Effect of Ghrelin Injection on Blood and Body Composition in Rats

A.S. Leaflet R1840

Michelle Bohan, graduate research assistant
Travis Knight, assistant scientist III of animal science
Aimee Wertz, postdoc of animal science
Allen Trenkle, distinguished professor of animal science
Donald Beitz, distinguished professor of animal science and biochemistry

Summary

Ghrelin has been reported to cause hyperglycemia in humans and adiposity in rodents. The objective of trial one was to test the effects of ghrelin on blood and body composition in rats. The objective of trial two was to evaluate the effect of two doses of ghrelin on blood and body composition. Trial One: Adult male Sprague Dawley rats were administered 1 μg/rat ghrelin in 0.15 M NaCl or vehicle (0.15 M NaCl) every morning for 30 days. The terminal blood sample was analyzed for glucose, urea nitrogen, and nonesterified fatty acids concentrations. The carcasses were analyzed for total lipid and nitrogen content. Blood urea nitrogen, nonesterified fatty acids, carcass total lipid, and carcass total nitrogen concentrations were similar for the control and ghrelin groups. However, blood glucose concentration tended to be higher in the ghrelin group than in the control. Ghrelin administered at 2.4 μg/kg did not cause adiposity in rats but did tend to cause hyperglycemia. Trial Two: Adult male Sprague Dawley rats were administered 1 μg/rat ghrelin, 20 μg/rat ghrelin, or vehicle every morning for 30 days. The terminal blood sample was analyzed for glucose and cholesterol concentrations. The carcasses were analyzed for total lipid and nitrogen content. Carcass total nitrogen concentrations were similar for the control and ghrelin groups. Carcass percent lipid was higher in the 1 μg/rat ghrelin group ($P = 0.04$). Feed intake among all three groups was the same. Blood glucose concentration, however, tended to be higher in the ghrelin groups than in the control. Blood cholesterol concentration was lower in the ghrelin treated animals. In the second trial, ghrelin injection of 1 μg/rat did cause an increase in adiposity whereas 20 μg/rat did not cause an increase in adiposity. Both ghrelin treatments tended to cause hyperglycemia in rats. In summary, ghrelin increased blood glucose concentration without changing body composition.

Introduction

Body composition is important to beef cattle producers. The hormone ghrelin is involved in regulating feed intake and body composition. Ghrelin is a newly discovered hormone that acts upon the growth hormone secretagogue receptor (GSH-R). The GSH-R was discovered in the 1970s. There are many synthetic compounds that act upon this receptor to change the animal's body composition. Since the discovery of the GSH-R, the search for an endogenous ligand has been conducted. In 1999, ghrelin was discovered (Kojima *et al.* 1999) to be an endogenous ligand of this receptor.

Ghrelin is composed of 28 acids with an octanoic acid attached at Ser 3. The octanoyl moiety is required for activity and gives ghrelin its specificity for the growth hormone secretagogue receptor (GHSR). Ghrelin is synthesized primarily in the fundus region of the stomach, although other tissues produce ghrelin in small amounts.

Since the discovery and isolation of ghrelin, many studies have been performed to determine the biochemical and physiological functions of ghrelin in the body. Ghrelin stimulates growth hormone release independent of the growth hormone releasing hormone (Kojima *et al.* 1999). Additionally, leptin activity is controlled by ghrelin. Ghrelin is an antagonist of leptin by acting upon the neuropeptide Y/Y1 receptor pathway (Shintani *et al.* 2001). Leptin causes satiety, whereas ghrelin stimulates nutrient intake (Nakazato *et al.* 2001). Leptin and ghrelin regulate the action of each other. Ghrelin has been reported to increase accretion of adipose tissue in rats. After ghrelin injection, hyperglycemia and decrease in insulin concentration was observed in humans.

The overall objective of our study is to investigate the effect of ghrelin on hormones involved in the metabolic pathways related to physiological changes. The objective of the first trial was to determine the effect ghrelin on blood and body composition. The main focus of second study was to evaluate the effect of two doses of ghrelin on blood and body composition. We hypothesize that ghrelin will cause hyperglycemia, insulinemia, and adiposity in the 20 μg/rat ghrelin group and slight hyperglycemia and insulinemia in the 1 μg/rat ghrelin group with no adiposity, with no change in the control group.

Materials and Methods

Trial One: Effect of ghrelin on blood and body composition.

Ten adult male Sprague Dawley rats (age 3 months) were assigned randomly to control (0.15M sodium chloride) or ghrelin (1 μg/rat) treatments. Treatments were administered daily by subcutaneous injection. Feed intake was measured daily for 2 weeks. For the remainder of the

trial, rats were fed the average daily intake plus 10%. Blood was collected from the saphenous vein on days 1, 7, 14, 21, and 28. On each of those days, blood was collected before injection, and at 5, 10, 15, and 20 minutes after injection. Serum samples were analyzed for glucose (Sigma) and insulin (Linco Research) concentrations. Rats were euthanized using CO_2 anesthesia. Terminal plasma samples were collected by using cardiac puncture. Carcass (body without head, skin, tail, feet, entrails, gastrocnemius muscle, and femurs), liver, femurs, and gastrocnemius muscle were harvested. Plasma samples were analyzed for glucose (Sigma), nonesterified fatty acids (NEFA, Wako Chemicals), and blood urea nitrogen (BUN, Sigma) concentrations. Gastrocnemius muscle was weighed. Carcass protein was determined by micro Kjeldahl assay. Carcass and liver lipids were measured using modified Folch wet tissue lipid extraction. Triacylglycerol content of liver was quantified colorimetrically (Pointe Scientific). Bone density was measured by using Archimedes' principle.

Trial Two: Effect of two doses of ghrelin on blood and body composition.

Sixteen adult male Sprague Dawley rats (age 3 months) were assigned randomly to one of three treatments: control (0.15M sodium chloride), 1 μg/rat ghrelin, or 20 μg/rat ghrelin. Treatments were administered daily by subcutaneous injection. Rats were fed standard rat chow *ad libitum*. Blood was collected from the saphenous vein on days 1 and 22 of the trial. On each of those days, blood was collected before injection, and at 5, 10, 15, and 20 minutes after injection. Serum samples were analyzed for glucose (Sigma) and insulin (Linco Research) concentrations. Rats were euthanized using CO_2 anesthesia. Terminal plasma sample was collected using cardiac puncture. Carcass (body without head, skin, tail, feet, entrails, gastrocnemius muscle, and femurs), femurs, and gastrocnemius muscle were harvested and stored at -20°C. Liver and adipose tissue were collected, frozen in liquid nitrogen and stored at -80°C for future analysis. Plasma samples were analyzed for glucose and cholesterol concentrations (Sigma). Carcass protein was determined by micro Kjeldahl assay. Carcass and liver lipids were measured by using modified Folch wet tissue lipid extraction.

Results and Discussion

Trial One

Femur density tended to be higher in the ghrelin group (3.68 g/cm^3 ± 0.17) than in control group (3.3 g/cm^3 ± 0.17, P = 0.17). Because growth hormone reverses loss of bone marrow (Tseng, 1998), the gain in bone density and weight could be effects of increased growth hormone release by ghrelin.

Table One: Effect of ghrelin injection on blood metabolites.

Metabolite	Control	Ghrelin	P-value
Glucose (mg/dl)	149.72 ± 4.43	172.32 ± 14.15	0.190
NEFA (μeq/l)	214.58 ± 12.7	216.03 ± 27.4	0.962
Urea Nitrogen (mg/dl)	9.69 ± 0.94	9.71 ± 0.62	0.988

A = mean ± SEM
Significant at P < 0.1

Table Two. Effect of ghrelin injection on carcass measurements.

Carcass Measurement	Control	Ghrelin	P-value
Total Lipid (% wet weight)	7.70 ± 0.55[A]	6.94 ± 0.34	0.917
Total Protein (% dry weight)	67.1 ± 0.96	67.93 ± 1.6	0.698

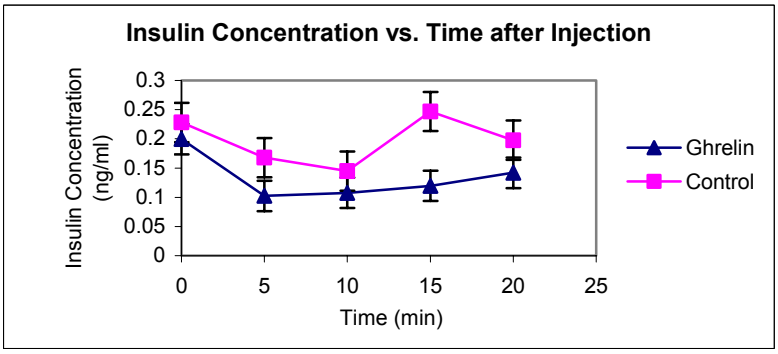
A = mean ± SEM
Significant at P < 0.1

Table Three. Liver Composition.

	Control	Ghrelin	P value
Liver Lipid (% wet weight)	4.25 ± 0.24[A]	3.80 ± 0.19	0.176
Liver Triacylglycerols (mg/dl)	30.88 ± 1.75	26.59 ± 1.56	0.110

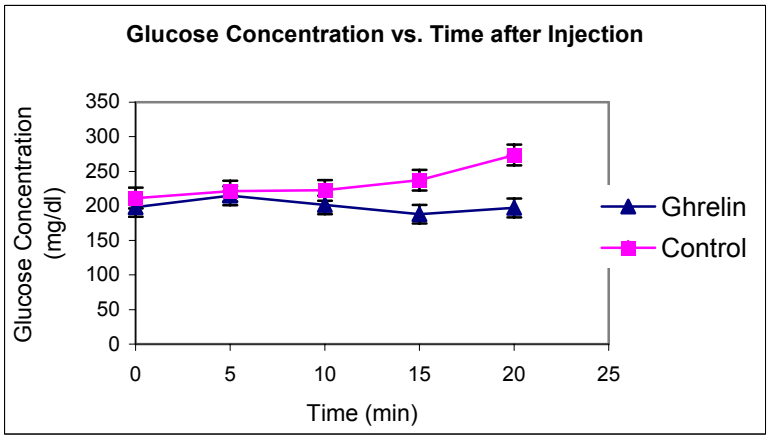
A = mean ± SEM

Figure One. Effect of ghrelin injection on insulin concentration.



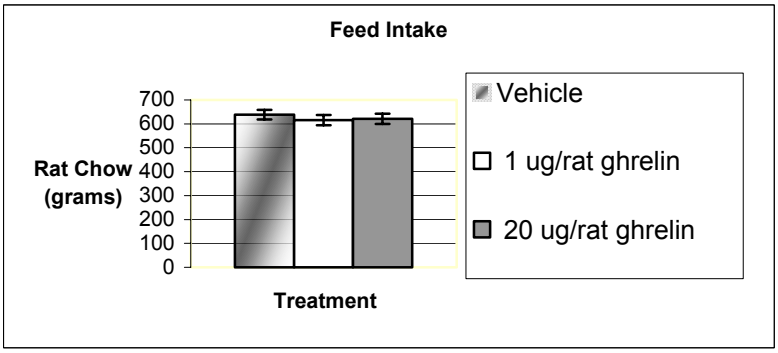
Mean ± SEM
Significant p<0.1
Treatment effect p = 0.17

Figure Two. Effect of ghrelin injection on glucose concentration.



Mean ± SEM
Significant p<0.1
Treatment effect p = 0.08

Figure 3. Total feed intake of treatment groups during injection period.



Mean ± SEM
Significant p<0.1
Treatment effect p = 0.18

Table Four. Effect of two doses of ghrelin on carcass measurements.

Treatment	Lipid (% of carcass)	Nitrogen (% of carcass)
Control	$4.68 \pm 0.34^{A\ a}$	12.6 ± 0.55
1 µg/d Ghrelin	5.84 ± 0.37^{b}	11.5 ± 0.6
20 µg/d Ghrelin	5.09 ± 0.37^{ab}	12.2 ± 0.6

A = mean ± SEM
different small letters indicate significant differences between groups at $P \leq 0.1$

Table Five. Effect of two doses of ghrelin on blood metabolites.

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)
Control	135.5 ± 6.5^{A}	113.1 ± 3.5^{a}
1 µg/d Ghrelin	149.2 ± 7.2	101.7 ± 3.9^{abc}
20 µg/d Ghrelin	150.6 ± 7.2	111.8 ± 3.9^{bc}

A = mean ± SEM
different small letters indicate significant differences between groups at $P \leq 0.1$

In previous studies, ghrelin has caused adiposity in rats (Tschop, 2000). In trial one, however, there was no significant difference in carcass lipid or protein. The gastrocnemius muscle weights of control and ghrelin treatments were similar (P = 0.738).

Liver lipids and liver triacylglycerols tended to be lower in the ghrelin group. Ghrelin stimulates growth hormone release. Growth hormone has been reported to decrease glucose production and increase lipid oxidation in the liver (Piatti, 1999). The increase in lipid oxidation could explain the decrease in liver lipids and triacylglycerols.

Blood urea nitrogen and NEFA were similar in the ghrelin and control groups. Blood cholesterol was higher in the ghrelin treatment. Terminal blood glucose tended to be higher in the ghrelin group than in the control, demonstrating a mild hypoglycemic effect of ghrelin.

The results indicate that ghrelin caused a decrease in insulin concentration and an increase in glucose concentration (Figures One and Two). Ghrelin injection caused hyperglycemia in rats over time while causing a decrease in insulin concentration. This result is consistent with the known physiological effects of ghrelin (Broglio, 2001).

Trial Two

In the second trial, the group administered the 1 µg/d ghrelin group had a significantly higher percent carcass lipid than the control (P =0.03). The 20 µg/d ghrelin group was not significantly different from the control (P = 0.43). Ghrelin has been reported to cause adiposity in rats. The increase in lipid percent in the 1 µg/d ghrelin group is consistent with those findings. However, the 20 µg/d ghrelin group did not have a higher percent lipid, which was not expected. The increase in adiposity in the 1 µg/d ghrelin group and not in the 20 µg/d ghrelin group is not does not have a clear explanation. The treatment groups did not significantly differ in carcass percent nitrogen ($P > 0.21$).

To determine if the rats with a higher percent lipid ingested more feed, the feed intake of the three treatments groups were measured. Because the three treatment groups did not significantly differ in feed intake, the effect of ghrelin on adiposity is not a result of greater dietary energy.

Blood glucose tended to be higher in the in the ghrelin groups versus the control (P = 0.18). The 1 µg/d ghrelin group was higher than the control (P = 0.18) but lower than the 20 µg/d ghrelin group (P =0.14). The daily injection of ghrelin seems to have elevated the blood glucose in a dose-dependent manner. Serum cholesterol was lower in the rats given 1 µg/d ghrelin (P = 0.04). Rats in the 20 µg/d ghrelin and the control groups were similar in serum cholesterol concentration (P = 0.82).

Implications

In trial one, ghrelin did not have an effect on adiposity. Bone density was greater in the ghrelin treated rats. Hyperglycemia and a decrease in insulin concentration were observed in ghrelin treated rats. In trial two, lean mass was not effected by ghrelin, but the rats given 1 µg/d ghrelin had an increase in adiposity. Blood cholesterol concentration was decreased by the 1 µg/d ghrelin treatment, but not the 20 µg/d ghrelin treatment. Both doses of ghrelin caused hyperglycemia; the greater dose caused the greater increase. The second study demonstrated an increase in adiposity in rats treated with 1 µg/d ghrelin. Both ghrelin treatments tended to cause hyperglycemia in rats. In summary, ghrelin increased blood glucose concentration without changing body composition.

References

Broglio, F., Arvat, E., Benso, A., Gottero, C., Muccioli, G., Papotti, M., van der Lely, A. J., Deghenghi, R., & Ghigo, E. (2001) Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab* 86: 5083-5086.

Piatti, P. M., Monti, L. D., Caumo, A., Conti, M., Magni, F., Galli-Kienle, M., Fochesato, E., Pizzini, A., Baldi, L., Valsecchi, G., & Pontiroli, A. E. (1999) Mediation of the hepatic effects of growth hormone by its lipolytic activity. *J Clin Endocrinol Metab* 84: 1658-1663.

Tschop, M., Smiley, D. L., & Heiman, M. L. (2000) Ghrelin induces adiposity in rodents. *Nature* 407: 908-913.

Tseng, K. F., & Goldstein, S. A. (1998) Systemic over-secretion of growth hormone in transgenic mice results in a specific pattern of skeletal modeling and adaptation. *J Bone Miner Res* 13: 706-715.