

## *The Role of Growth Hormone in Regulation and Secretion of Ghrelin*

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### **Abstract**

**Background:** Ghrelin is a novel growth hormone (GH) releaser acylated peptide that has recently been purified from stomach and potently binds to the GH secretagogue receptor. In rats, fasting leads to elevated serum GH concentrations. Age-related decreases in energy expenditure have been associated with the loss of skeletal muscle and decline of food intake, possibly through a mechanism involving changes of GH secretion and feeding behavior. Ghrelin releases GH in vitro and in vivo in animal models, however its actions and specificity in humans are unknown.

**Method:** We investigate the relationship between age-related changes of growth hormone secretion and/or food intake and ghrelin function. Ghrelin (10 nmol/kg body weight) was administered intravenously to male 3-, 12-, 24- and 27-month-old Wistar adult rats, after which growth hormone concentrations and 2 h food intake were measured.

**Results:** Intravenous administration of ghrelin to rats increased food intake in all generations. In addition ghrelin administration elicited a marked increase in plasma GH levels, with the peak occurring 15 min after administration.

**Conclusion:** These changes in serum ghrelin concentrations during fasting were followed by similar, profound changes in serum GH levels. These data indicate that ghrelin is the main driving force behind the enhanced GH secretion during fasting.

**Keywords:** Ghrelin, growth hormone, food intake

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## **Introduction**

Ghrelin, a 28-amino-acid peptide, was isolated from human and rat stomachs as an endogenous ligand of growth hormone secretagogue receptor (GHS-R)<sup>1</sup>. Ghrelin stimulates growth hormone release when peripherally or centrally administered to rats and when applied directly to rat primary pituitary cells<sup>1-3</sup>. Plasma ghrelin levels decline with ageing due to impaired function of the gastric mucosa reducing the thickness of the membrane, the length of the glands, and the number of the endocrine cells in mice<sup>4</sup>. Previous human studies indicated that stomach ghrelin secretion decreases with ageing<sup>5</sup> and that ghrelin-induced growth hormone (GH) secretion is reduced in aged subjects compared to younger subjects<sup>6</sup>. In contrast to human data, plasma ghrelin concentrations and stomach ghrelin contents in aged rats are significantly higher than in young rats<sup>7</sup>.

The decline in blood levels of GH with ageing are commonly referred to as the somatopause<sup>8, 9</sup>. Because GH changes are associated with declines in physical abilities, attempts are often made to save the decline of physical abilities with ageing by GH replacement. However, the relative ratio of risk to benefit in GH replacement requires further discussion. Underlying mechanism of age-related somatopause, therefore, has to be investigated to find an ideal method of intervention.

Anorexia is commonly associated with ageing<sup>10, 11</sup> and may be related to age-related decline of plasma ghrelin (5). Normal ageing is associated with a decrease in appetite and energy intake, which has been termed the anorexia of ageing<sup>12, 13</sup>. Generally, after age 70-75 years, the reduction in energy intake exceeds energy expenditure in humans, resulting in weight loss where loss of muscle (sarcopenia) predominates and predisposes older subjects to protein energy malnutrition<sup>13,14</sup>. Ghrelin stimulates rat GH secretion in vitro and in vivo in anaesthetised animals<sup>1</sup>, which suggests that ghrelin may well be the expected third factor involved in GH regulation. As this new peptide circulates in normal subjects with a considerable plasma concentrations, it has been postulated that ghrelin is secreted by the stomach, and that it circulates in plasma to stimulate pituitary and hypothalamic structures involved in the somatotrope cell function. In any case, the physiology and physiopathology of this compound have yet to be precisely assessed.

The observed malnutrition and sarcopenia correlates with increased morbidity, and the number of hospitalizations with extended stays<sup>15</sup>. The causes of the physiological anorexia typified during ageing are unknown; they are probably multifactorial and include a reduction in feeding drive with increased activity of satiety signals. Ghrelin stimulates food intake as well as GH secretion<sup>16-19</sup>. In addition, ghrelin-induced GH secretion is higher compared to young rats. However, since these findings were provided from a cross-sectional study, the relationship between age-related dynamics of ghrelin and somatopause remains undefined. The present work studies the capability of ghrelin for releasing GH in rats, as well as its specificity.

## **Materials and methods**

### **Chemicals and drugs**

In this study, anesthesia was carried out by an intraperitoneal (ip) injection of sodium pentobarbital (75 mg/kg body weight) that was purchased from Abbot (Abbot Lab., Chicago, IL). Rat ghrelin was obtained from (Peptide Institute Inc., Osaka, Japan) and GH Biotrak Rat GH RIA kit was purchased from (Amersham, Buckinghamshire, UK).

## **Animals and treatment**

The committee for animal experiments of the Dicle University Medical Research Center (Diyarbakir, Turkey) gave its approval for the project. All experiments were carried out according to local guidelines for the care and use of laboratory animals and the guidelines of the European Community Council for experimental animal care. Every effort was made to minimize animal suffering and the number of animals used. Experiments were carried out on male Wistar adult rats at 3, 12, 24, and 27 months of age ( $n = 10$ ). All the rats housed under standart conditions in a room under a constant 12 h light /dark cycle with a humidity  $50 \pm 10\%$ . All the experimental procedures were carried out between 07.00 and 19.00 a.m.

## **Experimental Design**

Rats were used as follows;

**1-** Sterilized intravenous (iv) cannulae were implanted into the right jugular vein 1 week before the experiments of feeding and GH response on rats at 3, 12, 24, and 27 months of age. **2-**All rats recovered from surgery within 1 week, showing food intake amounts similar to pre-surgery levels and progressive weight gain. These rats were then used in the experiments. **3-**Rat ghrelin or saline was administered iv to rats fed *ad libitum*. The 2 h food intake amounts were then measured. **4-**This feeding test was performed using a crossover design experiment in which animals were randomized to receive either test substance with a washout period of 2 days between each administration. **5-**Two days after the feeding test, ghrelin (10 nmol/kg body weight) was administered iv to these rats which were anesthetized by sodium pentobarbital for the GH response test. **6-**After these tests, the iv cannulae were removed from the rats using sterilized devices. **7-**To prevent suppuration by infection, we frequently disinfected the rat, and exchanged cages after the operation. **8-**Rats were bred in previously described conditions until reaching the age of the following test.

## **Food intake**

During 3 days before administration, 24 h food intake amount was measured each day. Ghrelin (10 nmol/kg body weight) or saline was administered iv to rats at 10:00 AM through an iv cannula. The 2 h food intake amount was then measured. Also, relative amount of ghrelin-induced food intake was evaluated by the ratio of ghrelin-induced food intake to average of 24 h food intake amount during the 3 days. All of the rats used in these experiments were satisfactorily acclimated to handling before iv injections.

## **GH response**

After anesthesia by an ip injection of sodium pentobarbital, ghrelin (10 nmol/kg body weight) was administered iv to rats at 11:30 AM through an iv cannula. Blood samples were obtained from the tail vein, which was cut 15 mm from the tail end at a depth of about 2 mm by knife, at 0, 15, 30 and 60 min after administration. Then the plasma concentration of GH was determined.

## Statistical analysis

All results are expressed as the mean  $\pm$  S.E.M. The behavioral effects of drug and vehicle treatments were evaluated statistically using the non-parametric Kruskal-Wallis analysis of variance by rank, followed by the Mann-Whitney *f*-test with Bonferroni correction. Statistical significance was set at the  $P = 0.01$  level.

## Result

### Changes of age-related body weight and food intake

Body weight increased gradually in Wistar adult rats from 3- to 24-month of age. The body weight in 27-month-old Wistar adult rats was significantly decreased compared to 24-month-old Wistar adult rats ( $p < 0.01$ ) (Fig.1a). Food intake for 24 h were also increased from 3- to 24-month-old Wistar adult rats, while 24 h food intake in 27-month-old Wistar adult rats was significantly decreased compared to 24-month-old Wistar adult rats (Fig. 1b).

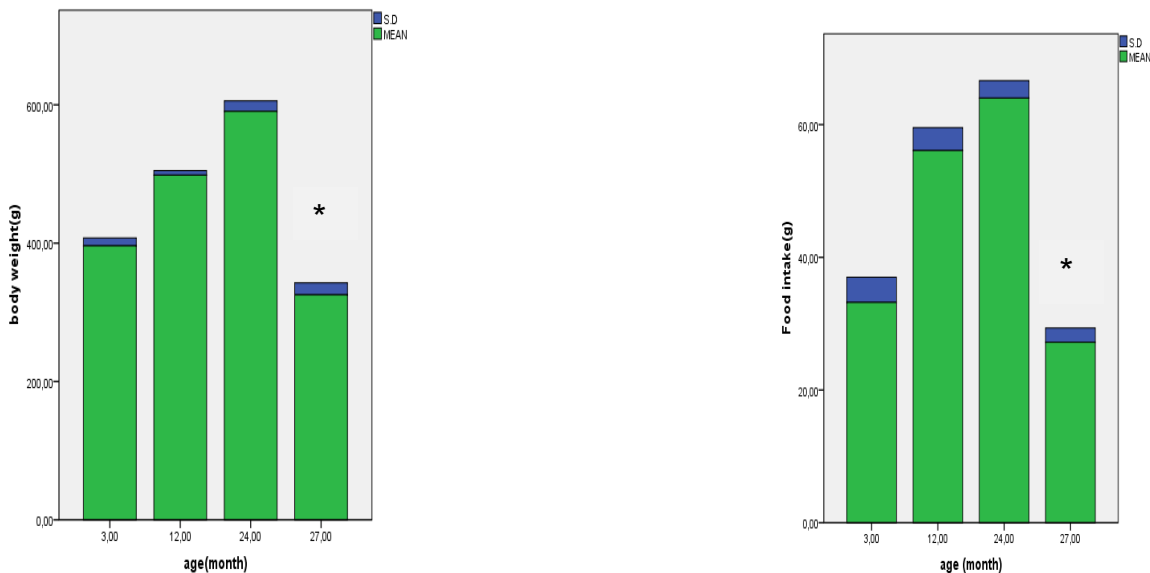


Figure 1.Changes of body weight (a) and 24h food intake (b) with ageing.

### Changes of age-related ghrelin-induced food intake

We examined the effects of ageing on ghrelin-induced food intake. While an iv administration of saline to Wistar adult rats did not induced food intake in all generations, an iv administration of ghrelin to Wistar adult rats increased food intake in all generations. The amounts of ghrelin-induced 2h food intake in 27-month-old Wistar adult rats were significantly decreased compared to the other generations ( $p < 0.01$ ) (Fig.2a). However, the ratio of ghrelin-induced food intake to 24 h food intake was the same among the generations (Fig.2b).

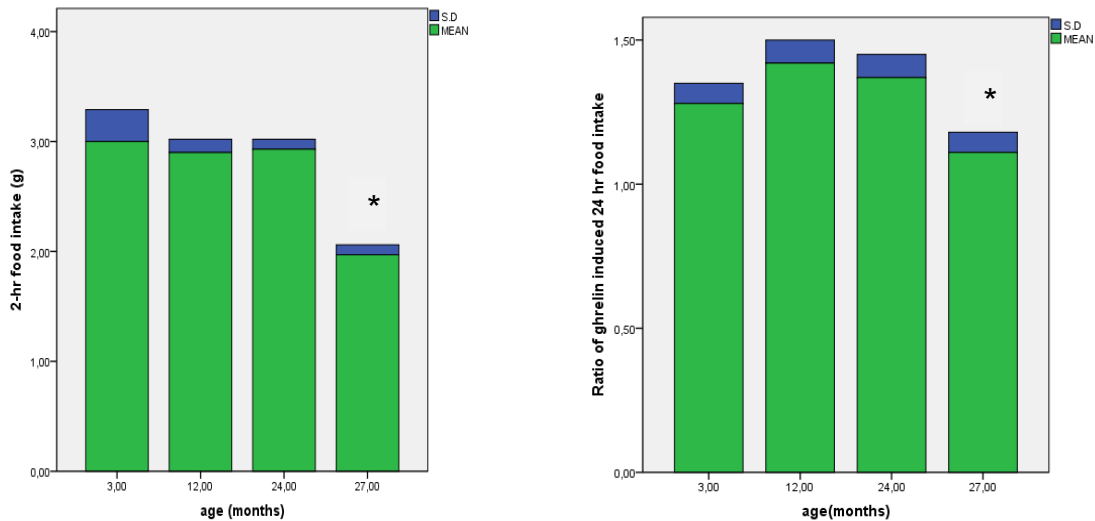


Figure 2. (a) Effect of i.v. administration of ghrelin (10 nmol/kg body weight) on 2h food intake in 3, 12, 24 and 27 month old rats. \* $p < 0.01$  vs. 3,12 or 24 months old rats  
 (b) No effect ageing on the ration of ghrelin induced 2 h food intake to 24 h food intake

### Changes of age-related ghrelin-induced GH secretion

We studied the release of GH in response to peripheral ghrelin administration at all generations of Wistar adult rats. Iv administration of ghrelin elicited a marked increase in plasma GH levels, with the peak occurring 15 min after administration (Fig.3). The level of ghrelin-induced GH secretion was not different among the generations.

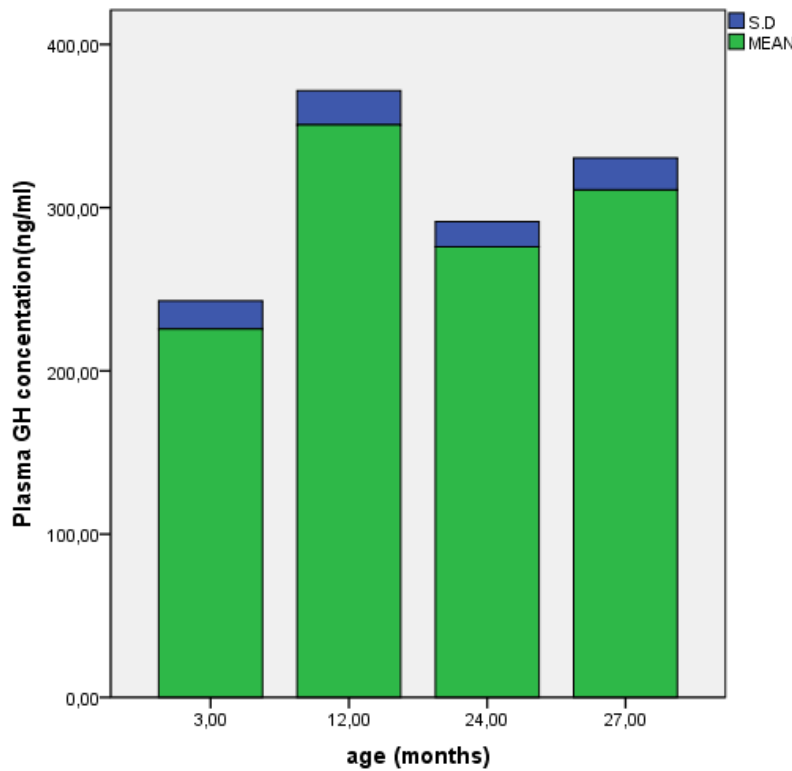


Figure 3. Effect of i.v administration of ghrelin (10 nmol /kg body weight ) After 15 minute on the plasma GH concentration in 3, 12, 24 and 27 months-old rats.

## **Discussion**

Longitudinal studies have demonstrated a decline in energy intake with ageing<sup>20,21</sup>. For example, a study involving a three-decade follow-up of 105 male humans aged 27-65 years demonstrated a decrease in daily energy intake of up to 25% (20). A 7-year longitudinal study in subjects aged 64-91 years also demonstrated a decrease in energy intake of 19.3 kcal/d per year in women and 25.1 kcal/d per year in men<sup>21</sup>. The reduction in energy intake with ageing exceeds energy expenditure, resulting in weight loss involved sarcopenia<sup>14-21</sup>. Indeed, the satiating effects of cholecystokinin (CCK), a gastrointestinal-derived anorectic peptide, increased with ageing and fasting and postprandial CCK concentrations are higher in healthy elderly subjects compared to young adults<sup>22</sup>. In contrast to age-related increase of CCK function, previous cross-sectional studies indicated that stomach ghrelin secretion and ghrelin-induced GH secretion decreased in aged subjects compared to younger subjects<sup>5,6</sup>. The efficiency of ghrelin and CCK signal transduction depend on the balance of their respective plasma concentration and/or on interactions between GHS-R and CCK type A receptor<sup>2</sup>. Thus, enhanced effects of CCK and/or reduced effects of ghrelin may contribute to the development of anorexia and in some cases protein malnutrition during ageing. Therefore, ghrelin coupled with its anabolic effects via the GH/IGF-1 axis indicate that rescue of reduced GHS-R activity by treatment with exogenous ghrelin or ghrelin mimetics may contribute to retard the progress of anorexia of ageing.

We indicate that iv administration of ghrelin increases food intake in all generations and that the ratio of ghrelin-induced food intake to 24 h food intake was the same among the generations. These results suggest that peripheral administration of ghrelin may prevent age-dependent decline in energy intake in animals.

The circulation level of insulin-like growth factor-1 (IGF-1) is increased by the increase in plasma GH concentration. GH and IGF-1 promote cell survival and proliferation through independent and different pathways<sup>23</sup>. The amplitude of pulsatile GH release from the anterior pituitary gland secretion is attenuated with ageing, and the attenuation of GH release induces decrease in IGF-1<sup>24,25</sup>. These age-related reductions are commonly referred to as the somatopause<sup>8,9</sup>.

The somatopause during ageing has been partially explained by the reduction in GH response to peptidyl or nonpeptidyl synthetic ghrelin mimetics, GH-releasing hexapeptide (GHRP-6) or MK-0677, and GH releasing hormone<sup>26-28</sup>. The GH responses to acute iv administration of ghrelin in elderly subjects were lower than those in young adult subjects<sup>6</sup>. In addition, expression of GHS-R messenger ribonucleic acid is reduced in the aged human hypothalamus, which is consistent with their reduced GH response to ghrelin<sup>28</sup>. Plasma ghrelin concentrations reduce in humans as they age<sup>5</sup>; therefore, lower ghrelin production in addition to reduced GHS-R levels suggest that somatopause may reflect impairment in the ghrelin signaling pathway. In contrast to humans, stomach ghrelin production and secretion are increased, and GH release in response to exogenous ghrelin is enhanced in aged rats<sup>7</sup>. Therefore, age-related decline in GH secretion may not be due to a reduction in stomach ghrelin secretion or a stimulatory action on GH release. The present study demonstrated that iv administration of ghrelin increased GH secretion in all Wistar adult rats investigated for 27 months at 15 min after administration. In addition, the levels of GH response to ghrelin were not affected with the months of age in rats.

Recent studies demonstrated that circulating ghrelin bound to the membranes of cardiomyocytes, adipocytes, and osteocytes dependently or independently of the GHS-R<sup>29</sup>. Ghrelin functions as an anti-catabolic agent in peripheral tissues, involving adipogenesis, osteogenesis, and cell

proliferation<sup>30-31</sup>. Therefore, ageing process represented by catabolic-anabolic imbalance in peripheral tissues may increase ghrelin utilization to maintain cell functions. The present study indicated the possibility of suppressing the age-related decline of GH secretion and food intake by ghrelin. Further studies will be necessary to clarify whether a chronic administration of ghrelin prevents age-related regression involved somatopause, sarcopenia, and anorexia.

These findings suggest that the aged rats maintain a high reactivity to ghrelin stimulation, and that aged rats secure storage of GH in the anterior pituitary gland.

In the present study we show that fasting rapidly induces an acute in systemic ghrelin concentrations. These changes in serum ghrelin levels during fasting are followed by similar changes in serum GH concentrations, indicating that ghrelin is the driving force of increased GH secretion during fasting.

In conclusion, our results indicate that peripheral administration of ghrelin increases GH secretion and food intake in all generations. Somatopause and anorexia of ageing are associated with declines in physical abilities. Therefore, ghrelin replacement may improve physical abilities to stimulate GH secretion and feeding in aged animals. The present study will provide novel insights into the physiological function of ghrelin in ageing process.

## **Acknowledgement**

The financial assistance of the research project unit of Dicle University, Diyarbakır, Turkey is gratefully acknowledged.

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