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Effect of exogenous ghrelin on body weight and hematocrit of male adult rats in chronic hypoxia

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ABSTRACT

Background: Ghrelin is a peptide predominantly produced by the stomach. Recent studies have shown its protective roles and plasma alterations during hypoxia.
Objectives: The aim of this study was to assess the effects of an exogenous administration of ghrelin on body weight and blood hematocrit during chronic hypoxia.
Materials and Methods: Twenty four adult male Wistar rats were divided randomly into 3 groups. Hypoxic rats with saline or ghrelin treatment were placed in a normobaric hypoxic chamber for 2 weeks. Controls remained in room air. Weight gain, hematocrit, and plasma ghrelin were measured.
Results: The rats showed significant ($P < 0.05$) weight loss in the hypoxic groups, and administration of ghrelin in hypoxic rats could prevent further weight loss. Interestingly, hypoxic animals that were treated with ghrelin were significantly more polycythemic than the controls and even the hypoxic rats treated with saline ($P < 0.001$). Plasma ghrelin significantly increased in the hypoxic animals at the end of the second week ($P < 0.05$).
Conclusions: It seems that exogenous administration of ghrelin may be useful in modulating metabolism in high-altitude situations and that polycythemia induced by ghrelin, to some extent, might be a beneficial compensation during hypoxia. However, more investigation is needed to confirm the beneficial effects of ghrelin to establish this peptide's status as a therapeutic agent.

▸ *Implication for health policy/practice/research/medical education:*
The fact that ghrelin might be a benifical compensation during hypoxia is of importance for modulation of metabolism in high - altitude situations. Thus the study can open new windows to establish this peptide's status as a therapeutic agent.

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1 Background

Loss of appetite and body weight are among complications of chronic hypoxia for individuals living in high altitudes (1). One of the modulators of appetite and body weight is ghrelin. Discovered in 1999, ghrelin was first assumed to be a GH-hormone secretagogue, which is released from the stomach (2). Ghrelin's role as an orexigenic peptide has been shown (3), whereas previous studies have demonstrated that chronic hypoxia, after 2 weeks, can lead to decreases in plasma levels of ghrelin in neonatal rats (4-6).

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On the other hand, some research groups are working to introduce ghrelin as a therapeutic agent in cardiovascular diseases (7). Its protective effect during hypoxia is also under scrutiny. For example, a positive effect of ghrelin related to hypoxic hypoxia has been shown in which a subcutaneous injection of ghrelin can protect the lungs against hypoxic pulmonary hypertension through a vasodilating mechanism (8). In this case the model is probably similar to living in high altitudes. Moreover, in one study, Taati and his colleagues concluded that 5 days of ghrelin treatment increased hematocrit moderately in normal rats (9). Although, in chronic continuous hypoxia, it is well documented that the situation causes an increase in hematocrit (10, 11), but high hematocrit levels inhibit endothelium-dependent vasodilation in response to ACh in patients with chronic hypoxemic lung disease (12).

2. Objectives

Taken together, in a clinical point of view, more studies are needed to validate ghrelin's probable beneficial roles. The present study aims to determine the combined effect of exogenous ghrelin and chronic hypoxia (CH) on body weight and hematocrit and to examine plasma ghrelin alteration profiles after CH.

3. Materials and Methods

3.1. Animals and chronic hypoxic protocol

All experiments were conducted in accordance with the ethical standards set forth by the faculty of medicine at the Tabriz University of Medical Sciences, Iran. Male adult Wistar rats (200–250 gr) were housed in cages in a temperature- and light-controlled environment and were provided with food and water ad libitum. Animals were randomly divided in 3 groups including control (C), hypoxic with saline (H+S), and hypoxic with ghrelin (H+G). Each group contains 8 rats. All animals were weighed on a digital scale on the first and the last day of the procedure. In hypoxic groups (H+S and H+G), hypoxia was induced by an Environmental Chamber System GO2 Altitude (Biomedtech Australia, Pty. Ltd), which generates hypoxic air without the need for a gas cylinder. H+S and H+G animals were placed in a ventilated chamber inflated by hypoxic air (O2 11%), simulated to 5150 m above sea level. An O2 sensor and controller was embedded in the chamber wall to monitor O2 concentration. Animals were kept in the chamber continuously for two weeks except for 20 min/day to clean the cages and perform daily injections.

3.2. Drug administration

Rats received a subcutaneous injection of either saline (0.1 ml) or ghrelin (150 μg/kg/day in 0.1 ml) (8) and were then placed into the hypoxic chamber. H+S and H+G rats continued to receive daily injections of either saline or ghrelin during the 2-week study period. Ghrelin was obtained from the Tocris Bioscience Co. (Bristol, UK) and was administered dissolved in saline.

3.3. Hematocrit measurement

Each animal's hematocrit was measured using the standard microhematocrit method. Blood samples were taken from the tails of the animals. Up to two-thirds the length of the microhematocrit tube was filled with blood sample and then sealed one end with a clay sealant. We used 2 tubes for each sample, a plain blue-ringed tube for anticoagulated blood and a heparinized red-ringed tube for finger stick. Blood-contained tubes were centrifuged at 12,000 g for 5 minutes in a microhematocrit centrifuge. Finally, the percentage of Hct was was taken with a microhematocrit reader.

3.4. Ghrelin measurement

All measurements were performed on pooled samples from each group. Ghrelin was measured by enzyme-linked immunosorbent assay (ELISA) using a reader (Statfax, Awarness, USA) and acylated ghrelin and unacylated ghrelin kits (cat. No. RD394062400R and cat. No. RD394063400R, Biorendor Co. Czech Republic). The detection limits were 0.2 and 0.7 pg/ml, respectively, for long and short immunological reaction. The intra-assay and interassay were 11.2 and 11.4%, respectively.

3.5. Statistical analysis

The collected data were analyzed using SPSS version 13.0. Results are reported as means ± SEM. Data were analyzed by one-way ANOVA to test for differences between groups. For statistically significant comparisons, post hoc analyses were performed using Tukey tests. P values of less than or equal to .05 were used as the level of significance for all statistical analyses. To compare weight changes, paired sample t tests were used.

4. Results

The average body weight of the C, H+S, and H+G groups before treatment were 212.62 ± 2.80, 214.37 ± 2.82, and 209.62 ± 2.05, respectively, which were not significantly

Table 1. Weight of animals in three experimental groups before and after treatment

	Weight [a]		
	Before	**After**	***P* value**
Control	212.62 ± 2.80	241.75 ± 4.80	<0.0001
Hypoxic + Saline	214.37 ± 2.82	206.25 ± 3.57	<0.001
Hypoxic +Ghrelin	209.62 ± 2.05	207.87 ± 3.30	NS
***P* value**	NS [b]	<0.0001	__

[a] Data are reported as means ± SE
[b] NS: Not significant

different. After 2 weeks their weight became 241.75 ± 4.80, 206.25 ± 3.57, and 207.87 ± 3.30. The H+S and H+G groups' weights were significantly lower than the weight of the C animals ($P < .05$), but compared with the pretreatment level, the weight of the H+G rats did not change significantly *(Table 1)*.

Average hematocrit of the C, H+S, and H+G groups after 2 weeks were 45.14 ± 1.01, 59.10 ± 1.37, and 69.57 ± 0.89 respectively, in which a significant polycythemia occurred in H+S and H+G animals compared with the C group ($P < .0001$; *Figure 1)*.

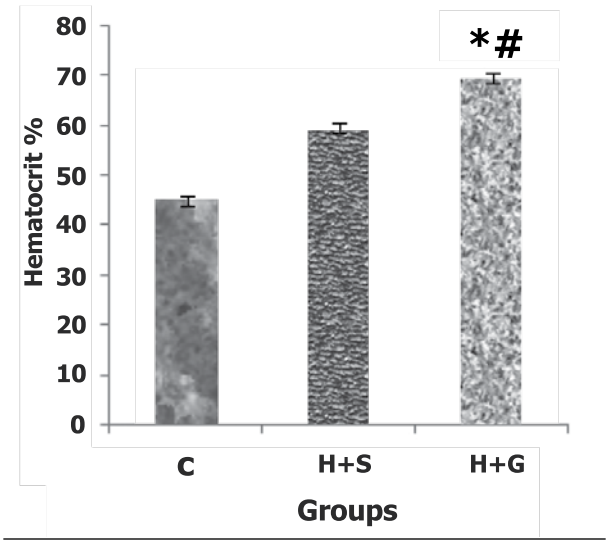

Figure 1. Average hematocrit after 2 weeks in controls (C), hypoxic rats treated with saline (H+S), and hypoxic rats with ghrelin (H+G)

*$P < 0.0001$, significant difference compared with the controls
≠$P < 0.0001$, significant difference compared with H+S. Ghrelin was injected subcutaneously (150 μg/kg/day in 0.1 ml).
Data are reported as means ± SE

Acylated ghrelin was measured in all three groups after 2 weeks. The average amounts of ghrelin in the C, H+S, and H+G groups were 86.50 ± 5.92, 136.43 ± 17.28, and 92.93 ± 7.29 pg/ml, respectively. In the case of the H+S animals, acylated ghrelin was significantly greater than that of the other two groups ($P < 0.05$; *Figure 2*). We also took measurements for unacylated ghrelin, which showed no significant difference between the three experimental groups.

5. Discussion

Hypoxic stress usually induces weight loss during living in high altitudes through decreased energy expenditure, increased metabolic rate (5, 13), and loss of appetite (1). Our data showed a clear weight loss in the hypoxic groups. It seems that administration of ghrelin in hypoxic rats could prevent further weight loss in the H+G group compared to animals with no treatment. Although exogenous ghrelin did not lead to a significant weight gain, at least it allowed the hypoxic animals' weight to be in the normal range. We assume that anorexia in the hypoxic condition could be overcome to some extent by

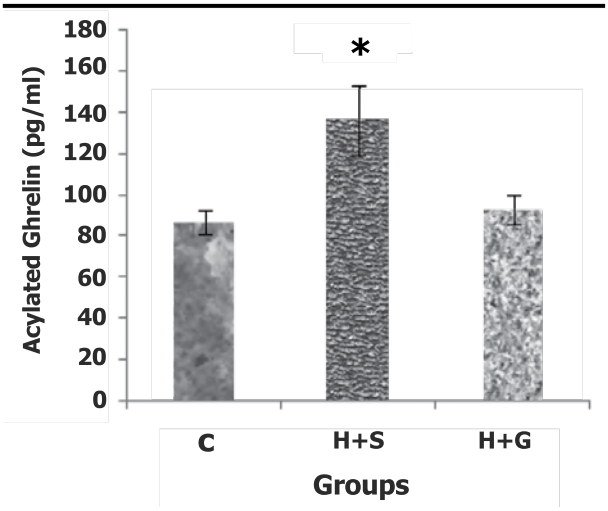

Figure 2. Average acylated ghrelin after 2 weeks in the control (C), hypoxic-with-saline (H+S), and hypoxic-with-ghrelin (H+G) groups

*$P < 0.05$, significant difference compared with the C and H+S animals. Ghrelin was injected subcutaneously (150 μg/kg/day in 0.1 ml).
Data are reported as means ± SE

ghrelin through increasing appetite and feeding. In this case, ghrelin can be taken into account as a potential useful agent in modulating metabolism in high-altitude situations and also in improving nutritional adaptations to balance metabolic needs for individuals living with chronic hypoxia. Certainly, more studies are required to elucidate this assumption.

The high blood hematocrit levels in hypoxic animals in our study are consistent with the reports from other researchers (5). It is interesting to mention that hypoxic animals that were treated with ghrelin were significantly more polycythemic than the controls and even the hypoxic rats treated with saline. Based on previous studies, ghrelin treatment after 5 days can increase hematocrit in normal rats (9), and subcutaneous injections of ghrelin can protect lungs against hypoxic pulmonary hypertension through a vasodilating mechanism (8). Another finding from the literature is that the endothelial NO production pathway can be activated by the sheer force exerted by circulating blood, which leads to flow-dependent vasodilation (13, 14). Because red blood cells are a major determinant of blood viscosity, polycythemia, which increases shear stress via an increase in viscosity, may also increase the release of NO. However, high hematocrit levels inhibit endothelium-dependent vasodilation in response to ACh in patients with chronic hypoxemic lung disease (12). Even one study has shown that phlebotomy can achieve normocythemia in chronically hypoxic rats and reduce pulmonary arterial blood pressure by 30% as compared with unphlebotomized hypoxic control rats (15). The present study is the first in the literature to show that ghrelin in a hypoxic model can increase hematocrit to an even higher level than the expected level in hypoxia alone. Although polycythemia is a common and to some extent beneficial compensation during hypoxia, the beneficial effect of this outcome of

ghrelin treatment is uncertain. More investigations are needed to explore this controversial issue. The first step to elucidate the mechanism responsible for this effect of ghrelin is to measure erythropoietin mRNA expression in the kidney of chronic hypoxic animals that are treated with ghrelin. However, although it seems that ghrelin increases blood viscosity, but supported by its well-known vasodilating effect, this adverse outcome will be neutralized and so little change will be inserted to total peripheral resistance (TPR).

According to our results, plasma ghrelin had significantly increased in hypoxic animals by the end of Week 2. Because of the existent proofs for protective roles of ghrelin, especially during hypoxia (8, 16, 17), it seems likely that the plasma level of ghrelin increased as a compensatory mechanism to protect different organs against hypoxia. Previous studies have demonstrated that chronic hypoxia, after 2 weeks, can lead to a decrease in the plasma level of ghrelin in neonatal rats (4-6). Because our study was performed in adult rats, the profile of plasma ghrelin change might differ for neonatal rats. On the other hand, some studies have found that gastric ghrelin gene expression in rats increases with age (18, 19). Although ghrelin plasma levels were not significantly different between controls and in the group treated with ghrelin, there was a small increase, so we speculate that some negative feedback mechanism might have prevented the enhancement that we observed in the hypoxic rats. Negative feedback in the gene expression of ghrelin in the stomach has been proposed previously (16), but the actual mechanism is unclear. Moreover, the acyltransferase that catalyzes ghrelin octanoylation has recently been identified as ghrelin O-acyltransferase (GOAT); (20). Furthermore, stomach GOAT mRNA levels have been correlated with circulating acylated-ghrelin levels (21). So, it seems that this is another way to decrease the endogenous production of acylated-ghrelin in the long term, when plasma ghrelin is higher. Nevertheless, we measured ghrelin at the end of study, and the proposed negative feedback that we discussed might not have been initiated in the beginning days to suppress both ghrelin and GOAT gene expression because this genomic reaction requires time, and in this time ghrelin would be able to have its effects. In addition, less is known about ghrelin clearance or metabolism (3), but some studies have shown that its plasma half-life, when exogenously administered, is short (22, 23).

It seems that exogenous administration of ghrelin may be useful in modulating metabolism in high-altitude situations. The polycythemia induced by ghrelin, can be, to some extent, a beneficial compensation during hypoxia, although more investigation is needed in this regard. It is suggested that, like present study, when plasma ghrelin alteration is hypothesized as an interfering factor, the life-span stage should be considered because it seems that ghrelin secretion is somehow age related.

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None declared.

Conflict of interest

This article is derived from part of my Ph.D dissertation entitled “Effects of ghrelin on gene expression of heme oxygenase, PKC, and RhO kinase in the lungs of chronic hypoxic Wistar rats.”

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