

IS DECREASED LEPTIN SECRETION AFTER ALCOHOL INGESTION CATECHOLAMINE-MEDIATED?

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Abstract — **Aims:** Catecholamines (CA) inhibit leptin secretion. Alcohol appears to have a similar effect. The mechanism underlying the inhibitory effect of alcohol is unknown, but CA may play a role as mediators. This hypothesis has never been tested. We decided to do so in the present investigation. **Methods:** Seven healthy subjects participated in two experiments (A and B), performed in random order, 1 week apart. In experiment A, three identical doses of ethanol (0.45 g/kg b.w.) were ingested at regular intervals between 09:00 and 12:00 hours. The alcohol doses were given against a background of oral placebo administered at 08:00 and 12:00 hours. In experiment B, identical doses of alcohol were ingested against a background of oral propranolol (40 mg at 08:00 and 20 mg at 12:00 hours). Pulse rates, and serum levels of ethanol, insulin, IGF-1 and leptin, were determined at regular intervals throughout the experiments. Urinary CA excretion was also determined. **Results:** Propranolol (experiment B) decreased the pulse rate significantly, compared with placebo (experiment A), but did not change the urinary excretion of adrenaline nor noradrenaline. Alcohol ingestion raised the serum ethanol levels similarly in the two experiments but did not affect the insulin or IGF-1 levels. The serum leptin levels declined similarly in the two experiments, as evidenced by the percentage serum leptin decline from baseline, which was $28.6 \pm 5.4\%$ in experiment A and $29.0 \pm 2.9\%$ in experiment B. **Conclusion:** The declining serum leptin concentration after acute ingestion of alcohol does neither appear to be CA-mediated nor to be caused by changed secretion of insulin or IGF-1. A direct inhibitory effect of alcohol on the adipocytes is possible, but increased disposal of leptin via hepatic metabolism or renal excretion could also contribute.

INTRODUCTION

Alcoholic drinks have been used for centuries as appetizers, but little is known about the mechanism behind their appetite-stimulating effect in man. Neuropeptide Y (NPY) and leptin may well be involved, as both are important regulators of hunger-satiety signals within the central nervous system (Dryden and Williams, 1996; Wang *et al.*, 1997). NPY stimulates food intake (Flood and Morley, 1991; Williams *et al.*), whereas leptin inhibits NPY (Blum, 1997), and consequently induces satiety. We have recently shown that ingestion of moderate amounts of alcohol decreases both the diurnal and the nocturnal secretion of leptin in healthy individuals (Röjdmarm *et al.*, 2001). As leptin is produced in adipocytes (Wabitsch *et al.*, 1996), the above-mentioned effect of alcohol could either be caused by direct or indirect inhibition of the adipocytes. Several indirect factors are plausible. Insulin and glucocorticoids have been found to stimulate leptin secretion (Larsson and Ahrén, 1996; Malmström *et al.*, 1996), whereas androgens (Wabitsch *et al.*, 1997), IGF-1 (Dagogo-Jack *et al.*, 1998) and CA (Fritsche *et al.*, 1998; Couillard *et al.*, 2002) appear to have opposite effect. In our above-mentioned study (Röjdmarm *et al.*, 2001), all these factors, except CA, were determined. If alcohol inhibits the secretion of insulin or glucocorticoids, one would expect to find decreased leptin levels after alcohol ingestion. However, we were unable to confirm that assumption in our previous study (Röjdmarm *et al.*, 2001). Moreover, alcohol did not have a stimulative influence on testosterone nor IGF-1

production (Röjdmarm *et al.*, 2001). In contrast, it decreased the bioavailability of IGF-1, as reflected by a declining IGF-1/IGFBP-1 ratio after acute intake of alcohol (Röjdmarm *et al.*, 2000, 2001). Thus if neither of these factors appear to mediate the inhibitory effect of alcohol on leptin secretion, it still remains to be elucidated whether CA could play a role in this context. The objective of the present investigation was therefore to focus on that particular issue. For that purpose three oral doses of alcohol were given to healthy subjects, as in our previous study (Röjdmarm *et al.*, 2001), but in the present study alcohol was given against background treatments of either placebo or nonselective β -adrenoceptor blockade (propranolol). By comparing serum leptin responses to alcohol with these two background treatments, it should be possible to determine whether or not CA mediate the alcohol-induced inhibitory effect on human leptin secretion.

SUBJECTS AND METHODS

Subjects

Seven healthy volunteers were included in the investigation: four women (aged 24.8 ± 2.1 years) and three men (21.7 ± 0.3 years old). Their BMI were normal (21.0 ± 1.0 and 22.7 ± 0.3 m²/kg, respectively). All were free of medication. They used moderate amounts of alcohol at social events, but none was addicted to liquor, and all refrained completely from using alcohol in any form for 3 days prior to the experiments. Flushing, after ingestion of alcohol, had not been experienced by any of them previously. They were informed of the purpose of the study and gave their voluntary consent to participate in the investigation which was approved by the ethics committee of Huddinge University Hospital, Stockholm.

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Protocol

Each individual took part in two experiments (A and B) which were performed in a metabolic ward, in random order, 1 week apart.

Experiment A. At 07:30 hours a catheter was inserted into one of the antecubital veins and kept patent by a slow drip of normal saline. After an equilibration period of 30 min, basal blood samples were collected from the catheter. At 08:00 hours placebo was given orally. Then three identical 150-ml doses of alcohol were ingested at 09:00, 10:30 and 12:00 hours. Each dose contained 0.45 g ethanol/kg b.w. The alcohol concentration in the solutions ranged between 16.5 and 25.6%/vol. (mean \pm SEM: $20.5 \pm 1.2\%$ /vol.). A second oral placebo dose was given at 12:00 hours.

Experiment B. In this experiment propranolol was substituted for placebo; 40 mg propranolol were ingested at 08:00 hours and an additional 20 mg at 12:00 hours. In all other details, experiment A and B were identical.

In both experiment A and B, pulse rates were recorded and serum concentrations of insulin, IGF-1 and leptin determined immediately before the first dose of placebo/propranolol (08:00 hours), and subsequently at 09:00, 11:00, 13:00 and 15:00 hours. Serum ethanol levels were analysed at intervals, as shown in Fig. 1. Adrenaline and noradrenaline excretion by the urine over a period of 7 h (08:00–15:00 hours) was also determined.

Assays

Serum leptin concentrations were measured radioimmunologically (Ma *et al.*, 1996) by use of a commercial kit from Linco (St Charles, USA). The intra- and interassay coefficients of variance (CV) were 3.8 and 6.5% at serum leptin concentrations ranging between 2.5 and 8.2 $\mu\text{g/l}$. The assay had a sensitivity of 0.5 $\mu\text{g/l}$. IGF-1 was also determined by RIA after separation of IGF from IGFBP by acid-ethanol extraction and cryoprecipitation. To minimize interference of remaining IGFBP, des(1–3)IGF-1 was used as a radioligand (Bang *et al.*, 1991). Intra- and interassay CV were 4 and 11%, respectively. CA excretion by the urine was measured by liquid chromatography according to Eriksson and Wikström (Eriksson, 1992). Intra-assay CV for adrenaline, at excretion values of 53 and 173 nmol/24 h, were 7 and 5%, respectively. Corresponding CV for noradrenaline, at 232 and 588 nmol/24 h, were 5 and 4%, respectively. Insulin levels were determined radioimmunologically (Livessy *et al.*, 1980). A commercial kit was used (Insulin RIA 100; Pharmacia and Upjohn, Stockholm, Sweden). An automated Hitachi 911 analyser from Roche Diagnostics (Bromma, Sweden) made it possible to measure serum ethanol levels. The principals of serum ethanol analysis has been described previously (Bucher and Redetzki, 1951).

Statistical analysis and calculations

To test whether placebo–alcohol would have different effect than that of propranolol–alcohol on pulse rates and serum levels of ethanol, insulin, IGF-1 and leptin, we used a two-way ANOVA for repeated measures followed by Turkey's post-hoc test.

Percentage changes of pulse rates and leptin concentrations from baselines were also determined (Figs 1, 4, left panels). These changes — measured over time — were used to calculate areas under the curve (AUC). AUC obtained in experiment A (AUC-A), were combined as means, and compared with those obtained in experiment B (AUC-B). The means \pm SEM are shown in Figs 1 and 4 (right panels). Serum ethanol increments over time were also calculated and presented as AUC (Fig. 1, right panel). However, the ethanol AUC were based on absolute serum ethanol changes over time, not on percentage changes. To determine whether AUC-A differed significantly from AUC-B, Student's *t*-test was applied (paired differences). This test was also used to compare CA excretion values of experiment A and experiment B. *P*-values of <0.05 were considered significant.

RESULTS

None of the participants flushed after ingestion of alcohol.

Pulse

When alcohol was consumed after administration of placebo (experiment A) the pulse rate increased significantly ($P < 0.02$ at 11:00 hours and $P < 0.001$ at 15:00 hours). The pulse-AUC_{08–15} (reflecting the percentage change of the pulse rate over time) was 103.5 ± 39.1 .

Propranolol, given alone in the initial part of experiment B, lowered the pulse rate by $14 \pm 4\%$ at 09:00 hours (from 68 ± 4 beats/min at 08:00 hours to 58 ± 3 beats/min at 09:00 hours; $P < 0.05$). When alcohol was ingested after propranolol

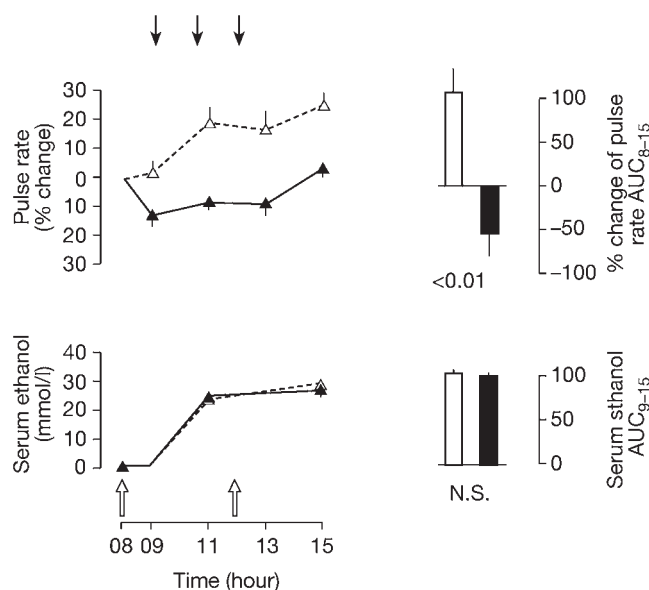


Fig. 1. (Top panel) Pulse rates and pulse-areas under the curve (AUC) in response to three oral doses of ethanol (\downarrow) given to seven healthy subjects on background treatments of either placebo (open triangles/open bars) or propranolol (closed triangles/closed bars). Placebo/propranolol was given at 08:00 hours. Percentage changes of pulse rates from baseline are shown to the left. Pulse-AUC are shown to the right. Pulse-AUC were calculated by use of \uparrow percentage changes of pulse rates from baseline over time (08:00–15:00 hours). Values denoted are means \pm SEM. (Bottom panel: left) Serum ethanol concentrations recorded in response to three oral doses of ethanol on backgrounds of either placebo or propranolol. (Bottom panel: right) Ethanol AUC. Symbols as above.

priming, the pulse rate did not increase as in experiment A; it tended to stay low for at least 4 h and did not reach levels above basal until the end of the experimental period (Fig. 1, left panel). This was reflected by the percentage change of the pulse rate $AUC-B_{08-15}$ which appeared below the baseline (Fig. 1, right panel). It differed significantly from the corresponding area in experiment A (-52.8 ± 25.9 vs. 103.5 ± 39.1 ; $P < 0.01$; Fig. 1, right panel).

Serum ethanol

In experiment A the serum ethanol level increased from 0 mmol/l at 09:00 hours to a maximum of 28.1 ± 0.8 mmol/l at 15:00 hours. The ethanol concentration curve was very similar in experiment B (Fig. 1, left panel). Consequently, the ethanol- AUC_{09-15} did not differ significantly in the two experiments (Fig. 1, right panel).

Urinary adrenaline

The urine excretion of adrenaline between 08:00 and 15:00 hours was 27.4 ± 5.0 nmol/7 h in experiment A and 28.2 ± 7.4 nmol/7 h in experiment B. These values did not differ significantly (Fig. 2).

Urinary noradrenaline

In experiment A, 82 ± 23.5 nmol/7 h was excreted and 92.3 ± 21.5 nmol/7 h was excreted in experiment B. The difference was not significant (Fig. 2).

Serum insulin

Basal serum insulin concentrations were similar in experiments A and B (5.3 ± 1.2 and 4.6 ± 1.0 mU/l, respectively). The small subsequent changes in insulin level were not significantly different in the two experiments (Fig. 3).

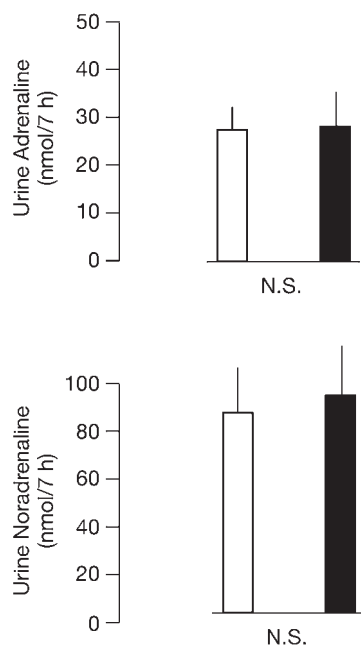


Fig. 2. Excretion of adrenaline and noradrenaline by the urine during 7 h (08:00–15:00 hours) on background treatments of either placebo (open bars) or propranolol (closed bars) in seven healthy subjects.

Serum IGF-1

Similar basal IGF-1 levels were obtained at 08:00 hours in experiments A and B (290 ± 38 and 315 ± 17 μ g/l, respectively), and the subsequent small IGF-1 changes were not significantly different (Fig. 3).

Serum leptin

Both experiments presented similar serum leptin concentrations at 08:00 hours (7.1 ± 2.0 μ g/l for A, 6.5 ± 1.5 μ g/l for B). Also, at 09:00 hours similar serum leptin concentrations were recorded in the two experiments (6.8 ± 2.0 and 6.3 ± 1.5 μ g/l, respectively). After ingestion of alcohol at 09:00 hours, the leptin level declined significantly in both experiments. The percentage leptin decline between 09:00 and 15:00 hours was $28.6 \pm 5.4\%$ in experiment A and $29.0 \pm 2.9\%$ in experiment B (Fig. 4, left panel). When the total leptin decline over a 6-h period (09:00–15:00 hours) was expressed by a percentage decremental area under the curve (AUC_{09-15}), the AUC obtained in the two experiments were not significantly different, as shown in Fig. 4 (right panel).

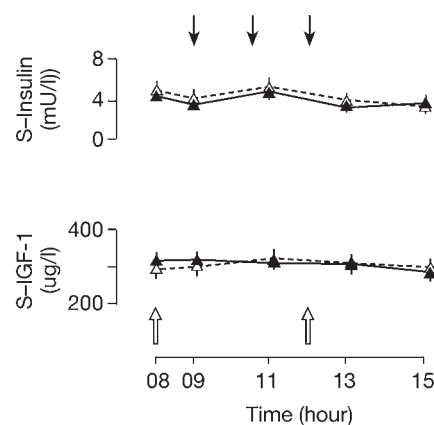


Fig. 3. Serum levels of insulin and IGF-1 after intake of three oral doses of alcohol, on backgrounds of either placebo or propranolol. Symbols as in Fig. 1.

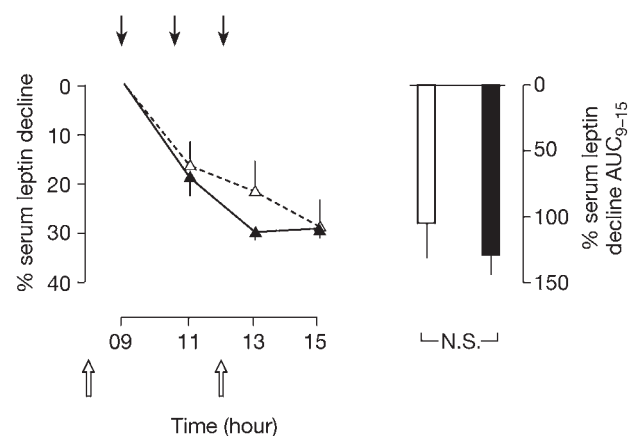


Fig. 4. (Left) Percentage changes of leptin concentrations from baseline in response to three oral doses of ethanol, on background treatments of either placebo or propranolol. (Right) Leptin-AUC obtained under the above mentioned conditions. Leptin-AUC were based on percentage serum leptin changes from the baseline over time (09:00–15:00 hours). Symbols as in Fig. 1.

DISCUSSION

We recently observed that alcohol had an inhibitory influence on leptin secretion in healthy subjects (Röjdmarm *et al.*, 2001). The underlying mechanism was unknown. Direct or indirect effects were possible, and several factors that could be mediators of the alcohol effect were tested, but none appeared to be of importance in this context (Röjdmarm *et al.*, 2001). One factor — catecholamines (CA) — known to inhibit leptin secretion in man (Scriba *et al.*, 2000; Couillard *et al.*, 2002), was left unexplored in the above-mentioned report. Therefore, the present investigation was undertaken with the intention of finding out whether alcohol inhibits leptin secretion indirectly, either via increased CA secretion or via changed responsiveness of the β -adrenoceptor. For that purpose we gave healthy subjects three small oral doses of alcohol against background treatments of either placebo (experiment A) or propranolol (experiment B). When alcohol was ingested against a background of placebo, the pulse rate increased markedly. One explanation of this could be increased serum acetaldehyde concentration after ingestion of alcohol. An alternative possibility could be enhanced CA secretion. It is well known that the clinical response to alcohol differs markedly between individuals. This might, at least in part, be attributed to genetic differences, as suggested by Luczak *et al.* (2002). Some individuals — in particular Asians (Wolff, 1972) — experience discomfort and flush after alcohol consumption. The ethanol-induced flushing syndrome is characterized not only by a raised facial skin temperature, but also by increased cardiac output and tachycardia (Wolff, 1972; Kupari *et al.*, 1983), and appears to be caused by increased serum acetaldehyde levels after alcohol intake (Ho *et al.*, 1988). However, none of the subjects included in our investigation had alcohol-induced flushing. This makes a crucial role of acetaldehyde less likely in this context. If so, increased CA secretion has to be considered. In some (Ireland *et al.*, 1984; Sjoquist *et al.*, 1985; Kovacs *et al.*, 2002) but not all (Potter *et al.*, 1986; Heikkonen *et al.*, 1991) previous investigations, increased CA secretion/excretion has been found after alcohol consumption. We noted similar CA excretion in urine after intake of alcohol regardless of background treatment. This implies that the increased pulse rate after alcohol was probably not a consequence of increased CA secretion, but could well be a reflection of changed adrenoceptor responsiveness to CA. Both α - and β -adrenoceptors have been found in white adipose tissue (Lafontan *et al.*, 1993), but little is known about the cellular pathway involving leptin secretion (Cone *et al.*, 2003). Three β -adrenoceptor subtypes (β_1 , β_2 , and β_3) are expressed in human adipocytes (Collins *et al.*, 2001). All of them are members of the large family of G-protein-coupled receptors, which stimulate intracellular cAMP levels (Collins *et al.*, 2001). The rapid effect of β -adrenoceptor stimulation of leptin release from adipose tissue suggests that leptin secretion is regulated by cAMP (Cone *et al.*, 2003). When the participants in our study were given propranolol before ingestion of alcohol, the pulse rate decreased significantly, and subsequent ingestion of alcohol did not overcome the propranolol-induced β -adrenoceptor blockade. If CA do mediate the effect of alcohol, different leptin responses to the drug should be found in experiments A and B. That was not the case; instead, similar leptin declines

were recorded in the two experiments, which suggests that factors other than CA should be investigated to explain how alcohol inhibits leptin secretion in man.

Leptin, like many other hormones, is secreted rhythmically throughout the day. High serum leptin concentrations prevail in the early morning and low serum leptin concentrations prevail around mid-day (Saad *et al.*, 1998). This means that the declining leptin level, which was found in the present investigation after ingestion of alcohol, could reflect a normal secretory profile of leptin, rather than an alcohol-induced leptin inhibition. However, this appears unlikely for the following reason. We recently studied healthy subjects of both sexes who, in almost all respects, were comparable to those included in the present investigation (Röjdmarm *et al.*, 2001). When these individuals were given three oral doses of water in the morning their serum leptin levels declined by $22.7 \pm 3.3\%$. After ingestion of three oral doses of alcohol the corresponding leptin decline was $30.3 \pm 3.9\%$ ($P < 0.05$). This leptin decline after alcohol was in close accordance with that found in both experiments A and B (a leptin decline of $\sim 29\%$). For that reason we believe that alcohol has an inhibitory effect on human leptin secretion. This assumption is further supported by the fact that alcohol also appears to inhibit leptin secretion during the night (Röjdmarm *et al.*, 2001), when serum leptin levels normally tend to increase (Sinha *et al.*, 1996).

If CA can be excluded from the list of plausible mediators of the alcohol effect, several other should be considered. Insulin, cortisol, testosterone and IGF-1, are all plausible mediators, as all have the potential of influencing leptin secretion (Williams *et al.*; Malmström *et al.*, 1996; Larsson and Ahrén, 1996; Wabitsch *et al.*, 1997; Dagogo-Jack *et al.*, 1998; Nyomba *et al.*, 1999). However, these plausible mediators were scrutinized in our previous study and none was found to mediate the leptin-inhibitory effect of alcohol (Röjdmarm *et al.*, 2001). Insulin and IGF-1 levels were also determined in the present investigation. Neither of them changed noticeably after ingestion of alcohol. This finding thus supports our previous observations.

It may be said that our results are at variance with those reported by Nicolas *et al.* (2001). They found increased serum leptin levels in chronic alcoholics, regardless of nutritional status or presence of liver cirrhosis. The discrepancy between their findings and ours is unexplained, but different disposal of leptin has to be considered. It has been maintained that the splanchnic organs and the kidneys cooperate in the disposal of leptin. By use of an arterio-venous technique, Garibotto *et al.* (1998) were able to determine not only the fractional splanchnic extraction, but also the fractional renal extraction of leptin in nonobese subjects. The splanchnic extraction was 16% and the corresponding renal extraction 9.5%, but only small quantities of native leptin were found in the urine. Although this implies that leptin is metabolized within the kidneys, Garibotto's findings suggest that more leptin may be metabolized in the normal liver than in the kidneys. In patients with liver insufficiency, this may not be the case, as such patients probably metabolize less leptin in the liver than do healthy subjects. If so, this could, at least in part, explain why chronic alcoholics appear to have higher serum leptin levels than healthy individuals.

Ghrelin is also of interest in this context, as this hormone stimulates NPY (Toogood and Thorner, 2001;

Cowley *et al.*, 2003), and increases the intake of food (Wren *et al.*, 2000). It is not known whether alcohol stimulates the secretion of ghrelin. If so, it still remains to be investigated how such alcohol-induced ghrelin secretion affects human adipocytes. Until such studies have been performed, we are left with the fact that alcohol decreases serum leptin levels. This effect does not appear to be indirect. It may be caused by direct inhibition of adipocytes, but changed hepatic and/or renal disposal of leptin are other possibilities that require further investigation.

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