

## INCREASED FASTING PLASMA GHRELIN LEVELS DURING ALCOHOL ABSTINENCE

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**Abstract** — **Aims:** Ghrelin is a peptide hormone that antagonizes the action of leptin and is thereby thought to regulate feeding behaviour. The actions of ghrelin and leptin appear to be mediated by the neuropeptide Y (NPY) and Agouti-related protein (AGRP) system. Recent studies have suggested that leptin and NPY play significant roles in the pathophysiology of alcoholism. The aim of this study was to determine whether ghrelin is associated with the state and duration of abstinence in individuals with alcohol dependence. **Methods:** Fasting plasma ghrelin levels were compared between 47 individuals with chronic alcoholism during a period of abstinence and 50 control subjects. **Results:** Fasting plasma ghrelin levels were higher in alcohol abstainers than those in controls. Furthermore, a positive correlation was observed between ghrelin levels and the duration of abstinence. In addition, daily alcohol intake prior to abstinence was inversely related to ghrelin levels. **Conclusions:** These findings suggest that ghrelin plays a role in the pathogenesis of alcohol dependence, particularly during the abstinence period, in individuals with chronic alcoholism.

### INTRODUCTION

Ghrelin is a recently isolated peptide hormone that is secreted by endocrine cells in the gastrointestinal tract and the hypothalamus (Kojima *et al.*, 1999; Nakazato *et al.*, 2001). It has been found to increase food intake, body weight and growth hormone secretion. The actions of ghrelin are thought to be mediated, at least in part, by the neuropeptide Y (NPY) and Agouti-related protein (AGRP) system (Horvath *et al.*, 2001). Hunger and food-seeking behaviours are stimulated by falling leptin concentrations and rising ghrelin levels (Williams *et al.*, 2001; De Ambrogi *et al.*, 2003).

Similar to food-seeking behaviour, hypothalamic mechanisms are thought to play a role in the regulation of alcohol-seeking behaviour (Samson and Hodge, 1996). Moreover, alcohol craving and hunger are similar in psychological qualities and they are thought to share common underlying neural circuitries. Both, for example, are influenced by the mesolimbic brain reward system and its endorphinergic inputs (Wise, 1998; Saper *et al.*, 2002).

The aim of the present study was to determine whether ghrelin is associated with the state and duration of abstinence in chronic alcoholism, when craving for alcohol is greatest. We compared fasting plasma ghrelin levels in chronic alcoholics, after an abstinence period of >30 days, with levels in controls subjects. Because elevated plasma cortisol levels during abstinence are thought to be caused by alcohol withdrawal rather than by abstinence per se (Kutscher *et al.*, 2002), plasma concentrations of cortisol were also measured

to assess and control for any possible longer term alcohol withdrawal effects on ghrelin levels.

### SUBJECTS AND METHODS

#### Subjects

Forty-seven male patients with chronic alcohol dependence admitted to the Department of Psychiatry in Chunchen National Hospital were included in the study. The sample was composed of inpatients admitted to the hospital since they had an ongoing 6-month alcohol dependence rehabilitation treatment programme. Patients who underwent the detoxification programme and remained abstinent for >30 days were included. The mean abstinence period was  $102.0 \pm 62.7$  days. DSM-IV diagnoses were determined in a consensus procedure involving two board-certified psychiatrists (S.-J.Y.; B.C.), using all available clinical material, including a semi-structured interview based on DSM-IV (American Psychiatric Association, 1994). Patients with comorbid psychiatric and medical illnesses, such as schizophrenia, other substance-related disorders, hepatic cirrhosis, and diabetes mellitus, were excluded.

Fifty healthy male volunteers who had no history of substance dependence, psychiatric or medical illnesses were included as control subjects. Individuals were excluded from participating if they reported on the Alcohol Use Disorders Identification Test (AUDIT) (Babor *et al.*, 1992) as drinking more than once or >40 g of alcohol each week. The control subjects were also required to be abstinent from alcoholic beverages for at least 72 h prior to participation in the study. All subjects provided written informed consent after a complete description of the study; the study was approved by the hospital ethics committee (Holy Family Hospital, The Catholic University of Korea, Seoul, Korea) and the

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procedures followed were in accordance with Helsinki Declaration of 1975, as revised in 1983.

### Procedures

Blood samples were collected from all subjects at 7:00 AM after an overnight fast. Body composition was evaluated using electrical impedance biometry (Bolanowski and Nilsson, 2001) on the same day the blood samples were obtained. Body weight and height were measured on the same day as well. Additional routine laboratory tests included hemoglobin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total protein, albumin and glucose. Subjects were interviewed using structured questionnaires to examine their history of alcohol intake in detail. The daily intake of alcohol, the total duration of abstinence, and the duration of history of alcoholism were documented using the AUDIT.

Blood samples for cortisol measures were drawn into EDTA-coated tubes, cooled and immediately centrifuged for plasma, which was then stored at  $-80^{\circ}\text{C}$ . Plasma cortisol concentrations were analysed using a commercially available radioimmunoassay kit by a coated tube technique (DiaSorin Inc. Stillwater, MN). Intra- and inter-assay coefficients of variation were  $<8$  and  $<10\%$ , respectively. The calculated sensitivity was  $0.21\ \mu\text{g/dl}$ . Blood for measuring ghrelin levels was drawn into chilled tubes containing disodium EDTA ( $1\ \text{mg/ml}$ ) and aprotinin ( $500\ \text{U/ml}$ ). Plasma ghrelin levels were measured using a commercially available RIA kit (Phoenix Pharmaceuticals, Inc. Belmont, CA) that utilizes  $^{125}\text{I}$ -labeled bioactive ghrelin as a tracer and a rabbit polyclonal antibody raised against full-length octanoylated human ghrelin. Intra- and inter-assay coefficients of variation were  $<6\%$  and  $<8\%$ , respectively. The limit of detection of this assay was  $40\ \text{pg/ml}$  of human ghrelin.

### Statistical Analyses

An analysis of covariance (ANCOVA) with body mass index (BMI) as a covariate was used to compare ghrelin levels between the alcoholic and control groups. Pearson's

correlation coefficients were calculated for correlation analyses. A two-tailed partial correlation coefficient assessed the association of ghrelin levels with alcohol-related data after adjusting for BMI. Differences of ALT and GGT between groups were analysed using appropriate non-parametric statistics, such as the Mann-Whitney *U*-test.

## RESULTS

Baseline characteristics of the study subjects are summarized in Table 1. Groups were similar in age, height, BMI, routine laboratory data and plasma cortisol levels. By design, individuals with chronic alcoholism had significantly higher mean scores on the AUDIT than control subjects ( $P < 0.001$ ). The daily intake of alcohol for the patient group averaged  $197.4 \pm 146.8\ \text{g/day}$ , or  $2.88 \pm 2.03\ \text{g/kg/day}$ . The mean duration of abstinence was  $102.0 \pm 62.7$  days, the duration of alcohol dependence was  $17.6 \pm 7.7$  years and the number of inpatient detoxifications was  $4.1 \pm 2.8$ .

Mean plasma ghrelin levels were higher in the abstinent chronic alcoholics ( $339.40 \pm 110.30\ \text{pg/ml}$ ) than in the control subjects ( $290.60 \pm 102.46\ \text{pg/ml}$ ,  $P = 0.03$ ). In all subjects, fasting plasma ghrelin concentrations correlated inversely with BMI ( $r = -0.23$ ,  $P = 0.02$ ). Covarying for BMI in an ANCOVA confirmed that the higher ghrelin levels in the alcoholic group were not simply caused by differences across groups in body mass ( $F = 4.57$ ,  $P = 0.03$ ). Cortisol levels did not differ significantly between the patient and control groups ( $P = 0.36$ ).

A significant positive correlation was observed between ghrelin levels and duration of abstinence in the group with alcoholism ( $r = 0.373$ ,  $P = 0.02$ ), a finding that persisted when covarying for BMI ( $r = 0.374$ ,  $P = 0.02$ ). In addition, daily alcohol intake and daily alcohol intake per kg prior to the period of abstinence correlated inversely with fasting ghrelin levels ( $r = -0.338$ ,  $P = 0.04$ ;  $r = -0.355$ ,  $P = 0.03$ , respectively), findings that were unchanged when adjusting for BMI (Table 2).

Table 1. Distribution of the subjects in this study

Characteristic	Alcohol abstainers		Control		P
	Mean	SD	Mean	SD	
Age (years) <sup>a</sup>	45.95	6.97	43.42	7.99	ns
Body weight (kg) <sup>a</sup>	67.82	8.51	71.09	12.17	ns
Height (cm) <sup>a</sup>	169.29	6.55	171.73	6.61	ns
BMI ( $\text{kg/m}^2$ ) <sup>a</sup>	23.76	3.54	24.30	3.85	ns
AUDIT <sup>a</sup>	28.47	6.84	10.82	5.61	$<0.001$
AST (U/l) <sup>a</sup>	31.44	13.85	28.14	8.83	ns
ALT (U/l) <sup>b</sup>	38.05	26.00	36.00	20.32	ns
GGT (U/l) <sup>b</sup>	52.02	39.32	42.46	26.09	ns
Glucose (mg/dl) <sup>a</sup>	93.82	21.42	95.92	11.00	ns
Haemoglobin (g/dl) <sup>a</sup>	14.61	1.57	15.50	0.84	ns
Protein (g/dl) <sup>a</sup>	7.62	0.55	7.68	0.49	ns
Albumin (g/dl) <sup>a</sup>	4.05	0.51	4.08	0.31	ns
Cortisol ( $\mu\text{g/dl}$ ) <sup>a</sup>	15.66	4.09	16.65	6.07	ns

ns, non-significant; BMI, body mass index; AUDIT, Alcohol Use Disorders Identification Test; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase.

<sup>a</sup>Statistical significance was tested using an unpaired *t*-test.

<sup>b</sup>Statistical significance was tested using a Mann-Whitney *U*-test.

Table 2. Correlations of ghrelin levels with clinical measures

Parameters	<i>r</i>	<i>P</i>
Age (years)	0.267	ns
Body mass index (kg/m <sup>2</sup> )	-0.230	0.02
Duration of alcohol use (years)	-0.018	ns
Total abstinence period (days)	0.373	0.02
Daily alcohol intake (g/day)	-0.338	0.04
Daily alcohol intake per kg (g/kg/day)	-0.355	0.03

*r*, Pearson's correlation coefficient; ns, non-significant.

## DISCUSSION

The main finding of this study was that fasting plasma ghrelin levels were elevated in patients with chronic alcohol dependence after a period of prolonged abstinence from alcohol. Furthermore, alcohol consumption per day prior to abstinence was correlated inversely with plasma ghrelin levels after the period of abstinence. Although plasma ghrelin concentrations correlated with BMI, which is consistent with findings from previous human studies (Tschöp *et al.*, 2001; Shiiya *et al.*, 2002), covarying for BMI in our statistical analyses did not alter our findings. The pathophysiological significance of our findings of increased ghrelin levels during alcohol dependence is not entirely clear. Nevertheless, findings from extensive clinical and preclinical studies provide compelling circumstantial evidence for the role that ghrelin may play during the period of alcohol abstinence.

Ghrelin is known to antagonize the action of leptin, a neuropeptide that is thought to play an important role in the pathophysiology of alcoholism (Roy *et al.*, 1990; Thiele *et al.*, 1998; Kiefer *et al.*, 2001a; Nicolas *et al.*, 2001; Heilig and Thorsell, 2002; Lappalainen *et al.*, 2002; Santolaria *et al.*, 2003). This antagonism of leptin by ghrelin appears to be mediated by the NPY and AGRP systems, the key hypothalamic peptides involved in the central regulation of energy homeostasis (Horvath *et al.*, 2001). Acute alcohol withdrawal in rats is associated with reduced NPY expression in the amygdala and hypothalamus (Ehlers *et al.*, 1998; Roy and Pandey, 2002). NPY in the amygdala is thought to help modulate anxiety via activation of Y1 receptors, and thus NPY may be an endogenous anxiolytic (Heilig and Widerlov, 1995; Oberto *et al.*, 2000). The reduced NPY in the amygdala during alcohol withdrawal would therefore be expected to increase anxiety and in turn influence the likelihood of relapse (Thiele *et al.*, 1998; Roy and Pandey, 2002). Furthermore, high concentrations of leptin have been reported recently in the plasma of alcoholic patients during acute alcohol withdrawal (Kiefer *et al.*, 2001a,b). Thus, given the antagonism of NPY expression by leptin within the hypothalamus and the presence of leptin receptors within the amygdala (Burguera *et al.*, 2000), it is possible that the high levels of leptin are responsible for the decreased NPY expression reported previously during acute alcohol withdrawal in animal models of alcoholism (Roy and Pandey, 2002). However, little is known about the possible NPY-leptin interactions within the amygdala.

In contrast to the reduced NPY levels reported during alcohol withdrawal, prolonged alcohol abstinence in rodents is associated with significantly increased NPY expression in the hypothalamus (Ehlers *et al.*, 1998; Roy and Pandey, 2002). Absent NPY production in knockout mice increases alcohol consumption, whereas NPY overexpression in transgenic mice reduces alcohol consumption (Thiele *et al.*, 1998). Thus, alcohol consumption is inversely related to NPY levels in these animal models. Given that (1) ghrelin antagonizes the actions of leptin, (2) the evidence that elevated leptin levels reduce NPY expression, and (3) the inverse association of NPY expression with alcohol use, we hypothesize that the increased peripheral ghrelin levels detected in our study antagonizes leptin and thereby increases hypothalamic activity of NPY and helps to reduce alcohol consumption during periods of abstinence. This hypothesis gains further credibility from the known direct interaction of ghrelin with NPY-containing neurons in the arcuate nucleus of the hypothalamus (Kohno *et al.*, 2003) and by our finding that ghrelin levels are correlated positively with the duration of abstinence in individuals with chronic alcoholism. The hypothesis is also consistent with our finding that the average daily intake of alcohol prior to the period of abstinence correlated inversely with ghrelin levels during prolonged abstinence, in that the correlation suggests that higher ghrelin levels may help to reduce intake of alcohol even during times of relatively heavy use. However, our sample included only inpatient groups; further prospective investigation is required to draw more reliable conclusions on the casual effect relating ghrelin with reduced alcohol intake.

The current study has several limitations. First, because states of malnutrition are known to increase ghrelin levels (Ariyasu *et al.*, 2001; Otto *et al.*, 2001), we cannot entirely exclude the possibility that nutritional abnormalities in the individuals with chronic alcoholism contributed to the findings, even though their body weight, BMI, protein, albumin and glucose were similar to the control subjects. The considerable duration of abstinence possibly helped to minimize the nutritional disturbances in the patient group as well. Second, no measures of craving for alcohol were obtained during the abstinence period, and the interpretation of our findings in the context of the neurobiology of leptin, NPY and alcohol use would predict that the elevated ghrelin levels would correlate inversely with measures of alcohol craving.

In conclusion, our findings suggest that ghrelin plays a role in the pathogenesis of alcohol dependence and the capacity to maintain abstinence. Further prospective studies are needed to evaluate ghrelin and leptin levels during the stages of alcohol use, withdrawal and abstinence, as well as the association between ghrelin levels and the degree of alcohol craving. Combining these longitudinal biochemical studies with neuroimaging studies of alcohol craving would further help to identify the neural substrate through which ghrelin and leptin may act to influence alcohol use.

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