Ethanol Induces a Paradoxical Simultaneous Increase in Circulating Concentrations of Insulin-Like Growth Factor Binding Protein-1 and Insulin

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The aim of this study was to examine the effect of acute alcohol intake on circulating concentrations of insulin, C-peptide, insulin-like growth factor (IGF) binding protein-1 (IGFBP-1), and plasma glucose levels. We measured these parameters for 12 hours after administration of 0, 0.5, or 1.0 g ethanol/kg body weight to nine healthy volunteers between 7:00 and 7:45 PM according to a randomized, double-blind, crossover design. Following a snack at 9:00 PM, plasma insulin ($P < .05$) and C-peptide ($P < .01$) concentrations were significantly increased at 10:00 PM in the 1.0-g group as compared with the control group. C-peptide to insulin molar ratios were significantly higher ($P < .05$) in both ethanol groups at 10:00 PM and 2:00 AM than in the control group. No differences were observed in plasma glucose levels between the three groups. Plasma IGFBP-1 levels showed a dose-dependent increase in the ethanol groups, and remained increased from 10:00 PM for 3 hours ($P < .05$ or less) at the lower dose and for 6 hours ($P < .05$ or less) at the higher dose. These observations indicate that ethanol-induced postprandial hyperinsulinemia is due to increased insulin secretion and that alcohol may increase hepatic insulin extraction. The lack of any effect on plasma glucose levels suggests that alcohol intake must be associated with decreased insulin sensitivity. Alcohol intake results in a paradoxical increase in peripheral concentrations of IGFBP-1 despite simultaneous hyperinsulinemia. This implies that ethanol has a direct stimulatory effect on hepatic IGFBP-1 synthesis.

ETHANOL HAS BEEN shown to increase circulating insulin concentrations after glucose loading in man; however, the mechanism involved remains unknown. Metz et al[1] suggested originally that ethanol exerts a direct "priming" effect on the β cell. Later it was proposed that ethanol enhances insulin secretion indirectly by stimulating the release of gastrointestinal hormones.[2,3] A third possibility is that ethanol ingestion decreases hepatic insulin extraction, leading to increased peripheral concentrations. However, Adner and Nygren[4] have observed that alcohol intake is followed by both increased circulating insulin and C-peptide concentrations after intravenous glucose, providing evidence for enhanced insulin secretion.

Insulin suppresses hepatic production of insulin-like growth factor (IGF) binding protein-1 (IGFBP-1),[5] one of the six distinct IGFBPs thus far cloned and sequenced.[6] Hyperinsulinemic conditions, like obesity[7] and polycystic ovarian disease,[8] are associated with reduced peripheral IGFBP-1 levels, whereas IGFBP-1 concentrations are increased in both insulin-dependent and non–insulin-dependent diabetes mellitus.[9-11] IGFBP-1 inhibits the biological actions of IGFs in most in vitro studies.[12,13] If this holds true in vivo as well, insulin may modify the effects of IGFs on target tissues by regulating IGFBP-1 synthesis.

In this study, we evaluated the dose-dependent effect of ethanol ingestion on circulating concentrations of insulin, C-peptide, glucose, and IGFBP-1 first to explore further the mechanisms behind the ethanol-induced hyperinsulinemia observed after carbohydrate stimulation, and second to find out whether ethanol influences the regulation of hepatic IGFBP-1 production by insulin.

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SUBJECTS AND METHODS

The study was performed at the Endocrinology Unit, Department of Physiology, University of Oulu. Nine healthy medical students (five women and four men aged 21 to 23 years) who provided written informed consent were recruited as subjects. A detailed history was taken and a routine medical examination was performed for all subjects before commencing the trial. None of the subjects were excessive drinkers. The protocol for the study was approved by the Ethics Review Board, Medical Faculty, University of Oulu. Subjects were not allowed to consume any alcoholic beverage 1 week before study or during the 3 test weeks. Consumption of caffeine-containing products was also prohibited during test days and limited to a maximum of 200 mg/d during test weeks. The use of any medication and smoking was also forbidden during test weeks.

The study was a double-blind, randomized, three-way crossover experiment in which subjects received single oral doses of 0, 0.5, and 1.0 g ethanol/kg body weight mixed in a sugar-free carbonated drink (~600 mL) at 1-week intervals. Subjects arrived at the site of the study at 5:00 PM, at which time a cannula was inserted into the dorsal vein of the hand. Heparin 5,000 IU/100 mL physiological saline (200 μL) was used to keep the cannulas patent between sampling. The drinks were given at 7:00 PM and were to be consumed at a constant pace within 45 minutes. Blood samples were taken at 6:00 PM before the intake of alcohol, at 8:00, 10:00, and 12:00 PM, and at 1:00, 2:00, 3:00, 4:00, and 7:00 AM. Lights were turned off at 11:00 PM, at which time subjects were asked to go to bed. Blood samples after that time were taken in the dark (under 2 lux). The subjects had been fasting since 3:00 PM and received a standardized snack containing bread, butter, cheese, 0.2 L orange juice, and a banana at 9:00 PM. All effects and adverse effects during the test session were recorded by one of the researchers (A.-C.E.).

Methods

Blood was taken into EDTA tubes for insulin, C-peptide, IGFBP-1, and glucose measurements and into glass tubes for serum ethanol analysis. The tubes were centrifuged within 30 minutes, and plasma and sera were stored at −70°C. Serum ethanol concentrations were determined by gas chromatography.

Plasma glucose levels were measured by a glucose dehydrogenase method (Merck, Darmstadt, Germany). Plasma concentrations of insulin and C-peptide were analyzed radioimmunologically as previously described.[14] Plasma IGFBP-1 levels were quantified with an enzymometric modification of an immunoradiometric method[15] with a sensitivity of 0.4 μg/L, an intraassay variation of less than 4%, and an interassay variation of less than 10%.

Statistical Analysis

Hormonal concentrations were transformed to logarithms before statistical analyses to normalize their distribution. Data were evaluated by repeated-measures ANOVA, using time and dose at each time point as a within-subject factor. Calculations were performed with the Primer program (McGraw-Hill, New York, NY). After ANOVA, the Neuman-Keuls test was used to assess significance of differences between time points and doses. Results are expressed as the mean ± SE. The figures are based on the untransformed arithmetic means.

RESULTS

All subjects completed the study. No serious adverse effects could be seen. Serum ethanol concentrations peaked at 8:00 PM, 1 hour after the start of ethanol intake (Fig 1A). Maximal levels were 13.1 ± 1.1 and 26.8 ± 1.8 mmol/L in subjects receiving 0.5 and 1.0 g ethanol/kg, respectively ($P < .01$ between groups). Thereafter, serum ethanol decreased to undetectable levels (<0.4 mmol/L) in both groups in 5 and 11 hours, respectively. Ethanol was undetectable in the sera of all subjects receiving ethanol-free drinks.

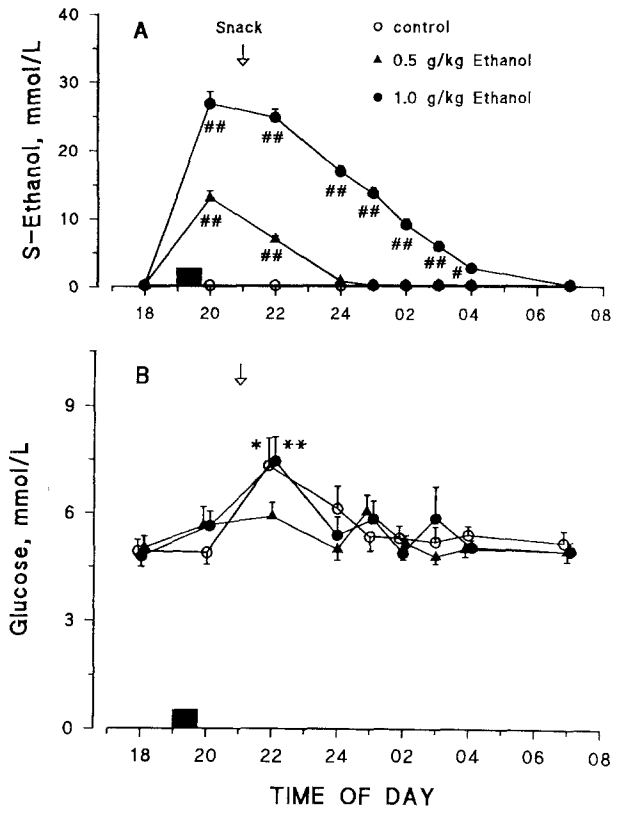

Fig 1. Concentrations of ethanol (A) and plasma glucose (B) after intake of 0, 0.5, or 1.0 g ethanol (■, taken in 600 mL beverage)/kg body weight. #$P < .05$ and ##$P < .01$ *v* controls; *$P < .05$ and **$P < .01$ *v* the starting level at 6:00 PM.

Plasma glucose (Fig 1B) levels were stable throughout the study, with a slight increase at 10:00 PM, reaching statistical significance in groups receiving no ethanol or 1.0 g ethanol/kg ($P < .05$ and $P < .01$, respectively). The groups did not differ from each other at any time point. Plasma insulin (Fig 2A) showed a clear diurnal rhythm with a decreasing tendency until 8:00 PM; from that point onward, an increase was seen due to the snack at 9:00 PM. This increase reached statistical significance at 10:00 PM ($P < .001$ *v* initial values in all groups). At midnight, values for both ethanol groups and the control group had decreased to levels that did not significantly differ from the initial values. At 10:00 PM, values for the 1.0-g group were significantly higher ($P < .05$) than for the 0.5-g and control groups. In all groups, plasma C-peptide (Fig 2B) showed a diurnal rhythm similar to that of insulin, with statistically significant peak values ($P < .001$) at 10:00 PM. Ethanol caused a dose-dependent increase in these levels, with the 1.0-g group having significantly increased levels at 10:00 PM ($P < .01$) as compared with the control group. No other differences were observed between the groups. The molar ratio of C-peptide to insulin showed a temporal change only in the 0.5-g group at 8:00 PM ($P < .05$), due to great variation in the values (Fig 2C). Both ethanol groups had higher ratios at 10:00 PM and 2:00 AM ($P < .05$) than the control group.

In the control group, IGFBP-1 levels increased toward the morning, reaching statistical significance ($P < .001$) at 7:00 AM (Fig 3). A clear diurnal pattern could be seen in both ethanol groups, with increasing values at 8:00 PM, reaching the highest levels 2 hours later, followed by a gradually descending pattern. At the lower dose, diurnal variation reached statistical significance from 10:00 PM to 7:00 AM ($P < .001$), and at the higher dose, from 8:00 PM to 7:00 AM ($P < .001$). IGFBP-1 levels in the 0.5-g group were significantly higher than in the control group from 10:00 PM to 1:00 AM ($P < .05$ or less), and in the 1.0-g group from 10:00 PM to 4:00 AM ($P < .05$ or less).

DISCUSSION

Results of the present study provide confirmation that ethanol ingestion potentiates meal-induced hyperinsulinemia[2,3] and support the view[4] that this is due to increased insulin secretion from the β cell, since the higher ethanol dose resulted in significantly enhanced postprandial plasma C-peptide concentrations and elevated insulin levels. By contrast, our observations do not lend support to the hypothesis that alcohol-induced postprandial hyperinsulinemia could be a consequence of decreased hepatic insulin extraction, since we actually found an increased postprandial molar ratio of C-peptide to insulin after ethanol ingestion, indicating increased insulin extraction in the liver. An alternative explanation to the increased C-peptide to insulin ratio could be a decreased peripheral C-peptide clearance. However, this is unlikely, since C-peptide is mainly eliminated through the kidneys, and alcohol intake

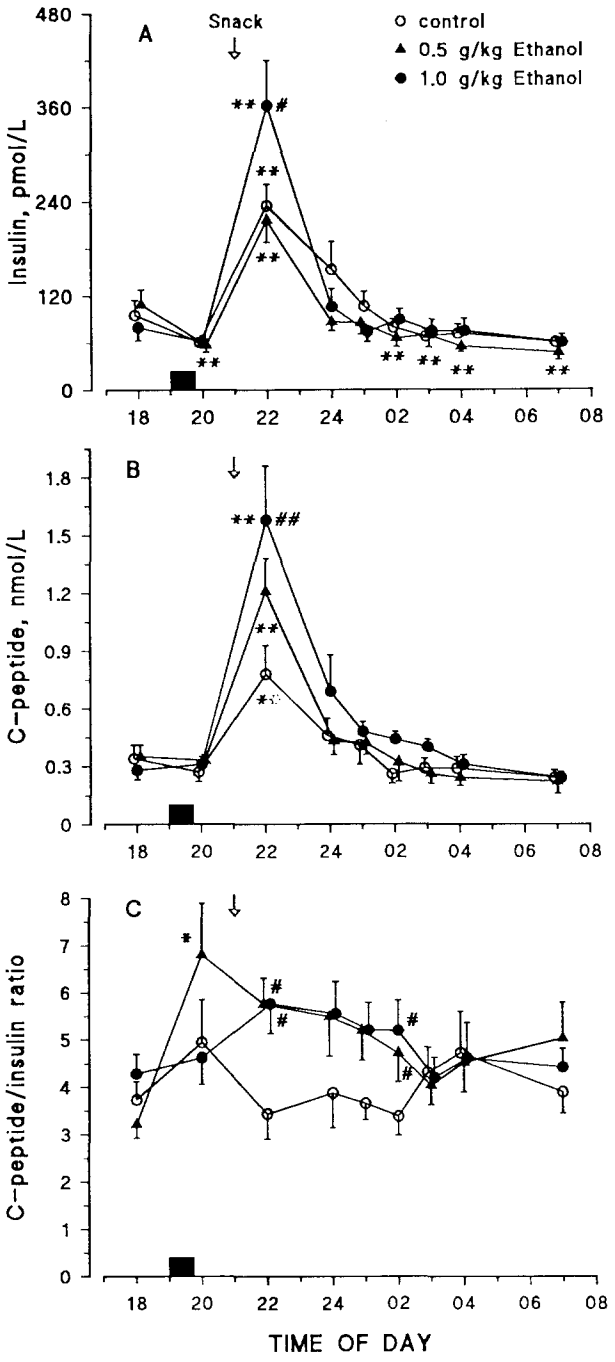

Fig 2. Concentrations of plasma insulin (A) and C-peptide (B) and molar ratio of C-peptide to insulin (C) after intake of 0, 0.5, or 1.0 g ethanol (■, taken in 600 mL beverage)/kg body weight. #$P < .05$ and ##$P < .01$ *v* controls; *$P < .05$ and **$P < .01$ *v* the starting level at 6:00 PM.

is associated with increased diuresis[16,17] due to a central effect, but ethanol does not affect glomerular filtration.[18]

Although causing postprandial hyperinsulinemia, alcohol intake did not induce a significant decrease in plasma glucose levels. This is in conflict with previous findings that ethanol ingestion results in decreased blood glucose levels in response to oral glucose.[2,3] On the other hand, there are more recent studies reporting impaired insulin sensitivity as a consequence of alcohol intake in healthy subjects,[19-21] which is in line with the present observations. The ethanol-induced decrease in insulin action has been claimed to be a consequence of peripheral insulin resistance caused by both a receptor and postreceptor defect.[20]

Since it has been demonstrated that insulin is the major regulator of circulating IGFBP-1 concentrations,[9] we expected to find decreased peripheral IGFBP-1 levels after alcohol ingestion as a consequence of ethanol-induced hyperinsulinemia. To our surprise, we observed a dose-dependent increase in plasma IGFBP-1 levels after ethanol intake. This increase occurred despite simultaneous postprandial hyperinsulinemia, implying that it could not be mediated by insulin. Accordingly, these data suggest a direct stimulatory effect of ethanol on hepatic IGFBP-1 synthesis. Another explanation could be that the increased secretion of IGFBP-1 would be mediated by an ethanol-induced suppression of growth hormone (GH) release. It has been shown that ethanol inhibits secretion of GH,[22,23] and on the other hand, an inverse relationship has been reported between GH and circulating IGFBP-1 concentrations.[24] However, this alternative is unlikely, since in the same study population a significant, dose-dependent suppression of GH secretion by ethanol was observed for 3 hours after midnight (A.-C. Ekman, O. Vakkuri, J. Leppäluoto, et al, unpublished observation, April 1994), whereas IGFBP-1 levels peaked before midnight.

The increased peripheral IGFBP-1 concentrations could also potentially be due to an ethanol-induced stimulation of the secretion of catecholamines.[25] Hooper et al[26] observed recently that both norepinephrine and epinephrine are capable of stimulating synthesis and release of IGFBP-1. The temporal pattern of increased IGFBP-1 release in the present study with a peak within 3 hours after alcohol intake argues against a mechanism mediated by catecholamines, since infusion of catecholamines resulted in peak

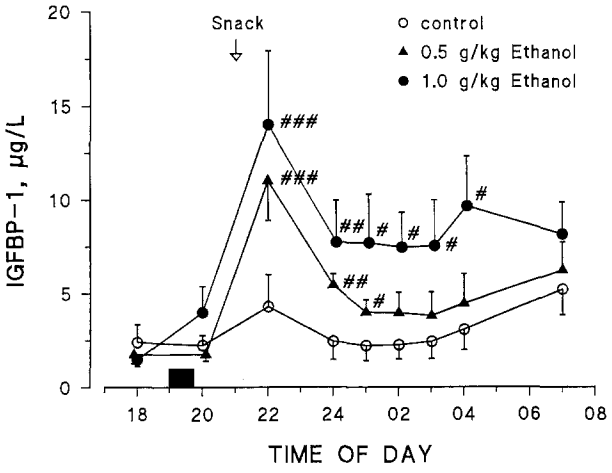

Fig 3. Concentrations of plasma IGFBP-1 after intake of 0, 0.5, or 1.0 g ethanol (■, taken in 600 mL beverage)/kg body weight. #$P < .05$, ##$P < .01$, and ###$P < .001$ *v* control.

IGFBP-1 concentrations at a considerably later time point, namely 12 hours.[26]

The conspicuous effect of ethanol on circulating IGFBP-1 levels indicates that ethanol may be a significant indirect modulator of peripheral IGF actions, which, according to most studies,[12,13] are inhibited by IGFBP-1. Since both IGF-I and IGF-II are involved in regulation of fetal growth,[27] alcohol may be a strong inhibitor of prenatal growth by increasing circulating IGFBP-1 concentrations. The fetal alcohol syndrome induced by maternal alcohol ingestion during pregnancy and characterized by severe intrauterine growth retardation[28] might represent a clinical manifestation. A recent study[29] actually demonstrated in a rat model that maternal ethanol intake led to increased levels of low–molecular-weight IGFBPs in the fetal circulation.

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