

THIRD VENTRICLE GHRELIN INFUSION EFFECT ON THE METABOLIC PARAMETERS UNDER DIFFERENT ENERGY LEVELS IN DIETS*

H. KHAZALI

College of Biological Sciences, Shahid Beheshti University, Tehran, I. R. Iran
E mail: hkhazali@hotmail.com

Abstract – The goal of this study was to determine whether ghrelin affects the mean plasma concentrations of metabolic parameters such as thyroxine (T4), triiodothyronine (T3), growth hormone (GH), insulin, glucagon, glucose, fatty acid and urea in the goats fed different energy content in diets. Sixteen goats were randomly divided into 4 groups. Animals in groups 1 and 2 were fed 100 % and animals in groups 3 and 4 were fed a 50 % energy content in their diet for 20 days. After 20 days, animals in groups 1 and 3 received a daily infusion of 1 ug ghrelin, while groups 2 and 4 received a daily infusion of 2 ug galanin into their third ventricle for 5 days. Blood samples were collected daily from the jugular veins before infusions on day 4 until 4 days after the last infusions of ghrelin. Samples were assayed for plasma T3, T4, GH, insulin and glucagon concentrations by double-antibody RIA. Glucose, fatty acid, and urea concentrations were also measured. Lower dietary energy intake and infusions of 1 and 2 ug ghrelin significantly ($P<0.01$) decreased the mean plasma concentrations of T3, T4, insulin, and glucose, and significantly ($P<0.01$) increased the mean plasma concentrations of GH, glucagon, fatty acid, and urea of the animals in groups 3 and 4. Different dosages of the ghrelin infusions did not change the plasma concentrations of the metabolic parameters in the animals fed a normal energy content in diets. The results of this experiment indicated that ghrelin may negatively affect the T3, T4, insulin, and glucose and increase GH, glucagon, fatty acid, and urea in the goats with a negative energy balance, but not in those with the positive energy balance.

Keywords – Ghrelin, Metabolic Hormones, Goat

1. INTRODUCTION

Ghrelin is a 28-amino-acid neuropeptide that is mostly found in the hypothalamus [1-3]. Based on its neuron distributions in the hypothalamus, ghrelin coexists with many other neurons. For example, in the hypothalamic area, ghrelin coexists with neurons secreting different neurotransmitters such as GHRH, GABA, noradrenaline, 5-hydroxytryptamine (5-HT), and NPY [4]. Therefore, ghrelin controls different physiological actions on many different glands [5-8]. One of the physiological actions is its effect on metabolism and feeding behaviors that make ghrelin an orexigenic hormone [6]. The orexigenic effect of ghrelin decreases or increases the plasma levels of insulin, glucagon, somatostatin, gastrin and increases the release of growth hormone [9-15]. Most of the above studies were conducted in human and rat as a nonruminant. Ruminants have different metabolisms from that of nonruminants [16]. For example, higher plasma glucose level, less insulin responsivity and fatty acid metabolism are some of the physiological peculiarities that make them different from ruminant [17-18]. It is assumed that the control of feeding behavior is different from that of nonruminants. There are very few reports about the orexigenic effect of ghrelin on the metabolic hormones in ruminants fed different energy contents in diet. Therefore, the first goal of this experiment was to determine whether ghrelin affects the mean concentrations of metabolic parameters in the goats fed a different energy content in diets.

*Received by the editor June 24, 2008 and in final revised form April 5, 2010

Among the many studies done on the effect of ghrelin on metabolic hormone, there are no reports about the effect of ghrelin on thyroid hormones under different energy intake. The importance of thyroid hormones in metabolism is well known. For example, thyroid hormones play an important role in the regulation of energy homeostasis via oxygen consumption and heat generation [19-20]. Changes in basal metabolic rate caused by different energy content in diet is accompanied by changes of thyroid hormones secretions. Therefore, the second goal of this study was to determine whether ghrelin alters the thyroid hormones secretion in the goats fed a different energy content in the diet.

2. MATERIALS AND METHODS

a) Experimental Design

Sixteen goats (weighing between 40 to 50 kg) were randomly divided into 4 groups. Animals in group 1 and 2 were fed 100 % energy (NE) and animals in group 3 and 4 were fed 50 % energy (LE) content in diet for 20 days. Gross energy and chemical compositions of feedstuffs consisting of dry material, crude protein, crude fiber, ether extract, total ash, NDF, ADF, calcium and phosphorous were analyzed in the Animal Science Research Institute of Karaj. Diets were formulated based on AFRC (1995) (Table 1) [21]. During the course of the experiment, daily feed was weighed based on body weight and individually given to each goat every morning. The goats had free access to fresh water. Diet 1 and 2 consisted of 100% and 50% of the maintenance energy requirements, respectively. Other requirements were balanced at maintenance level. After 20 days, all animals were prepared for surgery. Goats were anesthetized throughout the surgery for third ventricle cannulation under stereotaxic methods and jugular vein cannulations. Surgical procedures were done under general anesthesia induced by sodium pentobarbital and maintained by halothane in a closed circuit system. Each goat was kept in a single cage for a 4 day recovery period. During the recovery period, cannules were washed by PBS solution to prevent clotting. After surgery, on day 5, the goats in group 1 and 3 received 1 ug ghrelin, while goats in group 2 and 4 received 2 ug ghrelin into their third ventricles at 09.00 hour for 5 days. Body weight of animals was measured on day 1 and 20 of the experiment.

Table 1. Experimental rations and prepared energy and nutrients

Diet	100 % energy	50 % energy
Ingredients/nutrition		
Wheat straw(g/day)	10	260
Alfalfa(hay) (g/day)	50	50
Corn(grain) (g/day)	10	220
Corn gluten meal(g/day)	210	85
Bone meal (g/day)	1.34	0.47
Salt(g/day)	1.66	1.22
Magnesium oxide(g/day)	0.69	-
Vitamin and mineral supplement	3.50	3.50
Metabolizable energy (MJ/kg)	13.03	9.73
Crude protein(%)	42.00	13.72
Calcium(%)	0.52	0.24
Phosphorous(%)	0.52	0.24
Sodium(%)	0.45	0.21
Magnesium(%)	0.24	0.11
Dry material intake (g/day)	287	620
Metabolizable energy intake(MJ/kg)	3.74	6.03
Metabolizable protein intake (g/day)	56.00	55.37

b) Blood Collection

Blood samples were collected from cannules that were put into the jugular veins everyday from 4 days before the first infusion of ghrelin until 4 days after the last ghrelin infusion. Blood samples were kept at 4°C until centrifugation. A saturated sodium citrate solution (40 ul sodium citrate solution/ml blood) was added to the samples before centrifugation to prevent clotting of plasma during storage. Plasma was stored at -20 ° C until assayed for T3, T4, insulin, GH, glucagon, glucose, fatty acid, and urea.

c) Hormone Assays

Plasma T3, T4, insulin, GH, and glucagon were measured by a homologous double-antibody radioimmunoassay (RIA). For GH assay, ovine GH (TYN-OG), and antisera against GH were provided by Tabeshyarnoor Co. (Industrial City of Bu-Ali, Hamadan, Iran). Ovine GH (TYN-OG) was used for iodination. A seven-point standard curve ranging from 0.04 to 10 ng GH was used. An average assay binding of 40% was achieved using an initial 1:20000 dilution of GH antiserum for GH assays. The inter- and intra-assay variations were 6% and 9%. respectively. For the insulin assay, ovine insulin (TYN-OI), and antibody against insulin were provided by Tabeshyarnoor Co. (Industrial City of Bu-Ali, Hamadan, Iran). Ovine insulin (TYN-OI) was used for iodination. A seven-point standard curve ranging from 0.02 to 10 ng insulin was used. An average assay binding of 30% was achieved using an initial 1:5000 dilution of insulin antiserum for insulin assays. The inter- and intra-assay variations were 8% and 5%. respectively. For the glucagon assay, human glucagon (TYN-HC) and antibody against glucagon were provided by Tabeshyarnoor Co. (Industrial City of Bu-Ali, Hamadan, Iran). Human glucagon (TYN-HC) was used for iodination. A seven-point standard curve ranging from 0.02 to 10 ng insulin was used. An average assay binding of 35% was achieved using an initial 1:10000 dilution of glucagon antiserum for glucagon assays. The inter- and intra-assay variations were 7% and 6% respectively. For the T3 assay, T2 was purchased from Sigma Chemical Company and T3 antisera was purchased from Chemicon Co. (Temecula, Ca). T2 was used for iodination. A six-point standard curve ranging from 0.32 to 5.2 ng T3/ml was used. An average assay binding of 70% was achieved using an initial 1:5000 dilution of T3 antiserum for T3 assays. The inter- and intra-assay variations were 7% and 7% respectively. For the T4 assay, T3 was purchased from Sigma Chemical Company and T4 antisera was purchased from Chemicon Co. (Temecula, Ca). T3 was used for iodination. A six-point standard curve ranging from 2.2 to 25 ng T4/ml was used. An average assay binding of 60% was achieved using an initial 1:5000 dilution of T4 antiserum for T4 assays. The inter- and intra-assay variations were 7% and 5%, respectively. For the glucose assay, ELISA kits were purchased from Sigma Chemical Company. A six-point standard curve ranging from 20 to 250 mg glucose/dl was used. An average assay binding of 35% was achieved. The inter- and intra-assay variations were 4% and 6%. respectively. For fatty acid assay, ELISA kits were purchased from Sigma Chemical Company. A six-point standard curve ranging from 10 to 150 mg fatty acid/dl was used. An average assay binding of 45 % was achieved. The inter- and intra-assay variation were 5% and 8%, respectively. For the urea assay, ELISA kits were purchased from Sigma Chemical Company. A six-point standard curve ranging from 10 to 150 mg urea/dl was used. An average assay binding of 32 % was achieved. The inter- and intra-assay variations were 4% and 6% respectively.

d) Statistical Analysis

All analyses were conducted using General Linear Model procedures SAS, 1996. Data were analyzed using an analysis of variance for a repeated measure design. Mean comparisons were evaluated by least significant difference with single degree of freedom.

3. RESULTS

a) T3 & T4

Infusions of 1 and 2 ug ghrelin into the third ventricle did not change the mean plasma concentrations of T3 and T4 of the animals in groups 1 and 2 that were fed NE. Mean plasma T3 levels of the animals in group 1 and 2 were about 2.0, 2.1, 2.2 and 2.1, 1.9, 2.1 ng/ml before, during, and after infusion of ghrelin respectively (Fig. 1). Also, Mean plasma concentrations of T4 of the NE animals in group 1 and 2 were about 41, 40, 40 and 39, 42, 41 ng/ml before, during, and after infusion of ghrelin respectively (Fig. 2). Plasma T3 and T4 levels of LE fed animals in groups 3 and 4 were significantly ($P<0.01$) lower than that of the NE fed animals (Fig. 1 and 2). Ghrelin infusions significantly ($P<0.01$) decreased plasma T3 and T4 levels in the LE fed animals (Fig. 1 and 2).

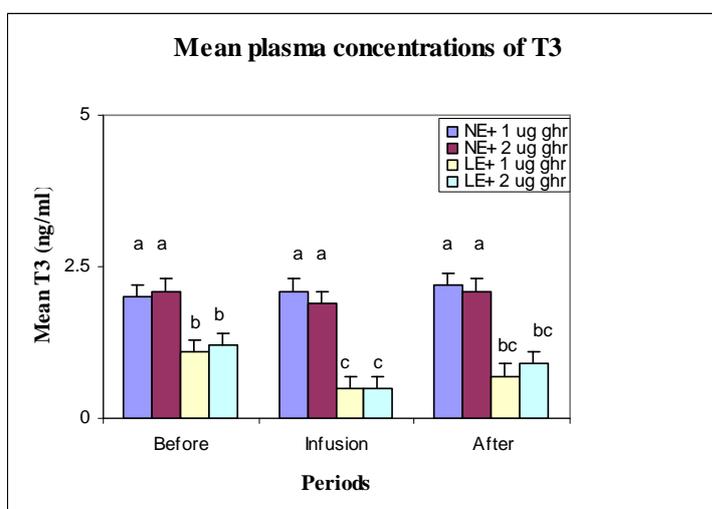


Fig. 1. Mean plasma concentrations of T3 of the animals in the different groups of 1 (NE and 1 ug ghrelin), 2 (NE and 2 ug ghrelin), 3 (LE and 1 ug ghrelin) and 4 (LE and 1 ug ghrelin) and before, during and after infusions of ghrelin. (NE = normal energy; LE = low energy). a, b, c,; Treatments with different letters are different at $p<0.01$

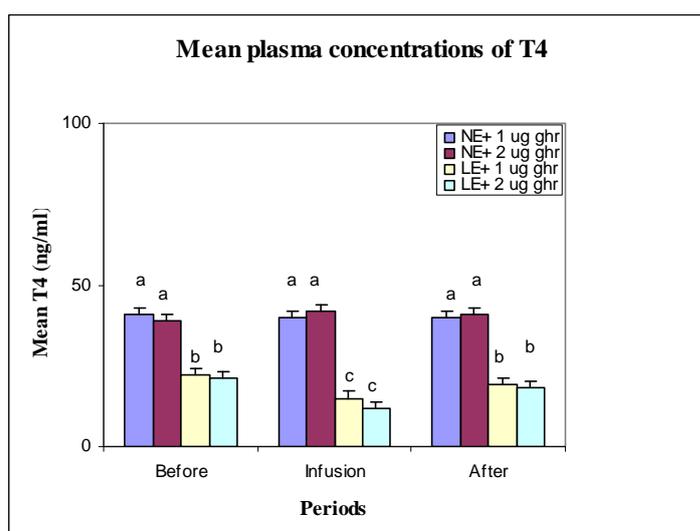


Fig. 2. Mean plasma concentrations of T4 of the animals in the different groups of 1 (NE and 1 ug ghrelin), 2 (NE and 2 ug ghrelin), 3 (LE and 1 ug ghrelin) and 4 (LE and 1 ug ghrelin) and before, during and after infusions of ghrelin. (NE = normal energy; LE = low energy). a, b, c,; Treatments with different letters are different at $p<0.01$

b) GH

Low energy content in diet increased the GH plasma levels of the animals in group 3 (0.7 ng/ml) and 4 (0.9 ng/ml) in comparison with plasma GH levels of those animals fed NE. Further, to the effect of lower energy dietary intake, infusions of 1 ug ghrelin significantly ($P<0.01$) increased the mean plasma GH levels in the animals of group 3 (from 0.7 to 2), followed by a declining GH level from 2 to 1.2 after infusion of ghrelin. Also, the mean GH level of the animals of group 4 significantly ($P<0.01$) increased from 0.9 to 3 by infusion of 2 ug ghrelin (Fig. 3). Infusions of 1 ug ghrelin did not change the mean plasma concentrations of the GH in the animals of group 1 that were fed 100% energy content in diets for 20 days. Mean plasma concentrations of the GH of group 1 were about 0.5, 0.6, and 0.6, ng/ml before, during and after infusion of ghrelin, respectively (Fig. 3). Two ug ghrelin did not change the mean plasma concentrations of the GH in the animals of group 2 that were fed NE. Mean plasma concentrations of the GH of group 2 were about 0.6, 0.6 and 0.6 ng/ml before, during, and after infusion of ghrelin, respectively (Fig. 3).

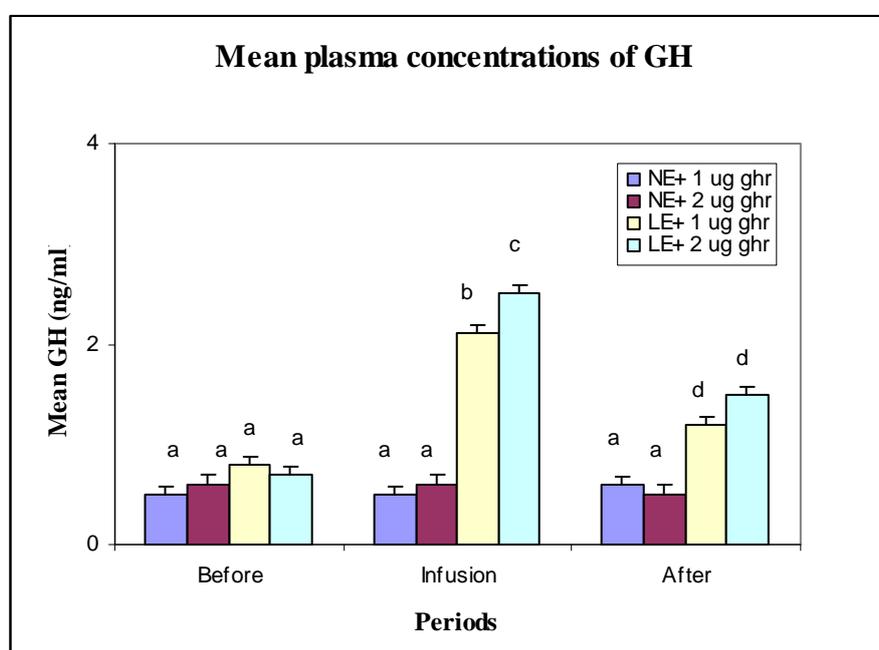


Fig. 3. Mean plasma concentrations of GH of the animals in the different groups of 1 (NE and 1 ug ghrelin), 2 (NE and 2 ug ghrelin), 3 (LE and 1 ug ghrelin) and 4 (LE and 1 ug ghrelin) and before, during and after infusions of ghrelin. (NE = normal energy; LE = low energy). a, b, c, d: Treatments with different letters are different at $p<0.01$

c) Insulin

Infusions of 1 and 2 ug ghrelin did not change the mean plasma concentrations of the insulin in the animals of groups 1 and 2 that were fed NE. Mean plasma concentrations of the insulin of the animals in groups 1 and 2 were about 45, 45, 44 and 42, 46, 44 ng/ml before, during and after infusion of ghrelin, respectively (Fig. 4). Mean plasma concentrations of insulin of the animals in groups 3 (30 ng/ml) and 4 (29 ng/ml) fed LE were significantly ($P<0.01$) lower than the plasma insulin levels of those animals in groups 1 (45 ng/ml) and 2 (42 ng/ml) fed NE (Fig. 4). Infusions of 1 ug ghrelin significantly ($P<0.01$) decreased the mean of the plasma levels of insulin in the animals of group 3 from 30 to 15, followed by rising plasma levels of insulin from 15 to 26 after the infusion of ghrelin. Also, the mean of the plasma levels of insulin of the animals in group 4 significantly ($P<0.01$) decreased from 29 to 13 by infusion of 2 ug ghrelin (Fig. 4).

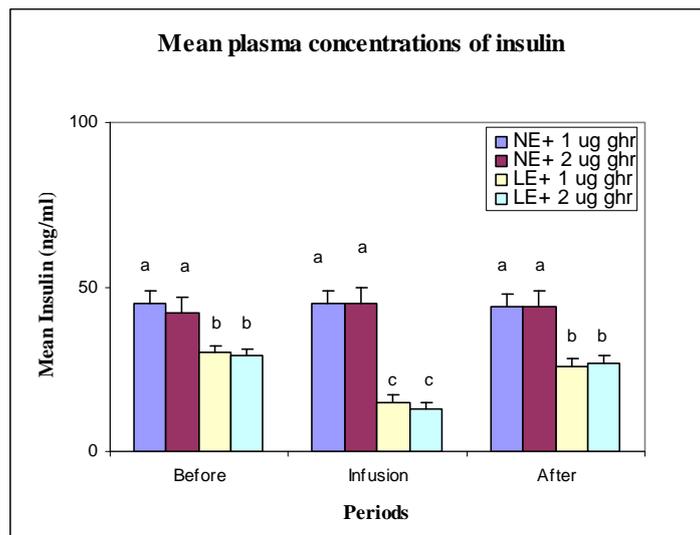


Fig. 4. Mean plasma concentrations of insulin of the animals in the different groups of 1 (NE and 1 ug ghrelin), 2 (NE and 2 ug ghrelin), 3 (LE and 1 ug ghrelin) and 4 (LE and 1 ug ghrelin) and before, during and after infusions of ghrelin. (NE = normal energy; LE = low energy). a, b, c,; Treatments with different letters are different at $p < 0.01$

d) Glucagon

Ghrelin infusions did not change the mean plasma concentrations of the glucagon in the animals of group 1 and 2 that were fed NE. Mean plasma levels of the glucagon of the animals in group 1 and 2 were about 3, 3.2, 3 and 3.1, 3.1, 3 ng/ml before, during, and after the infusion of ghrelin, respectively (Fig. 5). Mean plasma concentrations of glucagon of the animals in groups 3 (8.2 ng/ml) and 4 (8 ng/ml) were significantly $P < 0.01$ higher than that of the animals fed NE (Fig. 5). Infusions of 1 ug ghrelin significantly ($P < 0.01$) increased the mean of the plasma levels of glucagon in the animals of group 3 from 2.9 to 5, followed by decreasing glucagon plasma levels from 5 to 3.6 after infusion of ghrelin. Also, the mean of the plasma levels of glucagon of the animals in group 4 significantly ($P < 0.01$) increased from 3 to 126 by infusion of 2 ug ghrelin (Fig. 5).

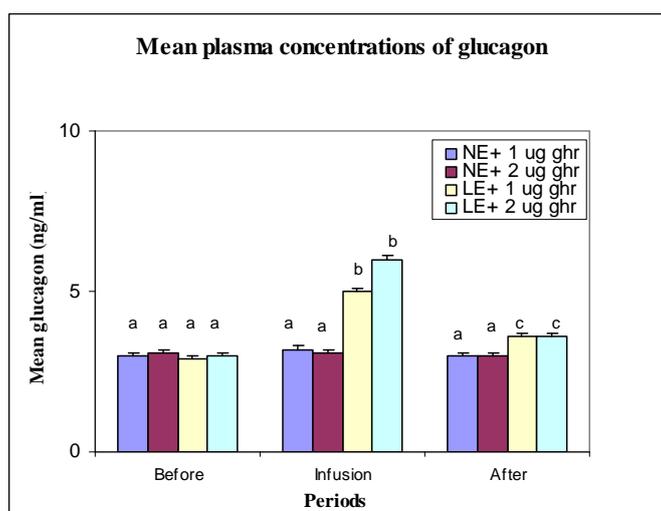


Fig. 5. Mean plasma concentrations of glucagon of the animals in the different groups of 1 (NE and 1 ug ghrelin), 2 (NE and 2 ug ghrelin), 3 (LE and 1 ug ghrelin) and 4 (LE and 1 ug ghrelin) and before, during and after infusions of ghrelin. (NE = normal energy; LE = low energy). a, b, c,; Treatments with different letters are different at $p < 0.01$

e) Glucose

Ghrelin did not change the mean plasma concentrations of the glucose of the animals in group 1 and 2 that were fed NE. Plasma concentrations of the glucose of groups 1 and 2 were about 50, 48, 50 and 52, 50, 50 mg/dl before, during and after the infusion of ghrelin respectively (Fig. 6). Plasma glucose levels of the LE fed animals in groups 3 (32 mg/dl) and 4 (28mg/dl) were significantly $P<0.01$ lower than the mean plasma concentrations of glucose of those animals in group 1 (50 mg/dl) and 2 (52 mg/dl) fed NE (Fig. 4). Infusions of 2, but not 1 ug ghrelin significantly ($P<0.01$) decreased the glucose levels among those animals of group 3 fed LE (Fig. 6).

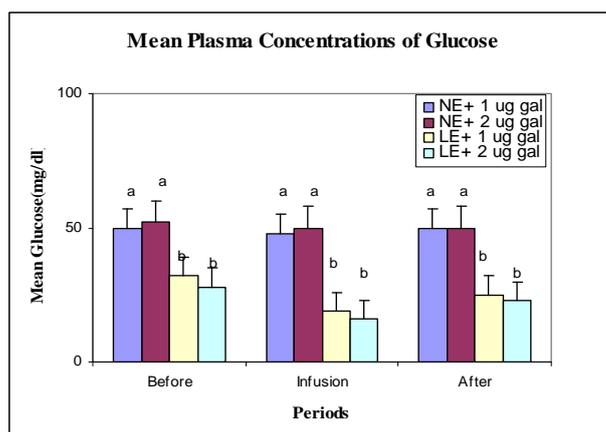


Fig. 6. Mean plasma concentrations of glucose of the animals in the different groups of 1 (NE and 1 ug ghrelin), 2 (NE and 2 ug ghrelin), 3 (LE and 1 ug ghrelin) and 4 (LE and 1 ug ghrelin) and before, during and after infusions of ghrelin. (NE = normal energy; LE = low energy)

f) Fatty acid

Infusions of 1 and 2 ug ghrelin did not change the mean plasma concentrations of the fatty acid of the animals in groups 1 and 2 that were fed LE. Mean plasma concentrations of fatty acid of the animals in group 3 (70 mg/dl) and 4 (68 mg/dl) fed LE were significantly $P<0.01$ higher than the mean plasma concentrations of fatty acid of those animals in groups 1 (40 mg/dl) and 2 (2 mg/dl) fed NE (Fig. 7). Infusions of 1 and 2 ug ghrelin significantly ($P<0.01$) increased the fatty acid levels among those animals fed LE (Fig. 7).

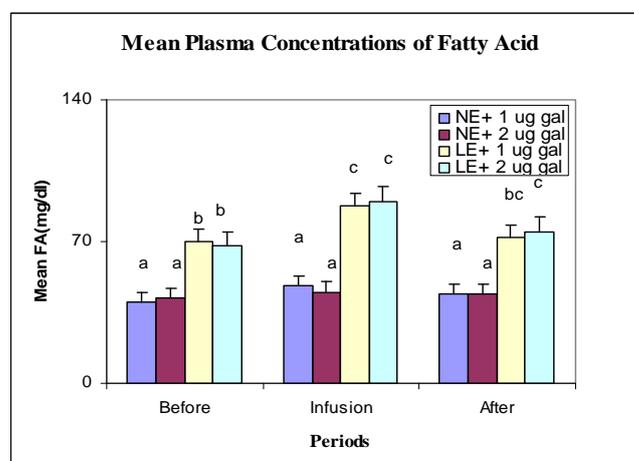


Fig. 7. Mean plasma concentrations of fatty acid of the animals in the different groups of 1 (NE and 1 ug ghrelin), 2 (NE and 2 ug ghrelin), 3 (LE and 1 ug ghrelin) and 4 (LE and 1 ug ghrelin) and before, during and after infusions of ghrelin. (NE = normal energy; LE = low energy)

g) Urea

Ghrelin did not change the mean plasma concentrations of the urea of the animals in all groups. Mean plasma concentrations of urea of the animals in group 3 and 4 fed LE were significantly $P < 0.01$ higher than the mean plasma concentrations of urea of those NE fed animals in groups 1 and 2 (Fig. 8).

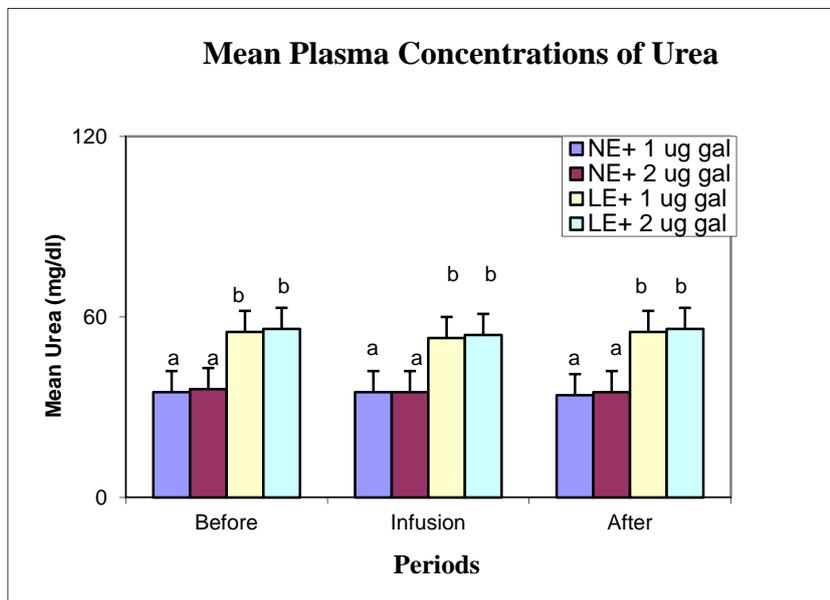


Fig. 8. Mean plasma concentrations of fatty acid of the animals in the different groups of 1 (NE and 1 ug ghrelin), 2 (NE and 2 ug ghrelin), 3 (LE and 1 ug ghrelin) and 4 (LE and 1 ug ghrelin) and before, during and after infusions of ghrelin. (NE = normal energy; LE = low energy)

h) Body weight

Low energy dietary intake for twenty days significantly ($P < 0.01$) decreased the mean body weight of the animals from 47 Kg to 35 Kg.

4. DISCUSSIONS

a) T3 & T4

Our study is the first to report the effect of ghrelin into the third ventricle on thyroid hormones in the ruminants. The results of the effect of ghrelin on mean plasma T3 and T4 levels of the goats fed LE is similar to the previous finding of [22] that reported the peripheral injection of ghrelin increased the plasma level of thyroid stimulating hormones (TSH) in nonruminants such as rat and human, but there was no data on the plasma level of T4 in that study. It is well established that increase of plasma TSH level is accompanied by a decrease of plasma T3 and T4 in an NE fed human [23-24]. Our results indicate that the NE fed goats as a ruminant are not sensitive to ghrelin as that of in nonruminant. Only when the ruminant animals are in a long-term fasting period, are they sensitive to the effect of ghrelin on plasma T3 and T4 levels. The hypothalamus pituitary thyroid (HPT) axis plays an important role in the regulation of energy homeostasis [19-20] via the effects of thyroid hormone to increase oxygen consumption and heat generation [19-20]. Thus inhibition of the HPT axis during fasting would appear to be an important adaptive mechanism to conserve energy stores [24-27]. The state of central hypothyroidism induced by fasting is orchestrated by changes of circulating levels of ghrelin which rise with fasting, and is restored to normal levels by refeeding ([24]. Thus, if ghrelin is administered exogenously to fasting animals, a higher decrease in the circulating levels of thyroid hormones can be observed [27].

b) GH

Our study is the first to report the effect of ghrelin into the third ventricle on GH in the ruminants fed LE. Our result about the effect of ghrelin on GH in the goats fed LE is similar to other studies, indicating that ghrelin is a hypophysiotropic hormone that elicits GH secretion [1-12]; and enhances the GH response to GHRH in NE fed nonruminant [12]. Furthermore, conflicting evidence exists in vitro about the direct effect of ghrelin on GH, with an inhibitory influence on GH secretion observed in rat [28] and a stimulatory one observed in rat [15]. Our finding about the effect of ghrelin on GH in the NE goats fed normal energy content in diet is different to the results of other studies that showed injections of ghrelin increase GH in rat and human [10-12]. This may be due to the normal plasma level of insulin and the inhibitory effect of normal concentrations of plasma glucose [29] in the goats fed NE on the GH secretions.

c) Insulin

Our data are different from the studies in nonruminants that indicated ghrelin may slightly decrease the plasma level of insulin [9, 13, 19]. In those studies, the effect of ghrelin was not on the long-term fasting subject. Our result is similar to the previous finding which reported that intravenous administration of ghrelin into fasted conscious dogs decreased plasma insulin levels [14]. The mechanism of inhibitory effect of ghrelin on insulin release most likely occurs through the inhibition of cyclic AMP [14].

d) Glucagon

Our results are different from the previous studies that reported ghrelin has no effect on glucagon level in rats [9]. This may be due to the plasma glucose concentrations in the fasted dog [30]. Also, some studies have indicated that ghrelin inhibited glucagon secretion in rat. All of the above studies were conducted to determine the effect of ghrelin on glucagon via in vitro or peripheral injections. In our study, decreased plasma levels of glucose were caused by a lower energy intake [31] and ghrelin infusions may be the reason for the increased level of glucagon and decreased level of insulin.

e) Glucose

It is well established that low energy content in diet decreases mean plasma concentrations of glucose in most mammals [31] as we observed in the goats fed LE. Also, there is a negative correlation between the ghrelin infusion and the mean plasma level of glucose in the fasted ruminants, whereas in another study it is reported that there is a positive correlation between these two parameters in nonfasted nonruminant [13].

f) Fatty acid

Infusions of 1 and 2 ug ghrelin significantly ($P < 0.01$) increased the fatty acid levels among those animals fed LE (Fig. 7). This may be directly due to the effect of a negative energy balance, which caused severe weight loss along with the lipolysis of adipose tissue [16].

g) Urea

Our result regarding the effect of ghrelin on urea in the LE fed goats is similar to another study done in the nonruminant that indicated low energy diet increased plasma urea level [32]. When energy intake is inadequate, proteins can serve as an energy source, and the plasma urea level is considered as an endproduct of protein catabolism [33].

h) Body weight

Low energy dietary intake for twenty days significantly ($P < 0.01$) decreased the mean body weight of the animals. This was similar to our previous finding which reported that negative energy balance decreases body weight in ewes [34].

5. IMPLICATION

The results of our studies indicated that the third ventricle infusion of ghrelin may increase the plasma levels of GH, glucagon, fatty acid and urea, and decrease the plasma levels of T3, T4, insulin and glucose in the goats with severe body loss. The effect of ghrelin infusion into the third ventricle on metabolic parameters is different from the effect of ghrelin injections in peripheral circulation. Also, different metabolic systems of ruminant and nonruminant animals offer different changes of metabolic status under ghrelin effect.

REFERENCES

1. Takaya, K., Ariyasu, H., Kanamoto, N., Iwakura, H., Yoshimoto, A., Harada, M., Mor, K., Komatsu, T., Usui, A., Shimatsu, Y., Ogawa, K., Hosoda, T., Akamizu, M., Kojima, K., Kangawa, K. & Nakao, K. (2000). Ghrelin strongly stimulates growth hormone release in humans. *J. Clin Endocrinol Metab*, 85, 4908-4911.
2. Kamegai, J., Tamura, T., Shimizu, S., Ishii, H., Sugihara, H. & Wakabayashi, I. (2001). Ghrelin increase hypothalamic neuropeptide Y and Agouti-related protein, mRNA levels and body weight in rats. *Diabetes*, 50, 2438-2443.
3. Holst, B., Holliday, A., Bach, C. E., Elling, N. D. & Cox, H. M. (2004). Ghrelin receptor family. *The J. Biological Chemistry*, 51, 53809-53817.
4. Kojima, M. & Kangawa, K. (2005). Ghrelin structure and function. *Physiological Reviews*, 85, 492-522.
5. Ghigo, E., Arvat, R., Giordano, F., Broglio, L., Gianotti, M., Maccario, G., Bisi, A., Graziani, M., Papotti, G., Muccioli, R., Deghenghi, F. & Camanni, F. (2001). Biologic activities of growth hormone secretagogues in humans. *Endocrine*, 14, 87-93.
6. Gi, X., Reed, J., Englander, E. W., Chandrashekar, V., Bartke, A. & Greelry, G. H. (2003). Evidence that growth hormone exerts a feedback effect on stomach ghrelin production and secretion. *Exp Biomed.*, 288, 1028-1032.
7. Barreiro, M. L. & Tena-sempere, M. (2004). Ghrelin and reproduction: A novel signal linking energy status and fertility. *Molecular and Cellular Endocrinology*, 226, 1-9.
8. Fernandez-fernandez, R., Castellano, E., Aguilar, E. & Andpinilla, L. (2005). Effect of ghrelin upon gonadotropin-releasing hormone and gonadotropin secretion in adult female rats in vivo and in vitro studies. *Neuroendocrinology*, 82, 245-255.
9. Adeghate, E. & Ponery, A. S. (2002). Ghrelin stimulates insulin secretion from the pancreas of normal and diabetic rats. *J. Neuroendocrinol*, 14, 555-560.
10. Arosia, M., Cappiello, S., Manenti, C., Gebbia, P., Epaminonda, C., Penati, P., Beck- Peccoz, V. & Peracchi, M. (2003). Stimulatory effects of ghrelin on circulating somatostatin and pancreatic polypeptide. *J. Clin Endocrinol Metab*, 88, 701-704.
11. Arvat, E., Vito, F., Broglio, M., Papotti, G., Muccioli, C., Dieguez, F. F., Casanueva, R., Deghenghi, F., Camanni, L. & Ghigo, E. (2000). Preliminary evidence that ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. *J. Endocrinol Invest*, 23, 493-495.
12. Arvat, E. M., Maccario, L., Di Vito, L. F., Broglio, A., Benso, C., Gottero, M., Papotti, G., Muccioli, C., Dieguez, F. F., Casanueva, R., Deghenghi, F., Camanni, H. & Ghigo, E. (2001). Endocrine activities of ghrelin, a

- natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. *J. Clin Endocrinol Metab*, 86, 1169-1174.
13. Broglio, F., Gottero, A., Benso, F., Prodam, C., Gauna, C. M. & Maccaric, M. (2003). Effect of ghrelin on the insulin and glycemic receptor to glucose. *The Journal of Clinical Endocrinology and Metabolism*, 88, 4268-4272.
 14. Egido, E. M., Rodriguez- Gallardo, R. A., Silvestr, J. & Marco, J. (2002). Inhibitory effect of ghrelin on insulin and pancreatic somatostatin secretion. *Eur J. Endocrinol*, 146, 241-244.
 15. Hataya, Y., Akamizu, K., Takaya, N., Kanamoto, H., Ariyasu, M., Saijo, K., Moriyama, A., Shimatsu, M., Kojima, K., Kangawa, T. & Nakao, K. (2001). A low dose of ghrelin stimulates growth hormone (GH) release synergistically with GH-releasing hormone in humans. *J Clin Endocrinol Metab*, 86, 45-52.
 16. Harrison, F. A. & Leat, W. M. (1975). Digestion and absorption of lipid in non-ruminant and ruminant animals: a comparison. *Proc. Nutr. Soc.* 34, 203-21030.
 17. Elmahdi, B., Sallmann, H., Fuhrmann, H., von Engelhardt, W. & Kaske, M. (1997). Comparative aspects of glucose tolerance in camels, sheep, and ponies. *Comp Biochem Physiol*, 118, 147-151.
 18. Kaske, M., Elmahdi, B., von Engelhardt, W. & Sallmann, H. (2001). Insulin responsiveness of sheep, ponies, miniature pigs and camels: results of hyperinsulinemic clamps using porcine insulin. *J Comp Physiol*, 171, 549-56.
 19. Data, Y., Nakazato, M., Hashiguchi, S., Dezaki, K. & Mondal, M. S. (2003). ghrelin present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. *Diabetes*, 51, 124-129.
 20. Lanni, A., Moreno, A., Lombardi, P., de Lange, M. & Goglia, F. (2001). Control of energy metabolism by iodothyronines. *J. Endocrinol. Invest*, 24, 897-913.
 21. Agricultural and Food Research Council. (1995). Energy and Protein Requirements of Ruminants. An advisory manual prepared by the AFRC technical committee on responses to nutrients. 2nd ed. CAB International, Wallingford, UK.
 22. Dalkjar, A. & Hansen, R. (2003). Hyperthyroidism Is Associated with Suppressed Circulating Ghrelin Levels. *The Journal of Clinical Endocrinology & Metabolism*, 88, 853-857.
 23. Felig, P. & Frohman, L. A. (2001) *The thyroid: physiology, thyrotoxicosis, hypothyroidism, and painful thyroid*. Endocrinol. and metabol. 4th ed. New York, *McGraw-Hill*, 261-329.
 24. Reasner, C. A. & Ralbert, R. L. (2002). Thyroid disorders. Pharmacotherapy and pathophysiologic approach. 5th ed. New York, *McGraw-Hill*, 1359-1378.
 25. van Haasteren, G. A., Linkels, E., Klootwijk, W., van Toor, H., Rondeel, J. M., Themmen, A. P., de Jong, F. H., Valentijn, K., Vaudry, K., Bauer, T. J., Visser, M. & de Greef, W. J. (1995). Starvation-induced changes in the hypothalamic content of prothyrotrophin-releasing hormone (proTRH) mRNA and the hypothalamic release of proTRH-derived peptides: role of the adrenal gland. *J. Endocrinol*, 145, 143-153.
 26. Le'gra'di, G., Emerson, R. S., Ahima, J. S., Flier, G. H. & Lechan, L. M. (1997). Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. *Endocrinol*, 138, 2569-2576.
 27. Le'gra'di, G., Emerson, R. S., Ahima, W. M., Rand, J. S. & Lechan, R. M. (1998). Arcuate nucleus ablation prevents fasting-induced suppression of ProTRH mRNA in the hypothalamic paraventricular nucleus. *Neuroendocrinol*, 68, 89-9.
 28. Kamegai, J. & Tamura, H. (2004). The Role of Pituitary Ghrelin in Growth Hormone (GH) Secretion: GH-Releasing Hormone-Dependent Regulation of Pituitary Ghrelin Gene Expression and Peptide Content. *Endocrinology*, 145, 3731-3738.
 29. Holl, R. W., Bucher, W., Sorgo, E., Heinze, J., Homoki, C. H. & Debatin, K. L. (1999). Suppression of growth hormone by oral glucose in the evaluation of tall stature. *Hormone Research*, 51, 20-24I.

30. Cherrington, A., Lacy, W. & Cchiasson, J. (1978). Effect of glucagon on glucose production during insulin deficiency in the dog. *Journal of Clinical Investigation*, 62, 664-677.
31. Marsoobian, V., Grosvenor, M., Jacob, M. & Ipp, E. (1995). Very-low-energy diets alter the counterregulatory response to falling plasma glucose concentrations. *American J. Clin. Nut.*, 61, 373-378.
32. Khazali, H. (1992). Postweaning anestrus in the sows fed different energy content in diet. Ph. D. thesis. University of Illinois, Champaign-Urbana.
33. Reese, D. E., Moser, E. R., Peo, J., Lewis, D. R., Zimmerman, J. E., Kinder, B. D. & Stroup, W. W. (1982). Influence of energy intake during lactation on the interval from weaning to first estrus in sows. *J. Anim. Sci.*, 55, 595-598.
34. Towhidi, A., Khazali, H. & Zhandi, M. (2007). Leptin is a metabolic signal for GnRH-LH/FSH axis in feed-restricted ewes. *Asian-Aust. J. Anim. Sci.*, 20, 1039-1048.