The Direct Effect of Ethanol on Human Vascular Function

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Running Head:
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Abstract

Background: Epidemiologic studies indicate that moderate ethanol consumption reduces cardiovascular mortality. Cellular and animal data suggest that ethanol confers beneficial effects on the vascular endothelium and increases the bioavailability of nitric oxide.

Objectives: The purpose of this study was to assess the effect of ethanol on endothelium-dependent, nitric oxide-mediated vasodilation in healthy human subjects.

Methods: Forearm blood flow, (FBF), was determined by venous occlusion plethysmography in healthy human subjects during intra-arterial infusions of either methacholine (0.3, 1.0, 3.0, and 10.0 mcg/min, n = 9), nitroprusside (0.3, 1.0, 3.0, and 10.0 mcg/min, n = 9), or verapamil (10, 30, 100, and 300 mcg/min, n = 8), before and during the concomitant intra-arterial infusions of ethanol (10% ethanol in 5% dextrose). Additionally, a time control experiment was conducted, during which the methacholine dose response curve was measured twice during vehicle infusions, (n=5).

Results: During ethanol infusion, mean forearm and systemic alcohol levels were 227±30 and 6±0 mg/dL, respectively. Ethanol infusion alone reduced FBF (2.5±0.1 to 1.9±0.1 ml/dL/min, p<0.05). Despite initial vasoconstriction, ethanol augmented the FBF dose response curves to methacholine, nitroprusside, and verapamil (p<0.01 by ANOVA for each). To determine whether this augmented FBF response was related to shear-stress induced release of nitric oxide, FBF was measured during the co-infusion of ethanol and N-nitro-L-arginine methyl ester (L-NAME), (n=8), at rest and during verapamil-induced vasodilation. The addition of L-NAME did not block ethanol’s ability to augment verapamil-induced vasodilation.

Conclusions: Ethanol has complex direct vascular effects, which include basal vasoconstriction as well as potentiation of both endothelium-dependent and – independent vasodilation. None of these effects appear to be mediated by an increase in nitric oxide bioavailability, thus disputing findings from pre-clinical models.

Keywords: Alcohol, ethanol, nitric oxide, endothelial function, humans, verapamil, methacholine, nitroprusside, plethysmography,
**Introduction**

Large epidemiological studies demonstrate that moderate alcohol consumption is associated with a 40 to 60% reduction of mortality from coronary artery disease (Goldberg et al. 1994; Klatsky et al. 1981a; Rimm et al. 1991; Stampfer et al. 1988; Handa et al. 1990; Doll et al. 1994). Several mechanisms may contribute to the decreased cardiovascular mortality, including favorable effects on lipoprotein metabolism such as increased HDL$_2$ and HDL$_3$ cholesterol subfractions (Suh et al. 1992; Gaziano et al. 1993), and antithrombotic effects mediated by decreased platelet aggregation (Rubin and Rand 1994) and increased tPA levels (Hendriks et al. 1994; Ridker et al. 1994). Furthermore, animal and cellular data suggest that ethanol confers beneficial effects on the vascular endothelium, increasing the bioavailability of nitric oxide (Davda et al. 1993; Diebolt et al. 2001; Fitzpatrick et al. 1993; Greenberg et al. 1993) and decreasing the synthesis of endothelin-1 (Corder 2001).

In humans, ingestion of an alcoholic beverage acutely increases heart rate, cardiac output, and cardiac stroke volume (Kelbaek et al. 1988a; Kelbaek et al. 1988b) and decreases blood pressure (Kelbaek et al. 1988a; Rosito et al. 1999). Further, systemic administration of alcohol is associated with increases in forearm and coronary blood flow (Kelbaek et al. 1988b; Pirwitz et al. 1995; Cigarroa et al. 1990). However, studies examining the effect of ethanol on nitric oxide-mediated vasodilation in humans have yielded inconsistent results (Hashimoto et al. 2001; Djousse et al. 1999). Moreover, such prior observations of ethanol’s hemodynamic effects were made in the setting of oral or intravenous ethanol administration, and hence are confounded by ethanol’s interactions with the central nervous system.

The purpose of this study was to test the hypothesis that the vascular actions of ethanol in humans are mediated by endothelium-derived nitric oxide. Forearm blood flow was measured in healthy human subjects during the intra-arterial infusion of endothelium-dependent and endothelium-independent vasodilators before and during concomitant intra-arterial administration of ethanol. Thereafter, the local vasomotor effects of ethanol were assessed during inhibition of nitric oxide synthase.

**Methods**

**Patient population**

Healthy subjects were recruited from the greater Boston area. Exclusion criteria included the following: smoking; systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg; serum cholesterol level above the 75th percentile for age and sex; history of vascular disease; diabetes; family history of premature coronary artery disease; or any clinical manifestation of atherosclerosis, such as coronary artery disease, peripheral artery disease, or carotid artery disease. The study protocol was approved by the Partners Joint Human Research Committee, and informed consent was obtained from each subject.
**Experimental protocol**

Each subject was studied in a 23˚C temperature-controlled room in the fasting state. Non-steroidal anti-inflammatory drugs, alcohol, and caffeine were prohibited for 12 hours before study initiation. Under local anesthesia and sterile conditions, a 20-gauge polyethylene catheter was placed in the brachial artery of each subject for determination of blood pressure and for infusion of drugs. The vascular research laboratory was kept quiet and the lights dimmed. All subjects rested at least 30 minutes after catheter placement to establish a stable baseline before data collection. Venous occlusion plethysmography (described below) was employed to measure the forearm blood flow response to endothelium-dependent and –independent vasodilators during co-administration of ethanol or vehicle. Methacholine chloride (Provocholine, Roche Labs, Nutley, NJ), a congener of acetylcholine that acts by stimulating nitric oxide release from the vascular endothelium, was administered intra-arterially at doses of 0.3, 1.0, 3.0 and 10.0 mcg/min (n=9). To distinguish abnormalities in endothelial function from those of vascular smooth muscle, sodium nitroprusside (Elkins-Sinn Inc, Cherry Hill, NJ), which acts as a nitric oxide donor, was administered intra-arterially at doses of 0.3, 1.0, 3.0 and 10.0 mcg/min (n=9). To evaluate the direct smooth muscle effects of ethanol, independent of nitric oxide, the calcium channel antagonist verapamil, (American Reagent Laboratory Inc, Shirley, NY) was administered intra-arterially at doses of 10, 30, 100, and 300 mcg/min (n=9). On each visit, only a single vasodilator was employed to test the mechanism by which ethanol affects forearm blood flow. Each vasoactive drug was infused for five minutes at a rate of 0.4 ml/min. A 5% dextrose solution served as a vehicle control for determining baseline flows.

After recording the blood flow response to incremental doses of methacholine, nitroprusside, or verapamil, at least 45-60 minutes were allowed to pass to ensure re-establishment of basal conditions. Then, ethanol solution (10% ethanol in 5%dextrose) or vehicle was infused in the ipsilateral brachial artery. The infusion rate for the ethanol solution was determined using the subject’s basal forearm blood flow, the concentration of ethanol in the solution, and the desired final blood concentration (200mg/dL) as computational variables. The total intra-arterial infusion rate (ethanol solution plus dextrose solution) was maintained at a constant throughout the experimental series for each visit. In all cases, the total amount of ethanol infused during any one visit was less than 10 gm ethanol, (i.e. less than the amount of ethanol in a single glass of wine). Forearm blood flow measurements were repeated after ethanol had been infused intra-arterially for thirty minutes. Thereafter, the dose response curve to the endothelium-dependent or –independent vasodilator drug was once again assessed, this time during co-infusion of ethanol. Additionally, a time control experiment was conducted (during which the methacholine dose response curve was measured twice during vehicle infusions) (n=5).

**Contribution of nitric oxide to verapamil-stimulated vasodilation**

Numerous endothelium-independent vasodilators have been shown to elicit nitric oxide release (perhaps as a result of enhanced shear stress) (Hein and Kuo 1999; Tawakol et al. 2002; Parent et al. 1992; Buus et al. 2001; Dawes et al. 1997). As such, to determine if nitric oxide contributes to verapamil-induced vasodilation in humans, forearm blood
flow was measured during intra-arterial administration of verapamil, during the concomitant administration of vehicle vs the nitric oxide synthase inhibitor, N^2^-nitro L-arginine Methyl Ester, (L-NAME, 2 mg/min over 10 minutes, Clinalfa, Läufelfingen, Switzerland) (n=8).

**Contribution of nitric oxide to ethanol-augmented vasodilation**

Thereafter, we sought to determine whether the vascular responses to ethanol were mediated by release of nitric oxide. We employed verapamil as the vasodilator with which to test this hypothesis for 3 reasons. First, the classic endothelium-dependent dilator, methacholine was *not* chosen in this sub-protocol since nitric oxide inhibition is known to significantly attenuate the blood flow response to this pharmacologic probe (Bruning et al. 1996). Secondly, our initial results (from the first series of experiments, above) suggested that ethanol’s potentiation of vasodilation was greatest during verapamil infusion. Thirdly, we anticipated that a portion of the blood flow response to verapamil is attributed to nitric oxide, perhaps due to shear stress release of nitric oxide after an initially endothelium-independent augmentation of blood flow. After re-establishing baseline blood flow in the 8 subjects who participated in the aforementioned verapamil/LNAME experiment, the forearm blood flow response to verapamil was measured again, during the concomitant administration of (L-NAME, 2 mg/min over 10 minutes) plus ethanol.

**Venous occlusion plethysmography:**

Bilateral forearm blood flow (FBF) was determined by venous occlusion strain-gauge plethysmography, using calibrated mercury-in-Silastic strain-gauges (D.E. Hokanson, Inc, Bellevue Wash). Each arm was supported above the heart level. Venous occlusion was produced by inflation of a sphygmomanometric cuff in the upper arm. The lowest venous occlusion pressure needed to obtain the maximum rate of increase in forearm circumference was determined at the beginning of each study. Circulation to the hand was prevented by inflating the wrist cuff to suprasystolic pressures during each blood flow determination. FBF measured during each experimental period was composed of at least five different measurements performed at 10-15 second intervals. By measuring blood flow in the infused arm, the direct effect of the drug can be measured. By measuring blood flow in the non-infused arm, confirmation can be made that the drug is not causing systemic effects. FBF was derived from the rate of change in forearm volume during venous occlusion and is recorded on a Gould physiologic recorder. Measurements were expressed as ml/100 ml tissue/min. Brachial arterial pressure is measured via an indwelling arterial cannula. The cannula was attached to a Gould pressure transducer and the pressure measurements recorded on a Gould physiologic recorder. Forearm vascular resistance, (FVR), was calculated as the ratio of mean BP to FBF. To measure heart rate, the electrocardiogram was recorded and continually monitored during the experiments.

**Laboratory analyses**

Immediately after each ethanol infusion, blood samples were obtained from the ipsi- and contra-lateral antecubital veins for plasma alcohol measurements. Plasma alcohol
was measured by gas chromatography (Hewlett Packard, Inc., Palo Alto, CA). Systemic alcohol concentration was also measured at the bedside by means of a portable breath alcohol analyzer (Lifeloc Technologies, Denver, Colorado).

**Statistical analysis**
All data are presented as mean ± SEM. Group comparisons with respect to clinical characteristics were made with unpaired and two-tailed t tests. FBF dose response curves for each drug before and during co-administration of ethanol were analyzed with two way repeated-measures ANOVA followed by post hoc two-tailed t tests adjusted with a Bonferroni correction for multiple comparisons. Statistical significance was accepted at the 95% confidence level (P<0.05).

**Results**

**Patient population**
Twenty healthy subjects, including 12 men and 8 women (age, 29.5 ± 1.6 years), participated in the protocols. Most subjects were studied in more than one protocol. The subjects’ mean total-, LDL-, and HDL-cholesterol were 161±6, 89±4, and 52±3 mg/dL, respectively.

**Blood alcohol concentrations**
Mean plasma alcohol concentration after ethanol infusion was 227±30 mg/dL in the veins of the infused forearm. Mean systemic plasma alcohol concentration in the contra-lateral antecubital veins was only 6±0 mg/dL. The systemic ethanol concentration remained undetectable by breath analysis in all subjects.

**Effect of ethanol alone on hemodynamic parameters**
Intra-arterial ethanol infusion did not affect either heart rate (49±5 to 53±5 min⁻¹, p=NS), or mean arterial pressure (76±2 to 77±2 mmHg, p=NS). However, ethanol reduced ipsilateral FBF (2.5±0.1 to 1.9±0.1 ml/dL/min, P<0.01, fig. 1) and increased FVR (36±3 to 47±4 mmHg/ml/dL/min, p<0.05). In contrast, ethanol infusion did not affect contralateral FBF (1.6±0.1 to 1.7±0.2 ml/dL/min, p=NS) or FVR (49±4 to 52±6 mmHg/ml/dL/min, p=NS).

**Effect of ethanol on endothelium-dependent and -independent vasodilation**
Ethanol infusion was associated with a significant upward shift in the ipsilateral FBF dose response curves for each of the endothelium-dependent and –independent vasodilators (P<0.05 by ANOVA, with methacholine, nitroprusside, and verapamil, figure 2A,B,C). During the highest dose of methacholine, FBF was 24.4±1.9 ml/dL/min when vehicle was co-infused and 28.6±1.4 ml/dL/min when ethanol was co-infused, (P<0.01). Similarly, during the highest dose of nitroprusside, FBF was 18.5±1.4 when vehicle was co-infused and 21.4±2.3 ml/dL/min when ethanol was co-infused, (P<0.05). Likewise, during the highest dose of verapamil, FBF was 20.0±2.6 when vehicle was co-infused and 25.0±2.6 ml/dL/min when ethanol was co-infused,
This effect was consistent; ethanol augmented FBF at the highest dose of vasodilator in 22 out of 27 subjects. In congruence with these findings, FVR dose response curves were reduced by a greater extent during ethanol infusion for each of the endothelium-dependent and –independent vasodilators (P<0.05 ANOVA, for methacholine, nitroprusside, and verapamil). In contrast to those findings, in the time control experiment, (during which the FBF response to methacholine was measured twice during vehicle infusions), there was a non-significant reduction in FBF during the second series of measurements (P=NS, figure 2D). Further, there were no significant changes in contralateral FBF or FVR during any of the intra-arterial infusions.

The effect of nitric oxide synthase inhibition

The nitric oxide synthase inhibitor, L-NAME, did not affect resting heart rate (58±8 vs. 58±10 seconds⁻¹, P=NS), or mean arterial pressure (76 ±11 vs. 75±10 mmHg, P=NS). However, L-NAME was associated with an approximate 19% reduction in basal ipsilateral FBF (2.6±0.4 to 2.1±0.4 ml/dL/min, p<0.01, fig 3A). This effect was consistent; L-NAME reduced FBF at rest in 8 out of 8 subjects. This was paralleled by a significant increase in forearm vascular resistance (30.4±6.9 vs. 38.2±10.1, mmHg/ml/dL/min, P<0.05). Furthermore, L-NAME infusion significantly attenuated the FBF dose response curves to verapamil (P<0.05 by ANOVA, fig. 3A). The effect of L-NAME on verapamil-stimulated vasodilation was consistent; FBF at the highest dose of verapamil was reduced in 8 out of 8 subjects.

During co-infusion of L-NAME, ethanol did not further reduce resting FBF (2.3±0.2 to 2.1±0.3 ml/dL/min, p=NS). Moreover, L-NAME did not blunt ethanol’s ability to upwardly shift the FBF dose response relationship to verapamil (P<0.05 by ANOVA, fig 3B). Despite co-infusion of LNAME, FBF increased in 7 out of 8 subjects during ethanol and high-dose verapamil infusion.

Discussion

The important new findings of this study are that: 1) intra-arterial ethanol infusion causes limb vasoconstriction at rest, and 2) ethanol enhances the forearm blood flow response to endothelium-dependent and -independent vasodilators via a mechanism that does not involve nitric oxide.

Ethanol’s effect on nitric oxide bioavailability

This study tested the hypothesis that ethanol directly affects vasomotor function by acutely enhancing nitric oxide bioavailability. Such a finding would have highlighted a potential mechanism by which ethanol reduces cardiovascular mortality, since an increase in nitric oxide might lead to a reduction in the manifestations of atherosclerosis (via a decrease in vasoconstriction, leukocyte recruitment, platelet aggregation, and vascular smooth muscle proliferation). Indeed, animal and cellular studies have suggested that ethanol may increase nitric oxide bioavailability. Vascular strips taken from alcohol-fed rats relax more readily in response to acetylcholine (a stimulant for nitric-oxide release) than vascular strips taken from control animals (Hatake et al. 1991) and endothelial cells incubated with alcohol express more nitric oxide synthase mRNA
Prior human studies that assessed the effect of ethanol on nitric oxide bioavailability have yielded conflicting results. The ingestion of red wine has been associated with an increase in serum NO (measured by fluorescent indicator), and improved flow-mediated vasodilation in some studies, whereas other studies found no improvement in nitric-oxide mediated vasodilation after red wine ingestion (Djousse et al. 1999; Matsuo et al. 2001). However, in each of those studies, measurements were made in response to systemic administration of ethanol. Ethanol is known to increase sympathetic activation via effects on the central nervous system (CNS) (Kelbaek et al. 1988b; Johnson et al. 1986; Randin et al. 1995; van de Borne et al. 1997), thereby confounding the observations.

Thus in the present study, we sought to examine the direct effect of ethanol on human vasomotor function. To accomplish this, ethanol was infused locally, via arterial catheter, into the vascular bed being studied, dosed to achieve a forearm concentration of 227 mg/dL, which is equivalent to that achieved after drinking 4-6 glasses of wine over one hour. It is notable that the mean local blood alcohol concentration attained in this study is not frequently encountered during casual ethanol consumption (for comparison, the typical legal blood alcohol limit for driving an automobile in the USA is 100 mg/dL). Nonetheless, in the present study, systemic alcohol concentrations remained negligible. Therefore, the data reflect the direct effects of ethanol and are not confounded by ethanol’s effects on the central nervous system.

**Ethanol’s effect on resting blood flow**
We found that ethanol acutely and directly reduces resting forearm blood flow and increases forearm vascular resistance in humans. This observation may be relevant to the noted association between chronic, heavy ethanol intake and hypertension (Klag et al. 1993; Klatsky et al. 1986; Klatsky et al. 1981b; Klatsky et al. 1977; MacMahon 1987). Prior observations suggested that alcohol induced pressor effects by sympathetic activation that appeared to be centrally mediated (Randin et al. 1995). The vasoconstrictor effect observed in the current study is unlikely to result from centrally mediated changes in sympathetic tone, since systemic alcohol concentrations remained negligible during the course of this study, and since contralateral FBF remained unchanged. The mechanism of ethanol’s direct, acute vasoconstrictor effect remains to be elucidated.

**Ethanol augments vasodilation**
Despite this initial vasoconstriction, ethanol increased the dilator response to methacholine, nitroprusside, and verapamil. This potentiating effect of ethanol was most apparent at the higher doses of vasodilators infused. In contrast, repeat infusion of vehicle (as a time control), did not change the vasodilator responses (a trend towards reduced vasodilation was instead seen).
The observation that ethanol augments both "endothelium-dependent" and "endothelium-independent" vasodilation does not, on its own, exclude the possibility that the mechanism of ethanol's effect involves endothelial release of nitric oxide. Indeed, several vasodilator drugs that are traditionally classified as "endothelium-independent" have been shown to elicit nitric oxide release (Hein and Kuo 1999; Tawakol et al. 2002; Parent et al. 1992; Buus et al. 2001; Dawes et al. 1997). In such cases, the initial endothelium-independent stimulus causes an increase in flow, which can potentially lead to additional increases in flow as a result of shear stress-mediated nitric oxide release.

**Nitric oxide contributes to verapamil's dilator effect**

To test the hypothesis that nitric oxide release contributes to the vasodilator response of the classically endothelium-independent vasodilator verapamil, forearm blood flow was measured during intra-arterial administration of verapamil, during the concomitant administration of vehicle vs. L-NAME. During this series of experiments, we made the novel observation that nitric oxide synthase inhibition attenuates verapamil-induced vasodilation (fig. 3a). This observation supports the hypothesis that nitric oxide release plays a role in verapamil's vasodilatory effect in vivo. It follows, then, that although verapamil initially causes relaxation on vascular smooth muscle by inhibiting calcium channels, additional vasodilation may occur in vivo as a result of NO release (possibly resulting from shear stress-mediated NO release).

**Ethanol's augmentation of vasodilation occurs independently of NO**

However, the same concentration of L-NAME did not prevent ethanol’s ability to augment vasodilation (fig 3b). Taken together, these data suggest that ethanol directly augments both endothelium-dependent and -independent vasodilation via a mechanism that is independent of nitric oxide. Moreover, the observation that nitric oxide does not play a role in ethanol's local vasoactive effects does not directly conflict with the prior observations that alcoholic beverages enhance nitric oxide bioavailability. Indeed, prior studies suggest that it is the non-alcohol components in wine that are responsible for the nitric-oxide release (Fitzpatrick et al. 1993; Soares De Moura et al. 2002).

**Potential mechanisms**

Although these data demonstrate that NO does not play a significant role in ethanol’s acute, direct effect on vascular tone, the precise mechanisms by which ethanol altered vascular function in humans remains unexplained. It is plausible that ethanol’s vascular effects may result from its unique biophysical properties: ethanol’s compact bipolar structure allows it to readily intercalate within lipid bi-layer (Chin and Goldstein 1977). As such, it may alter the actions of cell membrane channels (Chin and Goldstein 1977), an ability that is thought to be responsible for some of ethanol’s anesthetic properties (Chin and Goldstein 1977; Slater et al. 1993; Slater et al. 1997; Stubbs and Slater 1999). Therefore, an effect on membrane ion channels is an attractive potential mechanism that may explain the vasoactive effects of ethanol.

**Study Limitations**
Caution should be exercised when extrapolating the direct, acute effects of pure alcohol to the effects of chronic, moderate consumption of alcoholic beverages. First, the vascular effect of pure ethanol may differ from the effect of alcoholic beverages, since some alcoholic beverages contain compounds that may have beneficial vascular actions that are independent of ethanol (Fitzpatrick et al. 1993). Second, since by design, systemic ethanol levels in this study were negligible, this study’s findings will not account for potentially important indirect effects of ethanol or of byproducts of ethanol metabolism. Third, the study examined only the acute effects of ethanol, and may not reflect ethanol’s chronic vasomotor effects. Fourth, the lack of a significant fall in FBF after ethanol is added to L-NAME should be interpreted with caution, since the study was neither designed nor powered to examine the role of NO in ethanol-mediated vasoconstriction at rest. Finally, the current study examined the acute, direct effects of ethanol on healthy subjects. As such, some of the observed findings, (especially the magnitude of FBF change) may differ in a population with established atherosclerosis.

Clinical Implications
The divergent direct vascular effects of ethanol reported in this study (resting vasoconstriction and potentiation of vasodilation) may, at first glance, seem counter-intuitive. However, they coincide with two well-described clinical effects of alcohol consumption: hypertension (Klag et al. 1993; Klatsky et al. 1986; Klatsky et al. 1981b; Klatsky et al. 1977; MacMahon 1987) and potentiation of syncope (Fisher 1979). Indeed, a biphasic response to ethanol ingestion has been previously described (Rosito et al. 1999).

Conclusions
The results of this investigation indicate that in healthy humans, ethanol acutely: 1) induces vasoconstriction at rest, and 2) augments endothelium-dependent and – independent vasodilation via a mechanism that does not involve nitric oxide. Further studies are needed to determine the mechanisms underlying the vascular actions of this commonly used compound.

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References


Figure Legends

Figure 1.
**Effect of Ethanol on Resting Forearm Blood Flow**
Ethanol’s effect on forearm blood flow (FBF) was determined during the infusion of vehicle vs. ethanol. Ethanol infusion was associated with a reduction in resting FBF in 23 out of 27 subjects (p<0.01).

Figure 2A.
**Effect of Ethanol on Endothelium-dependent Vasodilation**
Ethanol’s effect on forearm blood flow (FBF) was determined in response to the endothelium-dependent vasodilator, methacholine. Ethanol infusion was associated with a significant upward shift in the FBF dose response curve for methacholine, (p<0.05, ANOVA).

Figure 2B.
**Effect of Ethanol on Endothelium-independent Vasodilation**
Ethanol’s effect on forearm blood flow (FBF) was determined in response to the endothelium-independent NO donor, nitroprusside. Ethanol infusion was associated with a significant upward shift in the FBF dose response curve for nitroprusside, (p<0.05, ANOVA).

Figure 2C.
**Effect of Ethanol on Nitric Oxide-independent Vasodilation**
Ethanol’s effect on forearm blood flow (FBF) was determined in response to the endothelium-independent vasodilator, verapamil. Ethanol infusion was associated with a significant upward shift in the FBF dose response curve for verapamil, (p<0.05, ANOVA).

Figure 2D.
**Time and Vehicle Control**
In order to assess the effect of time on FBF, the dose-response curve to methacholine was determined twice in response to vehicle. There was a non-significant reduction in FBF during the second series of measurements (P=NS, ANOVA)

Figure 3A.
**Contribution of Nitric Oxide Release to Verapamil-Stimulated Vasodilation**
Forearm blood flow (FBF) response to verapamil was determined in the presence of the nitric oxide synthase antagonist LNAME vs vehicle. LNAME infusion was associated with a significant reduction in resting FBF. Further, LNAME infusion was associated with a significant downward shift in the FBF dose response curve for verapamil, (p<0.05, ANOVA), thereby demonstrating a role for NO in verapamil-stimulated vasodilation.

Figure 3B.
**Nitric Oxide Synthase Inhibition and Ethanol-Augmented Vasodilation**
During co-infusion of verapamil and LNAME, forearm blood flow (FBF) was measured in the presence of ethanol vs. vehicle. Despite concomitant inhibition of nitric oxide...
synthesis, ethanol infusion resulted in a significant upward shift in the FBF dose response curve (p<0.05, ANOVA).
Figures
Fig. 1

![Graph showing FBF (mL/dL/min) for Vehicle and EtOH groups with a P<0.01 difference.]

Fig. 2A

![Graph showing FBF (mL/dL/min) in response to Methacholine for Vehicle and EtOH groups with a *p<0.01 significance.]

Fig. 3B

- ■ LNAME & Vehicle
- ○ LNAME & EtOH

EtOH Started

FBE (ml/dl/min)

Verapamil (µg/kg/min)

P<0.001

Base 0 10 30 100 300