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Alcohol ingestion does not affect serum levels of peptide YY but decreases both total and octanoylated ghrelin levels in healthy subjects

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Abstract

Alcohol has been reported to have appetite-stimulating properties in humans. The underlying mechanism is unknown. Gastrointestinal hormones, such as ghrelin and peptide YY (PYY), could be involved as mediators of the alcohol effect because ghrelin stimulates the appetite and PYY appears to induce satiety. This investigation was undertaken with the intention to study that issue. Twelve young and healthy volunteers of both sexes participated in 2 experiments (experiments A and B), which were performed in random order 1 week apart. Alcohol (0.55 g ethanol per kilogram) was ingested in experiment A, drinking water in experiment B. Venous blood samples were collected before and repeatedly after the drinks. Serum concentrations of total ghrelin, octanoylated ghrelin (the bioactive form of the hormone), PYY, and ethanol were determined over a period of 5 hours. In experiment A, the ethanol level increased from 0 to 12.5 ± 0.7 mmol/L in 1 hour ($P < .001$), and then began to decrease. In experiment B, the ethanol level remained at zero throughout the entire experiment. Alcohol induced significant declines in total and octanoylated ghrelin concentrations from 30 minutes on. The total ghrelin level reached its lowest point 5 hours after the alcohol intake (36% ± 4% below the basal level; $P < .001$). The octanoylated ghrelin level fell 48% ± 5% below the basal level in 2 hours ($P < .001$) and then tended to level out. Drinking water left both total and octanoylated ghrelin levels unaffected. The PYY level remained unchanged after both alcohol and water ingestion. Alcohol has a strong inhibitory influence on human ghrelin secretion, but has no effect on circulating PYY levels. This makes it unlikely that the orexigenic effect of alcohol is mediated by either of these 2 hormones.

1. Introduction

Oral intake of alcohol not only has an orexigenic effect in humans [1-3], but also a stimulatory effect on energy expenditure [4,5]. This means that alcohol has dual effects on human energy homeostasis. Depending on whether the predominating effect in a given situation favors energy intake or energy expenditure, body weight and body mass index measurements may increase, decrease, or remain unchanged after alcohol ingestion [4]. Several factors may modulate the effect of alcohol on energy homeostasis. Associated energy intake, food composition, eating pattern, fat mass, and liver disease (cirrhosis) are examples of such factors [4]. Gender may also be a potential modulator considering that it has been reported that alcohol-consuming men add energy contained in the drinks to the energy content in their ordinary food, whereas in women, alcohol energy appears to displace other energy sources [6]. How alcohol stimulates appetite and brings about increased energy intake is currently unknown. A number of different mechanisms may be involved because appetite is regulated by a complex interplay between pro-opiomelanocortin and neuropeptide Y (NPY) systems in the arcuate nucleus (ARC) on the one hand, and hormone-mediated signals from the periphery on the other [7-9]. It is believed that hormone signals from peripheral tissues convey information to the ARC about the energy balance and nutritional status of the body [10,11].

Leptin is of interest in this context because this hormone induces satiety via inhibition of NPY neurons [12]. If alcohol inhibits the secretion of leptin, such mechanism could perhaps explain why alcohol has an orexigenic effect in humans. Our research group has previously shown that oral intake of moderate amounts of alcohol inhibits leptin secretion significantly [13]. However, leptin is known for long-term rather than short-term effects on human energy homeostasis [14,15]. Therefore, other hormones may be of

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greater interest in this context. Ghrelin induces hunger [11,16]. Its orexigenic effect appears to depend on NPY neurons. This assumption is supported by the fact that ghrelin increases NPY expression in ARC neurons of laboratory animals, whereas antibodies against NPY and ablation of the ARC block ghrelin's orexigenic effect [17,18]. It is possible that an increase in secretion of ghrelin mediates the appetite-stimulating effect of alcohol. If so, this alcohol-induced ghrelin increase could affect the NPY neurons directly, or indirectly via the vagus nerve [19], as food deprivation–induced ghrelin increase is abolished by subdiaphragmatic vagotomy [20]. In contrast to that assumption, we recently observed that total ghrelin concentrations begin to decrease shortly after alcohol ingestion [21]. However, the major proportion of total ghrelin (des-octanoyl ghrelin) is biologically inactive [22]. Only the octanoylated form is bioactive due to posttranslational modification of the serine 3 residue [22,23]. How alcohol affects octanoylated ghrelin in healthy individuals has never been studied before. Until that has been done, it cannot be decided whether ghrelin has a role to play as mediator of the orexigenic effect of alcohol.

Peptide YY (PYY) is also of interest. This gut-derived hormone is released postprandially in proportion to the calories ingested [24,25]. It has been maintained that PYY inhibits feeding in rats [26] and induces satiety in humans [26]. However, not all investigators agree on that [27], and additional studies are needed to decide whether PYY has weight-reducing properties in humans. How alcohol influences the secretion of PYY has not been studied before. One objective of this investigation was therefore to do so. Another was to establish whether alcohol affects serum levels of total and octanoylated ghrelin similarly.

2. Subjects and methods

2.1. Subjects

Twelve healthy subjects (6 men, 6 women), aged 22.9 ± 1.0 years (range, 18-29 years), volunteered to participate. Their body mass index measurements were 22.2 ± 2.7 kg/m^2. None of the women used oral contraceptives. They were investigated in the follicular phase of the menstrual cycle. Most participants consumed moderate amounts of alcohol at social events, but none was addicted to liquor, and all completely refrained from using alcohol in any form during 3 days before the tests. All gave informed consent to participate in the investigation, which was approved by the ethics committee at the Karolinska University Hospital in Stockholm, Sweden.

2.2. Protocol

Each individual took part in 2 experiments (experiments A and B) performed in a metabolic ward, in random order and 1 week apart. Participants fasted overnight (8 hours) and rested in a supine position during the tests.

Experiment A. At 7:30 AM a catheter was inserted into one of the antecubital veins that was kept patent by a slow drip of normal saline solution. Basal blood samples were collected from this catheter after an equilibration period of 30 minutes. Alcohol was given orally at 8:00 AM at a dose of 0.55 g/kg (equivalent to 10 cL of whisky and yielding 1117 kJ in a 70-kg man). Blood samples for determination of total and octanoylated serum ghrelin and PYY levels were drawn immediately before the alcohol ingestion and subsequently at 8:30, 9:00, 10:00, 11:00 AM, and 1:00 PM. Serum ethanol levels were determined at 8:00, 9:00, 10:00 AM, and 1:00 PM.

Experiment B. Drinking water was substituted for alcohol in this experiment. All other details were identical in experiments A and B.

2.3. Assays

Total ghrelin levels were determined with a radioimmunoassay (RIA) kit (Ghrelin [total] RIA kit from Linco Research, St Charles, MO). The sensitivity of the assay was 93 pg/mL and the intra- and interassay coefficients of variation (CVs) were 10% and 14.7%, respectively, at a total serum ghrelin concentration of 1000 pg/mL.

A RIA kit from the same manufacturer also measured octanoylated ghrelin concentrations. The sensitivity of the assay was 7.8 pg/mL. Intra- and interassay CVs were 6.7% and 9.6%, respectively, at an octanoylated serum ghrelin level of 140 pg/mL.

PYY levels were determined radioimmunologically with a kit that was also provided by Linco Research. The sensitivity of the assay was 10 pg/mL and the intra- and interassay CVs were 2.9% and 7.1%, respectively, at a serum PYY level of 111 pg/mL.

An automated Hitachi 911 analyzer from Roche Diagnostics (Bromma, Sweden) made serum ethanol measurements possible.

2.4. Statistical analysis

Difference over time within treatments and interaction between time and treatment were analyzed by 2-way repeated-measures analysis of variance and, if significant, was followed by Newman-Keuls post hoc test. P values less than .05 were considered significant. Values denoted are means ± SEM. Statistical analyses were performed using Statistica, Statsoft, version 7.1 (Tulsa, OK).

3. Results

3.1. Serum ethanol

Experiment A. After intake of alcohol, the ethanol level increased from 0 to 12.5 ± 0.7 mmol/L in 1 hour ($P < .001$) and then began to decrease. It reached 2.7 ± 0.5 mmol/L after 5 hours (Fig. 1).

Experiment B. After ingestion of water, the ethanol level remained at 0 mmol/L throughout the entire study (Fig. 1).

3.2. Serum PYY

Experiment A. The PYY level was 124.3 ± 6.4 pg/mL at 8:00 AM. It was unaffected by alcohol as shown in Fig. 1.

Experiment B. The basal PYY level was 118.3 ± 6.0 pg/mL at 8:00 AM. It did not differ significantly from the corresponding level in experiment A. In addition, drinking water had no significant influence on PYY (Fig. 1), and hormone levels in experiments A and B did not differ significantly over time.

3.3. Total serum ghrelin

Experiment A. A total ghrelin concentration of 896.2 ± 108.0 pg/mL was recorded at 8:00 AM. After alcohol ingestion, this level declined rapidly and was significantly below the basal level after 30 minutes ($P < .001$; Fig. 2). It continued to fall during the experiment and reached its lowest point after 5 hours, 36% ± 4% below the basal level ($P < .001$).

Experiment B. The basal total ghrelin concentration was 944.3 ± 117.4 pg/mL. This value did not differ significantly from that in experiment A. Drinking water did not change the total ghrelin concentration over time (Fig. 2). When comparison was made between total ghrelin concentrations in experiments A and B, significant differences ($P < .001$) were obtained from 8:30 AM onward.

3.4. Octanoylated serum ghrelin

Experiment A. At 8:00 AM the octanoylated ghrelin concentration was 117.1 ± 20.9 pg/mL. Alcohol ingestion induced a rapid ghrelin decline, which was significant ($P < .001$) from 8:30 AM onward (Fig. 2). A nadir was reached at 10:00 AM, 48% ± 5% below the basal level. Thereafter, the octanoylated ghrelin level tended to level out.

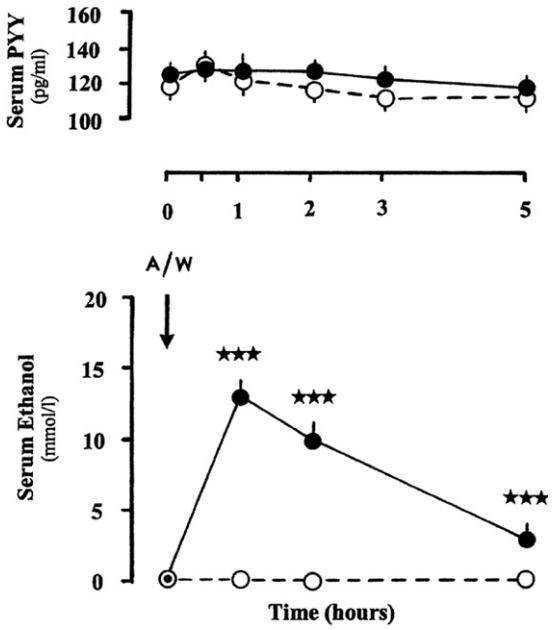

Fig. 1. PYY and ethanol concentrations in serum after ingestion of alcohol (A, ●–●) and drinking water (W, ○ - - ○) in 12 healthy subjects. Values are means ± SEM. ***$P < .001$ (compared with the basal level).

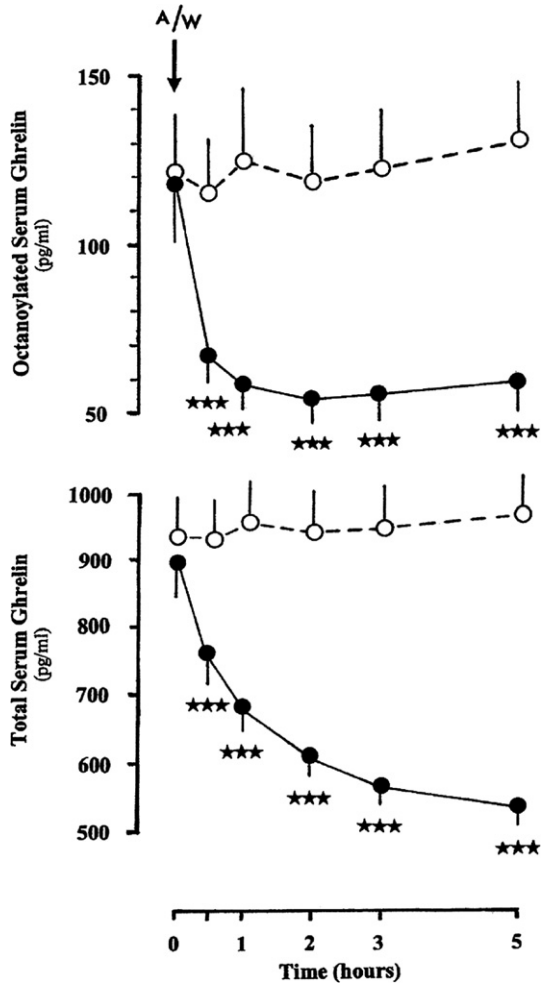

Fig. 2. Octanoylated and total concentrations of ghrelin after ingestion of alcohol (A, ●–●) and drinking water (W, ○ - - ○) in 12 healthy subjects. Values are means ± SEM. ***$P < .001$ (compared with the basal level).

Experiment B. The basal octanoylated ghrelin concentration in experiment B (121.8 ± 18.2 pg/mL) did not differ significantly from that in experiment A, and drinking water did not affect ghrelin levels over time (Fig. 2), but significant differences ($P < .001$) between octanoylated ghrelin levels in experiments A and B were obtained from 8:30 AM onward.

3.5. Gender

When gender differences were investigated by comparing laboratory values obtained in men and women before and after ingestion of alcohol/water, no significant differences were recorded between the sexes.

4. Discussion

Healthy, non–alcohol-dependent subjects of both sexes were investigated in this series. When they ingested a single dose of alcohol, which raised the ethanol level moderately, the ghrelin levels fell not only sharply, but also profoundly, over a long period (>5 hours). Other research groups have

studied patients with chronic alcoholism [28,29]. In these individuals, increased ghrelin levels were found during active drinking [28]. Elevated levels were also observed during periods of alcohol abstinence [28,29]. The discrepancy between our current findings in healthy subjects and those in patients with chronic alcoholism is unexplained. However, both alcohol abuse and alcohol withdrawal are stressful situations that may activate stress reactions in alcohol-dependent individuals. In mice, stress induced by both starvation and tail pinch has been found to increase gastric ghrelin gene expression [30]. It is possible that stress has a similar influence on human ghrelin producing X/A cells in the gastric oxyntic mucosa.

Two different ghrelin-specific RIAs have previously been developed: one recognizing the octanoyl-modified portion, the other the C-terminal portion of ghrelin [28]. By using these RIA systems, 2 major forms of ghrelin have been identified: octanoylated ghrelin, which is hormonally active, and total ghrelin, which is the n-octanoylated, inactive form of the hormone [31]. In our current investigation, alcohol decreased the serum levels of both these forms of ghrelin. This was an unexpected finding taking into account that both alcohol and ghrelin are known for appetite-stimulating properties. Suppressed ghrelin secretion is, therefore, hardly the mechanism underlying the orexigenic effect of alcohol. Other factors are probably more likely.

PYY is one such factor. Some investigators believe that it induces satiety via inhibitory signals from the gut to NPY neurons in the hypothalamus [26]. Provided that alcohol has a strong inhibitory influence on the secretion of PYY, the effect of such PYY inhibition would perhaps overshadow the effect of alcohol-induced ghrelin inhibition. If so, the net outcome would be appetite stimulation. However, this way of explaining the mechanism underlying alcohol-induced appetite stimulation is unlikely because this study shows that alcohol does not affect the secretion of PYY.

Gastrin-releasing peptide, cholecystokinin, and glucagon-like peptide 1 (GLP-1) are secreted by endocrine cells in the gastrointestinal tract. All of them are involved in the regulation of food intake by afferent satiety signals conveyed to the brain via vagal reflexes [32]. Previous studies have shown that alcohol is without influence on the secretion of human GLP-1 [33]. Hence, GLP-1 cannot be a mediator of the orexigenic effect of alcohol. Whether cholecystokinin and gastrin-releasing peptide act differently is unknown because studies in this field are missing.

In the present investigation, the dose of alcohol given to a 70-kg man yielded an energy provision of 1117 kJ. It is possible that this amount of energy, rather than the ethanol solution per se, could have suppressed the ghrelin secretion. Most previous studies have shown that ingestion of carbohydrates [34], fat [35], and protein [36] suppresses ghrelin secretion in humans, and Callahan et al [37] have found that a mixed meal containing 16% of the total daily energy expenditure decreases the circulating ghrelin concentration by approximately 20%. In the current investigation, an oral dose of alcohol, which contained approximately 10% of the total daily energy expenditure, doubled the ghrelin decline (36%-48%). This finding does not support the notion that energy provision is the sole cause of decreased ghrelin secretion after alcohol ingestion.

In rats, multiple ulcers arise when their gastric mucosa is exposed to ethanol [38]. In humans, alcohol appears to have a similar dose-dependent effect [39,40]. It has been suggested that ghrelin might be gastroprotective [38]. If so, our current results, demonstrating decreased ghrelin secretion after alcohol ingestion, could at least in part explain why alcohol often has a deleterious influence on the gastric mucosa. Provided that alcohol has a toxic effect on ghrelin-secreting cells in the stomach, one would expect to find a similar effect on other hormone-secreting cells in the gastrointestinal tract. This would mean that the PYY secretion should be suppressed by alcohol. As mentioned above, this does not seem to be the case. However, an unchanged serum PYY level after intake of alcohol does not necessarily mean that the drug lacks a toxic effect on PYY secretory cells in the gut because these cells are abundant in the distal part of the intestine, but scarce in the proximal part [24]. Moreover, a newly ingested alcohol solution is diluted considerably by digestive juices on its way through the body, which means that most PYY cells in the intestine are exposed to a much weaker alcohol solution than the ghrelin-secreting cells in the stomach.

5. Conclusion

In healthy individuals, alcohol exerts a suppressive effect on ghrelin-secretory cells as evidenced by rapidly decreasing serum levels of both octanoylated and total ghrelin after alcohol ingestion. By contrast, serum levels of PYY remain unchanged after alcohol ingestion. Considering that both alcohol and ghrelin stimulate appetite, whereas PYY appears to have the opposite effect, these findings imply that neither ghrelin nor PYY mediate the orexigenic effect of alcohol. Whether alcohol-induced ghrelin suppression is caused by a toxic effect of the drug on secretory cells in the stomach needs further elucidation.

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