School of Medicine, New Haven, CT, United States

Abstract

Background—Moderate alcohol consumption has been associated with both negative and favorable effects on health. The mechanisms responsible for reported favorable effects remain unclear. Higher (not necessarily elevated) concentrations of serum bilirubin, an antioxidant, have also been associated with reduced risk of cardiovascular disease and all-cause mortality. This study tests the hypothesis that single dose alcohol consumption elevates bilirubin providing a potential link between these observations.

Methods—18 healthy individuals (8 cigarette smokers) were administered alcohol, calibrated to achieve blood concentrations of 20, 80 and 120 mg/dL, in random order in 3 laboratory sessions separated by a week. Each session was preceded by and followed by 5–7 days of alcohol abstinence. Serum bilirubin was measured at 7:45 am prior to drinking, at 2 pm, and at 7:45 the next morning. Mixed effects regression models compared baseline and 24 hr. post-drinking bilirubin concentrations.

Results—Total serum bilirubin (sum of indirect and direct) concentration increased significantly after drinking from baseline to 24 hours in non-smokers (from Mean=0.38, SD=0.24 to

Corresponding Author: Stephanie O'Malley, Connecticut Mental Health Center, 34 Park Street, New Haven, CT 06509, Stephanie.omalley@yale.edu, Phone: 203-974-7490, Fax: 203-974-7606.

Contributors

SO and PJ designed and conducted the study; RG guided the data analysis plan and RW conducted the statistical analyses. All authors contributed to the drafting of the manuscript and approved the final version.

Conflict of Interest

Dr. O'Malley has served as a consultant to or advisory board member for Pfizer, Alkermes, Arkeo Pharmaceuticals, and the Hazelden Foundation; she is a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which is supported by Abbott Laboratories, Eli Lilly & Company, Lundbeck, Pfizer and Ethypharm; and she has received study supplies from Pfizer and a contract from Eli Lilly as a study site for a multi-site trial. None of these activities are related to the current report. No other authors report potential conflicts.

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Mean=0.51 SD=0.30, $F(1, 32.2) = 24.24, p < .0001$) but not in smokers (from Mean=0.25, SD=0.12 to Mean=0.26, SD=0.15, $F(1, 31.1) = 0.04, p = 0.84$). In nonsmokers the indirect bilirubin concentration and the ratio of indirect (unconjugated) to direct (conjugated) bilirubin also increased significantly.

Conclusions—Alcohol consumption leads to increases in serum bilirubin in nonsmokers. Considering the antioxidant properties of bilirubin, our findings suggest one possible mechanism for the reported association between alcohol consumption and reduced risk of some disorders that could be tested in future longitudinal studies.

Keywords

bilirubin; alcohol; cardiovascular disease; alcohol metabolism; smoking; UGT1A1

1. INTRODUCTION

Although the negative consequences of alcohol consumption are well documented, both moderate drinking and higher (not necessarily elevated) concentrations of serum bilirubin, an antioxidant, have also been associated with reduced risk for some chronic disorders and all-cause mortality. Numerous reports have found that moderate alcohol intake is associated with selected favorable health outcomes (Di Giuseppe et al., 2012; Doll et al., 2005; Freiberg et al., 2004; Mukamal et al., 2003; Rehm et al., 2003; Ronksley et al., 2011), although the mechanism for these benefits is still not understood (Carnevale and Nocella, 2012). At the same time, another line of research has indicated that higher concentrations (but still well within accepted reference ranges) of serum bilirubin, a powerful antioxidant (Rizzo et al., 2010; Stocker et al., 1987), correlate with better health outcomes or conversely lower bilirubin concentrations with higher morbidities (Cheriyath et al., 2010; Curtin and Fairchild, 2003; Fischman et al., 2010; Horsfall et al., 2011; Novotny and Vitek, 2003; Perlstein et al., 2008; Rigato et al., 2005; Vitek and Schwertner, 2008; Wu et al., 2011). Of note, any drinking has been reported to be associated with higher, but still within the reference range, concentrations of serum bilirubin as compared to abstainers (Tanaka et al., 2013). Experimental research systematically linking these two observations, however, is lacking.

While the overall clinical impact of light to moderate drinking remains controversial (Holmes et al., 2014), moderate alcohol ingestion has been associated with decreased risk for metabolic syndrome (Cheriyath et al., 2010; Freiberg et al., 2004), inflammatory illnesses (Di Giuseppe et al., 2012; Tabak et al., 2001) and cardiovascular disease (Brien et al., 2011; Corrao et al., 2004; Ronksley et al., 2011). A J-shape relationship for coronary heart disease has been found in which the minimum relative risk compared to abstainers was at 20gms of ethanol/day (1.5 standard drinks), and with poorer outcomes observed at 89gms/day (Corrao et al., 2004). A meta-analysis of 84 prospective cohort studies found that the relative risk of mortality from all causes was lower for drinkers compared to nondrinkers (Ronksley et al., 2011). Several mechanisms have been proposed including effects on high-density lipoprotein associated cholesterol, triglycerides, fibrinogen and antioxidant capacity (Brien et al., 2011; Covas et al., 2010; Rimm et al., 1999), but the possible impact of alcohol on bilirubin concentrations has yet to be considered as a potential mechanism.

Bilirubin concentrations, in turn, have also been inversely associated with risk for cardiovascular disease (Novotny and Vitek, 2003; Perlstein et al., 2008; Vitek and Schwertner, 2008), metabolic syndrome (Wu et al., 2011), inflammatory disease (Fischman et al., 2010), diabetes (Cheriyath et al., 2010), and some cancers (Horsfall et al., 2011). Unconjugated (indirect) bilirubin, the primary form of bilirubin circulating in healthy individuals, has antioxidant properties (Rizzo et al., 2010; Stocker et al., 1987), which have been suggested as possible mechanisms for its apparent protective effects. Higher concentrations of total serum bilirubin are associated with decreased risk for the aforementioned disorders. A meta-analysis of 11 studies in men, for example, showed a 6.5% decrease in coronary artery disease risk for each 0.06 mg/dl increase in serum bilirubin (Novotny and Vitek, 2003). Individuals with Gilbert's disease have a genetically determined deficiency in UDP-glucuronosyltransferase 1A1 (UGT1A1) activity (Bosma et al., 1995) associated with a moderate increase in indirect bilirubin concentrations, and are reported to have decreased risk for cardiovascular disease (Schwertner and Vitek, 2008). Despite the striking similarity between some reported effects of moderate alcohol intake and higher bilirubin concentrations, the link between these observations has been relatively unexplored.

In contrast to the hypothesized effects of alcohol, tobacco smoking has been associated with lower concentrations of serum bilirubin in numerous epidemiological studies (Hopkins et al., 1996; Madhavan et al., 1997; Tanaka et al., 2013; Van Hoydonck et al., 2001; Zucker et al., 2004) and a recent study suggests that concentrations rise following smoking cessation (O'Malley et al., 2014). Induction of UGT 1A1, the primary pathway for disposition of bilirubin (Bosma et al., 1995), by nicotine and/or other constituents of tobacco smoke has been suggested as a mechanism for the reduction in bilirubin among smokers (van der Bol et al., 2007).

In the current study, we tested the hypothesis that acute alcohol ingestion leads to elevations in bilirubin levels, and examined this by smoking status. This question was addressed within the context of a Phase I study designed to evaluate ethyl glucuronide elimination following low, medium, and high doses of alcohol (Jatlow et al., 2014). Eighteen participants received 1 of the 3 doses, on separate days, at least 1 week apart, in random order across 3 inpatient alcohol challenge laboratory sessions, preceded and followed by a period of documented abstinence from alcohol. We examined the effects of alcohol on mean bilirubin concentrations determined before and after alcohol consumption. A secondary analysis examined whether bilirubin concentrations measured following alcohol consumption returned to pre-drinking levels after 5 days of abstinence as further evidence of the causal influence of alcohol on bilirubin.

2. MATERIALS AND METHODS

2.1. Participants

Participants were 18 healthy male and female smokers and nonsmokers who reported a day of drinking sufficient to reach an estimated blood alcohol level (BAL) of ≥ 100 mg/dL in the past 6 months but who did not meet Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; American Psychiatric Association, 2000) criteria for alcohol dependence. The drinking criteria were used to recruit individuals who had experience

consistent with the highest dose of alcohol to be administered but who could comply with instructions to abstain prior to and following laboratory sessions.

2.2. Procedures

2.2.1. Intake—The Human Investigation Committee at Yale University approved this protocol which adhered to guidelines for the administration of alcohol to human subjects (National Advisory Council on Alcohol Abuse and Alcoholism, 2005). Written informed consent was obtained at screening prior to further assessment that included smoking history, self-reported drinking for the prior 90 days, breath samples for carbon monoxide and alcohol; urine sample for EtG, drug toxicology and pregnancy testing, and blood samples for routine testing. Participants received a physical exam (including an electrocardiogram if > age 45).

Eligible participants were scheduled for 4 laboratory sessions separated by at least 1 week. They were instructed to not consume alcohol or use ethanol containing products or foods for at least 5 days prior to and following each session. Participants were monitored daily using alcohol breathalyzer readings, self-report and urine for EtG to document abstinence. Monetary payments were provided contingent on abstinence.

2.2.2. Test Days Procedures

2.2.2.1. Baseline: Laboratory sessions occurred at the Hospital Research Unit of the Yale Center for Clinical Investigation. Subjects were instructed to fast after midnight prior to admission. After confirming compliance with dietary instructions, urine was obtained for EtG, toxicology screen, and pregnancy test, and vital signs and BrAC were measured. A blood sample was taken at 7:45 a.m. for measurement of bilirubin, plasma cotinine, and other markers. Subjects were then provided a light standardized breakfast.

2.2.2.2. Alcohol Administration: At 8:30 a.m., alcohol was administered as a mix of 80-proof vodka and a nonalcoholic mixer (diet cranberry drink with Sucralose). At each of 3 sessions, subjects received 1 of 3 alcohol doses. Order was counterbalanced within sex and smoking status using a Latin-squares design. At a fourth session, the medium dose was repeated; only data from the predrinking baseline was used in this study for the analysis of the effects of abstinence on bilirubin concentration.

The 3 doses were calibrated to achieve BALs of 20mg/dl, 80mg/dl and 120mg/dl using a formula that takes into account total body water (based on gender, age, height, weight), the duration of drinking, and the ratio of alcohol to mixer using the BAL calculator developed by Curtin (Curtin and Fairchild, 2003). To avoid dilutional effects as a source of variation we administered a constant total volume (alcohol + mixer) across doses for each subject based on the volume of the high dose. The ratio of alcohol to mixer was 1:3 in the high dose. The total volume was divided into 6 equal drinks, with each consumed over a 15-minute period.

Blood samples were collected to monitor alcohol levels at 9 a.m., 10:0 a.m., and every 30 minutes for 3 hours and thereafter hourly until concurrent BrACs were negative. Serum samples were assayed for bilirubin at baseline (7:45 a.m.), at 2 pm and at 7:45 a.m. the

following morning. Standardized meals were provided and subjects fasted from midnight until after the morning blood draw. Time of day and fasting condition were identical for collection of the baseline and 24 hour bilirubin samples. While in the hospital, smokers were permitted to use nicotine lozenges but were not allowed to smoke cigarettes. The number of 2 mg lozenges used (Mean = 5.2, SD = 3.4) did not differ by session ($p = 0.23$).

2.2.2.3. Blood Alcohol Concentration (BAC): Blood samples were collected in oxalate, fluoride tubes and concentrations determined by headspace gas chromatography employing a Teledyne Tekmar Headspace Autosampler (Teledyne Tekmar, 4736 Socialville Foster Rd., Mason, OH 45040) interfaced to an Agilent 7890A gas chromatograph (Agilent Technologies, 5301 Stevens Creek Blvd. Santa Clara, CA, 95051) equipped with a Restek Rtx-200 column (Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823).

2.2.2.4. Serum Bilirubin Concentration: Three mls of blood were drawn into heparinized tubes and immediately protected from light. Samples were centrifuged and plasma was transferred to black (opaque) tubes, initially frozen at -20° and subsequently transferred to a -70° C freezer within 24 hours. The 2 morning blood samples were also used to conduct selected liver enzyme tests before and 24 hours following the high alcohol dose. Assays for serum bilirubin and aminotransferases were performed on the Roche DPP Modular automated chemistry analyzer. Total and direct bilirubin were determined by a FDA approved diazo procedure (Jendrassik and Grof, 1938; Malloy and Evelyn, 1937) as adapted by the manufacturer (Roche Diagnostics International Ltd CH-6343, Rotkreuz, Switzerland). Total bilirubin involved addition of a detergent to solubilize the indirect (unconjugated) component. Indirect bilirubin was calculated by subtracting the direct bilirubin value from the total. For our primary outcome, we focused on the primary measurement (total bilirubin) rather than a derived measurement (indirect bilirubin). Moreover, published studies of the correlation of bilirubin concentrations with health outcomes have measured total bilirubin. However, secondary analyses examine indirect bilirubin concentration and the ratio of indirect to direct bilirubin.

2.3. Statistical Analyses

Baseline characteristics were summarized using descriptive statistics and compared for smokers and nonsmokers using Fisher's exact tests for categorical variables and t-tests for continuous variables. A mixed effects model with dose (low, moderate, high), time (0= pre-drinking, 1= 6 hours, and 2 = 24 hours from time 0) and smoking status (nonsmoker, smoker) was used to characterize changes in bilirubin. Dose, time and their interaction were within-subject factors in the model and smoking status was a between-subject factor. Period effects were included to control for order of administration of the 3 doses. The best-fitting correlation structure was selected based on Schwartz-Bayesian Information Criterion. We log-transformed bilirubin concentrations to approximate normality better. Total bilirubin concentrations below the detection threshold of 0.10 were coded as 0.05, i.e. at mid-point of the range between 0 and 0.10. This recoding was done for 12 of 162 values. We also performed sensitivity analyses with other imputation values in the range or after omitting 3 missing values (2–6hr and 1–24hr time point) and values below the detection threshold and verified that the conclusions were the same. Our primary focus was on change in bilirubin

concentrations from time 0 (pre-drinking) to time 2 (24 hours from baseline) because fasting and time of day conditions were identical for these time points. Thus, planned comparisons of time 0 and time 2 concentrations were examined.

A secondary analysis compared the post-alcohol bilirubin concentration (i.e., at 24hrs) for each dose to the subsequent bilirubin concentration measured following 5–7 days of abstinence (i.e., time 0 of the next session) to evaluate the effects of abstinence from alcohol on bilirubin. In most cases, the subsequent session occurred in the next week, but there were 7 instances in which there was more than 1-week between sessions. In all 7 cases, however, the second time-point was preceded by at least 5 days of documented abstinence. A similar mixed effects model with dose, time (post-drinking, following abstinence), smoking status and their interaction was used.

Finally, exploratory analyses of indirect bilirubin and the ratio of indirect to direct bilirubin were conducted in the nonsmokers [these measurements could not be derived in a large number of the samples (37 of 48 values) from smokers who generally had lower bilirubin concentrations] using mixed models with dose and time (time 0: pre-drinking and 24 hours from time 0) as within-subject factors. Both outcomes were log-transformed to approximate normality better. Observations corresponding to direct bilirubin concentrations below the quantitation threshold of 0.10 were coded as missing for 15 of 60 samples.

3. RESULTS

3.1. Participants

Ten nonsmokers (5 men, 5 women) and 8 smokers (6 men, 2 women) participated. Table 1 presents their characteristics. Smokers and nonsmokers were similar on all characteristics except age ($p = 0.01$) and drinking history ($p = 0.02$). Smokers were older and drank alcohol on fewer percentage of the baseline days. Total bilirubin levels were somewhat lower in smokers ($M = 0.38$, $SD = 0.46$) compared to nonsmokers ($M = 0.50$, $SD = 0.231$), although the difference was not statistically significant ($p = 0.13$). Seventeen participants completed all sessions. One nonsmoker vomited following the high dose and so bilirubin was not measured after the first morning (pre alcohol) time point for this subject. One nonsmoker was noncompliant with the requirements for abstinence and had slightly elevated levels of EtG on 2 outpatient days and on the morning of the high dose session suggesting consumption of a small quantity of alcohol on the prior day. We considered these exposures acceptable because they could have biased against our hypotheses by elevating the baseline bilirubin concentrations.

3.2. Alcohol Exposure

Grams of alcohol administered in the low, moderate and high dose on average were 26.3 ($SD = 5.91$), 57.8 ($SD = 13.01$), and 78.8 ($SD = 17.74$) respectively. Peak BACs achieved were 28.3mg/dl ($SD = 8.85$), 93.1 ($SD = 20.74$), and 138.8 ($SD = 27.62$) for the low, medium, and high doses respectively.

3.3. Effects of acute alcohol consumption on total bilirubin

Figure 1 presents the mean serum bilirubin concentrations before and after drinking for nonsmokers and smokers by alcohol dose. There was a significant interaction between time and smoking status ($F(2, 32) = 5.82, p = 0.007$) for bilirubin concentrations suggesting that the change in total bilirubin following drinking differed significantly between smokers and non-smokers. Pairwise comparisons between pre-drinking and 24 hours post drinking initiation showed that total bilirubin increased significantly following alcohol consumption in nonsmokers ($F(1, 32.2) = 24.25, p < 0.0001$) but did not change in smokers ($F(1, 31.1) = 0.04, p = 0.84$). In nonsmokers, bilirubin concentration increased from a mean of 0.38 mg/dL ($SD = 0.24$) to a mean of 0.51 mg/dL ($SD = 0.30$) across the 3 doses; whereas in smokers, the mean concentration remained essentially the same (baseline Mean = 0.25, $SD = 0.12$; post-drinking Mean = 0.26, $SD = 0.15$; to convert to $\mu\text{mol/L}$, multiply the mg/dL value by 17.104). At 24 hours, smokers had significantly lower total bilirubin on average than non-smokers ($F(1, 18.2) = 8.45, p = 0.01$). The differences between smokers and non-smokers were not significant at the other time points (p -values > 0.10). The effects of alcohol dose on bilirubin concentrations were not statistically significant (p -values > 0.18) and the pre-drinking morning levels did not differ by session ($F(2, 75) = 1.64, p = 0.20$).

3.4. Effects of abstinence from alcohol on total bilirubin

The analysis comparing post-drinking bilirubin concentrations to concentrations measured after abstinence yielded a borderline significant interaction between time (post-drinking, after abstinence) and smoking status ($F(1, 16.5) = 3.85, p = 0.07$). For nonsmokers who as a group showed an increase in bilirubin concentrations following alcohol consumption, pairwise comparisons between the 24-hour time point during each drinking session and the baseline time point for the following session after at least 5 days of alcohol abstinence showed that total bilirubin concentration decreased significantly following abstinence from drinking ($F(1, 16.4) = 11.31, p = 0.004$). Among smokers who as a group did not show increases in bilirubin concentrations following drinking, bilirubin concentrations did not change following abstinence ($F(1, 16.6) = 0.14, p = 0.72$).

3.5. Effects of alcohol on indirect bilirubin and the ratio of indirect to direct bilirubin

Using data from the nonsmokers, we examined the effects of alcohol on indirect bilirubin and on the ratio of indirect to direct bilirubin. There was a significant main effect of time for indirect bilirubin ($F(1, 19.3) = 15.64, p = 0.001$) and for the indirect/direct ratio ($F(1, 30.8) = 8.81, p = 0.006$). Both indirect bilirubin and the indirect/direct ratio increased from pre-drinking to post-drinking (baseline mean = 0.31, $SD = 0.22$, post-drinking mean = 0.40, $SD = 0.25$ for indirect; and baseline mean = 1.92, $SD = 0.79$, post-drinking mean = 2.21, $SD = 0.63$ for the ratio of indirect to direct bilirubin). There were no significant interactions between dose and time (p 's > 0.31) and no significant dose effects (p 's > 0.07).

3.6. Effects of alcohol challenges on liver enzymes

To explore the possibility that increases in bilirubin were a consequence of acute hepatotoxicity following acute alcohol consumption, we compared serum aspartate aminotransferase (AST) and alanine transaminase (ALT) activity at time 0 (pre-drinking)

and time 2 (24 hours) for the high dose session using mixed models with time as a within-subject factor and smoking as a between subject factor. ALT levels were log-transformed to better approximate normality. Alcohol consumption did not lead to increases in ALT or AST in nonsmokers or smokers (p-values > 0.3). The mean change from baseline to 24 hours (Time 0–Time 2) was 1.35 U/L (SD=4.11) for AST and –0.11 U/L (SD=4.71) for ALT.

4. DISCUSSION

Despite their similar impact on several common clinical disorders, studies examining the effects of acute alcohol consumption on bilirubin concentrations are lacking. In this study, we found that alcohol elevates concentrations of total bilirubin 24 hours after oral ethanol administration in nonsmokers, irrespective of the dose of alcohol. The magnitude of this effect is not meaningful in the context of hepatotoxicity, but of a size that has been associated with reduced risk of cardiovascular disease, respiratory illness and mortality in epidemiological studies (Horsfall et al., 2011; Novotny and Vitek, 2003; Perlstein et al., 2008; Vitek and Schwertner, 2008; Wu et al., 2011). Similar increases were not seen in smokers.

In nonsmokers, the increase in mean serum total bilirubin following alcohol consumption averaged across the 3 doses was 0.13 mg/dL. At the lowest dose, the increase was 0.08mg/dL and is likely to be physiologically meaningful. Differences in total bilirubin concentrations, similar to that seen in our study, have been associated with lower risk of illnesses in epidemiological studies. For example, a 0.06mg/dL increase in bilirubin was associated with a 6.5% lower risk of coronary artery disease in a meta-analysis of 11 studies (Novotny and Vitek, 2003). Moreover, other studies found that an increase of 0.1 mg/dL was associated with at least a 6% reduction in the risk of peripheral vascular disease (Perlstein et al., 2008; Vitek and Schwertner, 2008), a 17% reduction in the risk of metabolic syndrome (Wu et al., 2011), and an 8% (men) and 11% (women) reduction in lung cancer risk (Horsfall et al., 2011). The positive association of bilirubin concentrations with various favorable health outcomes has been attributed to, but not proven to, be a consequence of its antioxidant properties. Determining whether multiple days of light drinking alcohol would show a cumulative or persistent effect on bilirubin levels is a critical next step. A recent epidemiological study has documented increased bilirubin levels in association with any level of alcohol consumption (Tanaka et al., 2013).

A possible mechanism for the observed increases in total bilirubin among nonsmokers is that alcohol competitively inhibits bilirubin conjugation. Although the major routes for the metabolic disposition of ethanol involve oxidation by cytosolic ADH and microsomal *CYP* 2E1, minor pathways (< 0.1%) include conjugation with glucuronic acid and sulfate. The pathway for glucuronidation of ethanol involves UGT 1A1, which is also primarily responsible for glucuronidation of bilirubin. Gilbert's syndrome is a more dramatic example wherein reduced UGT 1A1 activity results in an increase in total bilirubin predominately composed of indirect bilirubin. The physiological significance of this pathway of alcohol metabolism has not been previously considered, although the resulting minor metabolite ethylglucuronide (EtG) has received interest as a longer term marker of alcohol use for monitoring abstinence (Jatlow and O'Malley, 2010).

Although glucuronidation is a minor pathway for the disposition of ethanol, the concentration of alcohol substrate is 2 or more orders of magnitude greater than that of bilirubin. Thus, alcohol may be a competitive inhibitor of bilirubin conjugation. Consistent with this possibility, both indirect (unconjugated) bilirubin and the ratio of indirect to direct (conjugated) bilirubin increased significantly following alcohol consumption. Moreover, alcohol has been reported to inhibit the glucuronidation of morphine (Bodd et al., 1986a, 1986b), and conversely, bilirubin to inhibit the glucuronidation of ethanol (Foti and Fisher, 2005). However, ethanol failed to inhibit glucuronidation of estradiol in vitro in our preliminary studies (unpublished data-assay performed by Cyprotex, 313 Pleasant St., Watertown, MA 02472). Moreover, we cannot rule out the possibility of slight sub-clinical hemolysis following alcohol consumption as responsible for the increase in bilirubin levels. And while AST and ALT did not increase after alcohol consumption, these enzymes may not be sufficiently sensitive to detect minimal acute hepatotoxicity (Hashimoto et al., 2013). Thus, the mechanism responsible for the small increase in bilirubin following alcohol consumption remains uncertain.

Smokers did not experience comparable increases in bilirubin concentrations suggesting that smokers may not derive the same benefit from moderate drinking as nonsmokers. This is not all together surprising in that epidemiological studies have shown that smokers have lower bilirubin levels than nonsmokers and former smokers (Hopkins et al., 1996; Madhavan et al., 1997; Tanaka et al., 2013; Van Hoydonck et al., 2001; Zucker et al., 2004), possibly through induction of UGT 1A1 by nicotine and/or other constituents of tobacco smoke (van der Bol et al., 2007) which may compensate for alcohol's effects. However, the differential effects of alcohol on bilirubin concentrations for smokers and nonsmokers in our study must be considered very preliminary given the small sample size and differences in baseline characteristics between smokers and nonsmokers.

Although there was no placebo alcohol condition, we compared bilirubin concentrations prior to alcohol consumption and at the same time the following morning. In both instances samples were obtained prior to eating. The selection of these 2 time points controlled for time of day and fasting status. For nonsmokers, total bilirubin levels increased following alcohol consumption and returned to baseline levels following 5–7 days of abstinence suggesting that alcohol ingestion was responsible for these increases. In addition, the increase following drinking is unlikely due to day-to-day fluctuations because the pre-alcohol bilirubin concentrations measured across the 3 testing days did not differ significantly from each other.

Finally, our study evaluated the effects of alcohol on bilirubin concentrations, a known antioxidant (Rizzo et al., 2010; Stocker, 2004; Stocker et al., 1987), but did not evaluate the antioxidant effects of alcohol consumption or of bilirubin. Acute alcohol consumption has a range of effects both positive and detrimental that were not the focus of this study, and several mechanisms have been proposed previously for the beneficial effects of alcohol including effects on high-density lipoprotein associated cholesterol, triglycerides, fibrinogen and antioxidant capacity (Brien et al., 2011; Covas et al., 2010; Rimm et al., 1999). Change in bilirubin concentration is likely not the only contributor to the overall anti-oxidant effects

of alcohol; however, bilirubin itself has antioxidant activity and small increases in bilirubin have been associated with salutary changes in markers of inflammation (Ong et al., 2013).

In conclusion, our results provide preliminary evidence that in nonsmokers acute alcohol consumption increases serum bilirubin, an antioxidant previously shown to be associated with a number of health benefits such as reduced cardiovascular risk. Future studies will need to determine whether repeated low-dose drinking produces greater and more sustained increases in bilirubin than that found with a single low dose surrounded by abstinence, and longitudinal studies of alcohol consumption will need to evaluate the hypothesized, but yet untested, link between alcohol-related increases in bilirubin concentrations and health. This report should not be construed as an endorsement of drinking. Rather, it offers a possible explanation for some of the health benefits that have been reported to be associated with low to moderate alcohol consumption.

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- Serum bilirubin was measured before and after acute alcohol consumption
- Bilirubin, an endogenous antioxidant, increased in nonsmokers but not in smokers
- Bilirubin concentrations correlate with favorable health outcomes
- Effects on bilirubin may explain some of the positive effects of alcohol on health

Figure 1.

Unadjusted Total Serum Bilirubin Concentrations Before and After Drinking for Nonsmokers (n=10) and Smokers (n=8) by Alcohol Dose. Nonsmokers showed a significant increase in bilirubin concentration from baseline; whereas nonsmokers did not (Smoker $\times$ Time, $p < 0.0001$). The effect of dose were not statistically significant. Across the 3 doses, mean bilirubin concentrations for nonsmokers prealcohol = 0.38mg/dL (SD=0.24) and post drinking = 0.51 mg/dL (SD=0.30); mean concentrations for smokers prealcohol =0.25, SD=0.12 and post-drinking =0.26, SD=0.15; to convert to umol/L, multiply the mg/dL value by 17.104). One data point was missing (24hr, High Dose, Smoker).

Table 1

Baseline Participant Characteristics (n = 18)

Variable	Nonsmokers (n = 10)	Smoker (n=8)	p-value
Age in years, Mean(SD)	26.3(8.67)	41.5(12.68)	0.01
Race			0.18
White, N (%)	5(50)	1((10)	
Black, N (%)	4(40)	0	
Other, N (%)	1((10)	2(25)	
Male, N (%)	5(50)	6(75)	0.37
Weight in lbs., Mean(SD)	176.3(38.88)	181.6(43.25)	0.79
Baseline alcohol consumption			
Baseline-percent days drinking, Mean (SD)	33.6(18.68)	14.2(9.41)	0.02
Standard drinks per drinking day, Mean (SD)	4.7(2.55)	4.4(1.23)	0.78
Peak standard drinks on a day, Mean (SD)	9.6(4.49)	8.2(4.12)	0.52
Baseline smoking characteristics			
Cigarettes per day, Mean (SD)	n/a	15.3(5.61)	
Carbon monoxide level, Mean(SD), ppm	n/a	16.9(4.85)	
Tests for liver function and injury			
Bilirubin total, Mean (SD), mg/dL	0.50(0.231)	0.38(0.046)	0.13
Aspartate aminotransferase, Mean (SD), U/L	26.4(12.56)	19.9(4.29)	0.15
Alanine aminotransferase, Mean (SD), U/L	26.3(12.76)	25.9(16.23)	0.95

Note: To convert to umol/L, multiply the mg/dL value by 17.104