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Acute alcohol consumption elevates serum bilirubin: An endogenous antioxidant

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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 (eight cigarette smokers) were administered alcohol, calibrated to achieve blood concentrations of 20, 80 and 120 mg/dL, in random order in three 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 a.m. prior to drinking, at 2 p.m., and at 7:45 the next morning. Mixed effects regression models compared baseline and 24 h post-drinking bilirubin concentrations.
Results: Total serum bilirubin (sum of indirect and direct) concentration increased significantly after drinking from baseline to 24 h in non-smokers (from $M = 0.38$, $SD = 0.24$ to $M = 0.51$, $SD = 0.30$, $F(1, 32.2) = 24.24$, $p < .0001$) but not in smokers (from $M = 0.25$, $SD = 0.12$ to $M = 0.26$, $SD = 0.15$, $F(1, 31.1) = 0.04$, $p = 0.84$). 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.

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

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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 20 g of ethanol/day (1.5 standard drinks), and with poorer outcomes observed at 89 g/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 20 mg/dL, 80 mg/dL and 120 mg/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 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-min period.

Blood samples were collected to monitor alcohol levels at 9 a.m., 10:0 a.m., and every 30 min for 3 h and thereafter hourly until concurrent BrACs were negative. Serum samples were assayed for bilirubin at baseline (7:45 a.m.), at 2 p.m. 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 h 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 ($M = 5.2$, $SD = 3.4$) 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 millilitres 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 h. The 2 morning blood samples were also used to conduct selected liver enzyme tests before and 24 h following the high alcohol dose. Assays for serum bilirubin and aminotransferases were performed on the Roche DPP Modular automated chemistry analyzer.

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