An exploration of the gastrointestinal hormone ghrelin: from discovery to implementation

BY KATLIN HORNIG
COLORADO STATE UNIVERSITY

Abstract

Ghrelin is a naturally occurring 28-amino acid brain-gut peptide with growth hormone-releasing and appetite-inducing capabilities. It is primarily secreted in the stomach mucosa but also has been expressed widely in different tissues, including the hypothalamus. This has led to the idea that it may have both endocrine and paracrine effects. It is a endogenous ligand of the G protein-coupled growth hormone secretagogue receptor. Ghrelin levels increase when subjects are fasting or hypoglycemic. Levels decrease in chronic obesity and positive energy balance. Ghrelin stimulates growth hormone release and regulates energy homeostasis by binding to ghrelin receptors in the anterior pituitary and possibly the mediobasal and mediolateral hypothalamus. This review progresses through a general analysis of the information pertinent to ghrelin's history, functional roles, structure, analytical aspects, clinical significance, and application. Eating disorders, gastrointestinal diseases, growth hormone deficiency, and growth hormone deficieny diagnosis will be analyzed. Special analysis of ghrelin in ovines will be briefly discussed.

Introduction

Ghrelin, a gastric-derived peptide, is an endogenous ligand for growth hormone secretagogue (GHS) receptor that may play an important role in energy homeostasis and the control of feeding behavior.[1] Ghrelin production is primarily in the endocrine cells in the gastrointestinal tract.[2] This orexigenic hormone's levels fluctuate over the course of the day in relation to food intake. Pharmacalogical administration of ghrelin has demonstrated increased feeding in multiple species.[2] Due to the hypophagia and hyperphagia that result from the removal of the lateral hypothalamus and ventromedial hypothalamus, respectively, it is demonstrated that feeding is regulated by a balance of the stimulatory and inhibitory regions of the hypothalamus.[3] Close interaction between the brain and gastrointestinal gland in the regulation of feeding is probable due to the gastrointestinal gland being the main site of food digestion and nutrient absorption.[4] Ghrelin is the first food-intake-stimulating signal originating from the stomach.[5] The following review will work to elucidate the most current knowledge foundation we have about ghrelin.

History

Kojima initially discovered Ghrelin in 1999 through investigation of rat stomach tissue.[6,7] Since then, it has also been discovered in mammals including humans, cattle, and sheep. The term ghrelin descends from the Proto-Indo-European root ghre – meanding "grow".[6] Bowers' study identified that synthetic peptide analogues of the opiate met-enkephalin specifically released growth hormone *in vitro*.[8] Human data later suggested that several peptide and non-peptide compounds have potent growth hormone (GH) releasing activity when administered parenterally or orally.[9] Peptides include the following: growth hormone-releasing peptide (GHRP)-6, GHRP-2, hexarelin, and ipamorelin. Non-peptide compounds include the following: L-692, L-429, MK-0677, and NN-703.[9] Demonstrated by computer-assisted overlays, non-peptide and peptide growth hormone secretagogues (GHSs) show three-dimensional similarities and many synthetic GHS have been synthesized by numerous companies since the initial appearance of that similarity.[10] Good oral bioavailability and negligible effects on other pituitary hormones are two of the effects of potent GH-release.[11] A specific G-protein coupled receptor was identified in 1996. This receptor was the growth hormone secretagogue receptor (GHS-R) which is expressed primarily in the hypothalamus and pituitary gland.[12] An endogenous ligand for this receptor was swiftly revealed and called ghrelin.[7] This so-called 'reverse pharmacology' is similar to the characterization and recognition of endogenous opiates and endocannabinoids.[13,14] Ghrelin's originally identified source of the stomach in combination with its presence in the hypothalamus makes it a newer member of the brain-gut peptide family.[9] Ghrelin's importance in body weight regulation was heightened with the observation that circulating ghrelin levels show both rapid and long-lasting effects on weight management.[9]

Structure

Ghrelin is synthesized as a preprohormone that is proteolytically processed to a 28-amino acid peptide.[5] Necessary for biological activity, a post synthetic modification takes place in which an n-octanoic acid residue is bound to one of the amino acids.[14] The primary source of circulating ghrelin is the gastrointestinal tract, primarily the stomach. Slighter amounts also originate from the intestine, hypothalamus, placenta, kidney, and pituitary gland.[16] Ghrelin is an example of a bioactive peptide with acyl (general formula: -C(O)R) modification.[3]

Chemical/biological structure and synthesis

In succession with the discovery of ghrelin was the identification of a stomach-derived mRNA sequence that codes for a protein with similarities in sequence to motilin and named motilin-related peptide m46.[17] Though the fatty acid modification was not recognized, the aforementioned peptide was found to be identical to ghrelin.[9]

Mass spectrometry was accompanied by high-pressure liquid chromatography (HPLC) to identify the amino-acid sequence of ghrelin and a discrepancy between the observed and calculated molecular weight.[9] This pointed to the presence of a post-translational modification; there is no other naturally occurring peptide that has been previously shown to have this acyl group as a post-translational modification.[15] The hydroxyl group of Ser3 is octanoylated, meaning the hydrogen atom of the hydroxyl group of the third N-terminal amino-acid serene residue is replaced by a hydrophobic moiety, C7H15CO. Another discovery was a splice variant of ghrelin with 27 amino acids all missing the fourteenth amino acid, glutamine. Biologically active analogues of ghrelin were later described in much smaller amounts with acyl chains of 10 or 11 C atoms or with a peptide chain that contains a missing twenty-eighth position amino acid, arginine.[18] At the Ser3 of the ghrelin molecule, the n-octanoyl group seems to be essential for a quantity of the hormone's bioactivity, which includes growth hormone release and appetite. Non-acylated desoctanoyl or non-acylated desacyl ghrelin circulates in much larger amounts than does the acylated form, and it does not displace ghrelin from its hypothalamic and pituitary binding sites.[19] The non-acylated forms are unable to stimu-

late GH release in vivo in such animals as rats and humans.[20] Numerous common features are shared among ghrelin and the gastrointestinal peptide motilin.[9] Both are synthesized within the upper gastrointestinal tract, exhibit prokinetic activity on gut motility, and both demonstrate the ability to stimulate GH release from the pituitary.[21] The structures of these genes are similar. The motilin receptor is the closest relative of the ghrelin receptor.[22]

Ghrelin was originally synthesized *in vitro*.[23] It has been shown to be mainly produced by X/A-like cells of the oxyntic stomach mucosa.[23] In humans, the ghrelin gene is located on chromosome 3 (3p25-26). Originally described were four exons and three introns that are at positions 141 (2000 bp intron), 258 (3000 bp intron), and 367 (800 bp intron) of the 511 bp long cDNA.24 Prepro-ghrelin contains a 23 amino-acid signal peptide and pro-ghrelin contains a 94 amino-acid peptide; this includes the 28 amino-acid mature ghrelin and a tail that is 66 amino acids. Gly-Ser-Ser-(n=octanoyl)-Phe-Leu, the first 4 or 5 residues of ghrelin, are satisfactory for calcium mobilization in vitro.[9,25] Ghrelin activity is not affected by the short, hydrophobic acylation of the hydroxyl group of Ser3 with longer aliphatic chains or with unsaturated or branched octanoyl groups. However, ghrelin activity is dramatically decreased as a result of the acetyl groups replacing the natural 8-carbon group. Recent studies show that shortened ghrelin molecules may show GH-releasing activity *in vitro*, but not *in vivo*.[26]

The 5` flanking region of ghrelin has been an elevated area of study. The 2000 bp region that is upstream in relation to the start codon contains some binding sites for transcription factors including the following: AP2, basic helix-loop-helix (bHLH), PEA-3, Myb, NF-IL6, NF-kB, hepatocyte nuclear factor-5, and half sites for estrogen and glucocorticoid response elements.[26] Ghrelin has been shown to co-purify with a high-density lipoprotein (HDL) that is associated with the plasma paraoxonase.[9,27] A calcium-dependent esterase, paraoxonase, breaks down oxidized lipids in low-density lipoproteins (LDL). Low paraoxonase activity is linked with coronary disease. The octanoyl group of ghrelin binding to the peptide with an ester bind suggests a possible role of the enzyme in ghrelin, desoctanoyl ghrelin conversion.

Origin

Ghrelin is expressed and identified in a number of different types of tissues. It has been shown that ghrelin is expressed in the pituitary, immune cells, lungs, placenta, cyclical expression in the ovaries, testes, and kidneys.[9] It can be identified at the mRNA level, protein level, or both. Ghrelin is most prominent in the stomach, small intestine, and hypothalamus.[9]

Originally, ghrelin was isolated from the stomach. Out of the numerous different types of endocrine cells in the stomach, about 20% of the chromogranin A-immunoreactive endocrine cells contain ghrelin mRNA.[28] Ghrelin cells are identified by being round or ovoid in shape and are not in contact with the lumen of the stomach but are positioned closer to the capillaries. Ghrelin is found in the fundus of the stomach in the oxyntic gland, which is the acid secreting part of the stomach.[9] The majority of circulating ghrelin is found to originate from the stomach, and a minor amount in comparison is found in the small intestine.[29] There are two types of ghrelin cells observed in the gastrointestinal tract: those that are closed and have no contact with the lumen and those that are open or elongated and have contact with the lumen.[30] When the acid-producing part of the stomach of rats was surgically removed, the amount of circulating ghrelin within the animals decreased by 80%, suggesting that the oxyntic mucosa is a major source of ghrelin.[31]

Ghrelin peptide is expressed in the hypothalamus and is released *in vitro* from the hypothalamic blocks spontaneously after stimulation of a depolarizing concentration of potassium chloride.[32] Hypothalamus immunostaining studies exhibited ghrelin expression in the internuclear space amid the lateral hypothalamus, arcuate nucleus (ARC), ventromedial nucleus (VMN), dorsomedial nucleus (DMN), paraventricular nucleus (PVN), and the ependymal layer of the third ventricle.[9]

Functional roles

Ghrelin holds a considerable role in the regulation of GH secretion.[5] GH release is stimulated when ghrelin activates Ghrelin Receptor (GHS-Rs) located on the pituitary and GH-releasing hormone-containing neurons in the hypothalamic arcuate nucleus. Studies have proven that ghrelin stimulates food intake and is very involved with energy homeostasis regulation.[5]

Secretion and release mechanism

Ghrelin signals are integrated with growth hormone releasing hormone and somatostatin to control the timing and magnitude of growth hormone secretion.[16] Cells in the anterior pituitary have a receptor (named after GHS-R) that when activated by the binding of ghrelin to GH stimulates the secretion of GH.[5] Acyl modification of ghrelin is pertinent for its activity; thus, enzymes that catalyze acyl modification are important in regulating the activity of ghrelin.[3] Amino acid sequences of ghrelin are well conserved; the ten amino acids at the N terminus of the sequence are identical. The structural conservation and the requirement for acyl modification indicate that the N-terminal section might be of central importance in regulating ghrelin's activities.[7]

Ghrelin secretion is controlled by the act of feeding.[3] Plasma ghrelin concentration increases throughout periods of fasting and decreases after food is ingested. This correlation has been noted in numerous animals including rats, humans, sheep, dairy cows, and beef steers.[6] The factors that are responsible for mediating the regulation of ghrelin secretion are unclear, but blood glucose levels may be critical.[33] If glucose is administered intravenously or orally it does decrease plasma ghrelin concentration. Gastric distension caused by increased water intake does not change ghrelin concentration. Thus, mechanical distension of the stomach cannot be the cause of ghrelin release.[33] High lipid concentrated diets decrease plasma ghrelin concentration; low protein diets increase plasma ghrelin concentration. Plasma ghrelin concentration also varies depending on body structure; lean people have high plasma ghrelin concentration and obese individuals have low plasma ghrelin concentration.[3]

Patients that undergo a gastric bypass surgery lose nearly 36% of their weight along with a concurrent decrease in their plasma ghrelin concentration.[34] Ghrelin concentration alterations with food intake diminish in these patients, thus suggesting that the main site of production of ghrelin is the stomach.[3] Plasma ghrelin concentration decreases for patients with short bowel syndrome.[35] The loss of ghrelin-producing tissues in these patients is a cause for lowered ghrelin concentration levels. Ghrelin secretion and concentration is influenced by such things as; feed intake, body condition, nutritional status, and physical framework.

Ghrelin regulation

Ghrelin regulation and effects occur at numerous points including: transcription and translation of the ghrelin gene; addition of post-translational modification; secretion rate of ghrelin from cells in the stomach, hypothalamus, and other sites; binding proteins in the circulation; transport across the blood-brain barrier; clearance of ghrelin by liver/kidney; influence of the other ligands of the ghrelin receptors; expression of ghrelin receptors; and intracellular signaling of ghrelin receptors.[36] Ghrelin changes throughout the day depending on amount and time of food intake. Factors that influence the upregula-

tion of ghrelin secretion include: fasting, low body mass index (BMI) or body score, leptin, growth hormone releasing hormone (GHRH), thryroid hormones, testosterone, and parasympathetic activity. Ghrelin secretion downregulation is influenced by: food intake, high BMI or body score, glucose, insulin, somatostatin, GH, GHS, ghrelin, PYY3-36, and urocortin-1.[9]

Appetite and feeding stimulant

Feeding intake is stimulated by the activation of GHS-Rs by ghrelin on NPY/agoutirelated peptide (AGRP)-producing neurons that are located in the arcuate nucleus.[37] Ghrelin has the capability to increase total fat tissue by decreasing fat oxidation. Motility stimulation and stimulation of gastric emptying that is induced by ghrelin can involve a local effect as well as some central mechanisms.[38] Adult *Homo sapiens* exhibit a two-fold increase in plasma ghrelin concentrations before a meal and decrease to trough concentrations within a single hour after eating.[5]

Ghrelin has been shown to be appetite-stimulating when it is administered both centrally and peripherally.[3] The main site of ghrelin is the arcuate nucleus. Due to the fact that peptide hormones in the blood do not generally pass through the blood-brain barrier, there has to be a mechanism through which peripherally administered ghrelin can trigger the central nervous system (CNS), which could possibly be the vagus nerve.[39] The appetite-stimulating effect of ghrelin is blocked by an neuropeptide Y (NPY) receptor (Y1) antagonist. Ghrelin stimulates NPY and agouti-related protein (AgRP) secretion, indirectly enhancing feed intake activity.[6]

Growth hormone releasing

Ghrelin can cause growth hormone (GH) release both *in vitro* and *in vivo*.[5] Ghrelin acts directly on the pituitary gland, as indicated by ghrelin specifically stimulating GH release from primary pituitary cells in a dose-dependent manner. In both rats and humans, an intravenous injection of ghrelin induces potent GH release.[7] Intravenous injection of ghrelin and intracerebroventricular (ICV) administration of ghrelin demonstrated an increase in rat plasma GH concentrations in a dose-dependent style. As little as 10 pmol of ICV ghrelin was shown to be enough to release GH.[40] This is a lesser amount than what is needed if ghrelin is administered intravenously, proving that ICV injection is much more potent. *In vivo* results, *in vitro* results, and ghrelin detected in blood samples implies that ghrelin is secreted from the stomach into the bloodstream and then acts directly on the pituitary gland to release GH.[3]

In cases where negative energy balance is generally prominent, such as low-calorie diets, chronic exercise, cancer anorexia, anorexia nervosa, and Prader-Willi syndrome, ghrelin concentrations were reported to have increased.[41]

Other functions

More than a mere natural GH secretagogue, ghrelin also acts on other central and peripheral receptors and exhibits an abundance of other actions.[5] Some of those actions include the following: stimulation of lactotroph secretion, stimulation of corticotroph secretion, influences gastroenteropancreatic functions, and has orexigenic, metabolic, cardiovascular, and antiproliferative effects.

Ghrelin concentration is low in human obesity, which could be correlated with high caloric intake. A reduction of body weight in obese individuals causes ghrelin concentrations to increase. Despite the conclusion that ghrelin levels are elevated in individuals engaging in dieting, those who undergo stomach-bypass surgery demonstrate decreased ghrelin concentrations. This leads to the idea that the size of the stomach may correlate directly with ghrelin concentrations.[42] A number of experimental observations have shown that ghrelin could possibly be a strong gastrokinetic agent.[5]

Analytical aspects

There are several assays available for ghrelin measurement. Linco Research, Inc., developed an analysis system typically applied when measuring human ghrelin levels.[5] With this analysis antibody was raided against a human ghrelin epitope that was carrying an octanoyl group on the serine-3 position. This position determines the biological function of the hormone via enabling binding to the receptor. Precautions must be taken with this test due to the acidification of the sample to stabilize the labile side chain.[43] The specifications for this analysis from Linco Research are the following: the lower limit of detection is 10 ng/L, linear range is 10-2000 ng/L, intraassay CV is 7.4%, and interassay CV is 13.5%.[43] A less preferred method of analysis was developed by Phoenix, Inc. It provides a lyophilized preparation that must first be dissolved in assay buffer and then diluted repeatedly.[43] There is also a calcium influx assay which has been used in rodents to detect ghrelin levels.[40] The Linco Research, Inc., ghrelin assay is also applicable to numerous other animals, including bovines.[6]

In ovines, ghrelin concentration has been measured by a competitive solid-phase immunoassay that makes use of Europium (Eu)-labeled synthetic rat ghrelin and polystyrene microtiter strips coated with anti-rabbit gamma-globulin.[1] Another method for ovine GH measurement is the GH; TR-FIA system that was developed using the RIA kit for ovine GH radioimmunoassay supplied by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).[1] In ovines there is another assay that can be used for ghrelin known as the time-resolved fluoro-immunoassay (TR-FIA). It, too, uses reagents supplied by NIDDK.[44]

Ghrelin and other hormones

The arcuate nucleus is the target site of leptin, ghrelin, neuropeptide Y (NPY), and agouti-related protein (AgRP). Leptin is an appetite-suppressing hormone from adipose tissues.[45] NPY and AgRP are appetite-stimulating peptides that are inhibited directly by leptin. An ICV injection of ghrelin caused the expression of Fos protein in NPY-containing neural cells and increased the amount of NPY mRNA in the arcuate nucleus.[3] An NPY receptor (Y1) antagonist blocks the appetite-stimulating effect of ghrelin. Appetite-stimulating effects were also inhibited by ICV injections of an AgRP inhibitor, anti-NPY IgG, or anti-AgRP-IgG. An intravenous injection of ghrelin was found to stimulate neurons that contained NPY and/or AgRP in the hypothalamus. Neuron fibers directly protrude onto these neurons as shown by immunohistochemical analysis.[3] From this information we can conclude that ghrelin increases feeding activity by stimulating NPY- and AgRP-containing neurons in the hypothalamus. This promotes the production and secretion of NPY and AgRP peptides. It can be deduced that the hormone ghrelin is a natural antagonist to the appetite-repressing, protein hormone, leptin.[3]

Clinical significance and application

Ghrelin has distinct orexigenic, adipogenic, and somatotropic properties.[5] The wide and various tissue distribution of ghrelin suggests that it may have multiple functionalities. Via vagal afferent-mediating ghrelin signaling, the brain-gut axis is the effector of anabolism by regulating growth, metabolism, and feeding. Ghrelin also has the ability to enhance immune responses and potentially down-regulate anti-inflammatory molecules.[5] Ghrelin's role as a brain-gut peptide highlights the significance of afferent vagal fibers as a major pathway to the brain, thus maintaining physiologic homeostasis. Ghrelin and growth hormone secretagogues (GHSs) will be particularly important due to the number of effects they have including: increased growth hormone release, increased ACTH and cortisol release, increased prolactin release, increased appetite, regulation

of carbohydrate metabolism, increased gastric motility, immune function regulation, increased sleep, increased bone density, increased heart rate, increased vasodilation, proliferative regulation, autonomous nervous system regulation, and decreased thermoregulation.[9]

The characterization of ghrelin will be important due to the knowledge that it is involved with feeding regulation, nutritional homeostasis, and metabolic processes. Inevitably, the future will show the generation of new approaches to the diagnosis and treatment of different disease categories, particularly those related to over nutrition and the catabolic response to surgical trauma.[46] Some of the various implementations for ghrelin associated clinical response include matters of: cardioprotective effects, diagnostic or therapeutic tool in GH deficiency, marker for neuroendocrine tumors, treatment of catabolic states, enhancing immune function in cachexia or AIDS, obesity, and anorexia nervosa.[5]

Catabolic states

A prevalent and potential application of ghrelin is in relation to osteoporosis, aging, and catabolic states. Upregulation of ghrelin has shown benefits for many degradative metabolic conditions. This includes those seen in postoperative patients and in AIDS-associated and cancer-associated wasting syndromes.[47] Though ghrelin levels in GH-deficient subjects are not vastly different from controls, it still is in question whether some subjects can lack ghrelin and have growth-retarded phenotype and whether ectopic production of ghrelin can lead to acromegaly.[48]

Eating disorders

Ghrelin has the ability to serve as an orexigenic agent for the treatment of such eating disorders as anorexia nervosa.[49] A mere administration of ghrelin orally or intravenously could stimulate appetite and improve the nutritional state of patients in this situation. Sensitivity to ghrelin is severly disturbed in individuals with anorexia nervosa due to very high plasma ghrelin levels.[50]

Gastrointestinal disease

Ghrelin could be a candidate for the treatment of postoperative gastric ileus due to its stimulation of gastric motility.[50] Ghrelin counteracts gastric ileus as has been shown by its strong prokinetic effect, accelerating gastric emptying, the small intestinal transit of liquid meals, and reversing delayed gastric evacuation.[51]

Growth hormone deficiency

Circulating GH in humans can be increased by intravenous injection of ghrelin dose-dependently. The co-administration of ghrelin and GHRH has a synergistic effect on GH secretion. The combined administration of the two is the most potent inducer of GH release yet identified. Ghrelin supplementation may have the ability to give beneficial effects to GH deficient adults and children.[50]

Growth hormone deficiency diagnosis

Ghrelin could be applied to the diagnosis and treatment of GH deficiency because of its potent GH-releasing activity and specificity. GH deficiency diagnosis is done through insulin-induced hypoglycemia. During this practice blood glucose level is decreased to less than 40 mg/dl. Side effects may result from the hypoglycemic action of insulin. Intravenously injected ghrelin in humans does not show any side effects, suggesting that ghrelin could be useful as a diagnosing tool for GH deficiency.[50]

Exploration of significance in ovines

Ghrelin in reproductive organs

There has been significance evidence of ghrelin presence and its receptor's presence in the various reproductive tissues of adult and fetal sheep. It has been indicated that testicular expression of ghrelin, along with its receptors, is physiologically regulated in adult sheep and developmentally regulated in a fetus. This has shown that the ghrelin ligand and receptor system could have a endocrine or paracrine role in the cellular proliferation, development, and function of ovine reproductive axis.[52]

In the tissue of adult sheep, ghrelin and growth hormone secretagogue receptors (GHSR-1a) immunostaining was distinguished in the stomach (abomasum in ruminants), anterior pituitary gland, testis, ovaries, and the hypothalamic and hindbrain regions of the brain. The adult testis experiences a significant effect on its level of immunostaining for ghrelin and GHSR-1a due to the season or photoperiod. For fetal sheep testis, there was a pertinent effect of gestational age on the level of immunostaining for ghrelin, SCF, PCNA, and GHSR-1a.[52]

Transient ghrelin surges

It has been demonstrated that ovines experience a transient surge of plasma ghrelin in the pre-feeding period. Just before a scheduled-meal feeding, drastic increases in plasma ghrelin occur. In sheep fed twice daily, a transient surge of ghrelin secretion occurred before each feeding, leading to the suggestion that a diurnal rhythm of plasma ghrelin would be dependent on feeding regimen.[1]

Another experiment has been conducted using pseudo-feeding with ovines. It found that transient ghrelin surges still occurred when pseudo-feeding. This has led to the idea that psychological factors stimulate ghrelin secretion prior to feeding as a result of their conditioned emotional response (CER).[1]

Conclusion

The peptide hormone ghrelin is found in numerous tissues throughout the body. It is extremely noticeable in the stomach and hypothalamus. Ghrelin is the endogenous ligand of the G protein-coupled growth hormone secretagogue receptor. Two of ghrelin's primary functions are the stimulation of growth hormone (GH) secretion and appetite inducing activities. Ghrelin will prove to be a very applicable hormone in the future as it is already demonstrating a number of possible clinical applications. Ghrelin is a very promising hormone that holds a positive and potential impact for the future of animals.

Acknowledgements

I would like to express appreciation to Dr. Archibeque for supplying me with the wonderful opportunity to further my comprehension on such an intriguing subject as ghrelin and for encouraging me to excel. Much appreciation is extended to Dr. Han for giving me the opportunity to work on a dynamic undergraduate research project with immense potential for scientific advancement. These relationships have provided abounding support for my future endeavors. Also, I would like to thank my father for encouraging me to strive to further my scientific endeavors while always exhibiting integrity. I thank my mother for always being the supportive force behind all my decisions.

References

[1]Sugino, T., Hasegawa, Y., Kikkawa, Y., Yamaura, J., Yamagishi, M., Kurose, Y., Kojima, M, Kangawa, K. and Terashima, Y. (2002) "A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep." *Biochemical and Biophysical Research Communications* 295. Pg 255-260.

[2]Williams, D.L. and Cummings, D.E. (2005) "Regulation of ghrelin in physiologic and pathophysiologic states." *American Society for Nutritional Sciences* 22. Pg 1320-1325.

[3]Kojima, M. and Kangawa, K. (2002) "Ghrelin, an orexigenic signaling molecule for the gastrointestinal tract." *Current Opinion in Pharmacology* 2. Pg 665-668.

[4]Havel, P.J. (2001) "Peripheral signals conveying metabolic information to the brain: Short- term and long-term regulation of food intake and energy homeostasis." *Experimental Biology and Medicine* 226. Pg 963–977.

[5]Meier, U. and Gressner, A.M. (2004) "Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghre-

lin, adiopectin, and resistin." *Clinical Chemistry* 50. Pg 1511-1525.
[6]Field, M. (2008) "Effects of nutrient restriction and heat stress on ghrelin secretion in dairy cattle and their implications for reproductive success." The University of Arizona. Pg 1-87.
[7]Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. and Kangawa K. (1999) "Ghrelin is a growth-hormone-releasing acylated peptide from stomach." *Nature* 402. Pg 656-660.
[8]Bowers, C.Y., Chang, J., Momany, F.A. and Folkers, K. (1977) "Effect of the enkephalins and enkephalin analogues on release of pituitary homones in vitro." *Molecular Endocrinology.* Pg 287-292.
[9]Korbonits, M., Goldstone, A.P., Guerorguiev, M. and Grossman, A.B. (2004) "Ghrelin – a hormone with multiple functions." *Frontiers in Neuroendocrinology* 25. Pg 27-68.
[10]Schoen, W.R., Pisano, J.M., Pendregrats, K., Wyvratt, M.J., Jr., Fischer, M.H., Cheng, K., Chan, W.W.S., Butler, B., Smith, R.G. and Ball, R.G. (1994) "A novel 3-substituted benzazepinone growth hormone secretagogue." *The Journal of Medical Chemistry* 37. Pg 897-906.
[11]Hansen, B.S., Ankersen, M., Hansen, T.K., Raun, K., Nielsen, K.K., Lau, J., Peschke, B., Lundt, B.F., Thogersen, H., Johansen, N.L., Madsen, K. and Andersen. P.H. (1999) "Pharmakokinetics of NN703." *European Journal of Endocrinology* 141. Pg 180-189.
[12]Howard, A.D., Feighner, S.D., Cully, D.F., Liberator, P.A., Arena, J.P., Rosenblum, C.I., Hamelin, M.J., Hreniuk, D.L., Palyha, O.C., Anderson, J., Paress, P.S., Diaz, C., Chou, M., Liu, K., Kulju-McKee, K., Pong, S.S., Chaung, L.Y., Elbrecht, A., Dashkevicz, M., Heavens, R., Rigby, M., Sirinathsinghji, D.J.S., Dean, D.C., Melillo, D.G., Patchett, A.A., Nargund, R., Griffin, P.R., De Martino, J.A., Gupta, S.K., Schaeffer, J.M., Smith, R.G. and Van der Ploeg, L.H.T. (1996). "A receptor in pituitary and hypothalamus that functions in growth hormone release." *Science* 273. Pg 974-977.
[13]Elphick, M.R. and Egertova, M. (2001) "The neurobiology and evolution of cannabinoid signaling." *Philosophical Transactions of the Royal Society of Biological Sciences* 356. Pg 381-408.
[14]Olson, G.A., Olson, R.D., Kastin, A.J. and Coy, D.H. (1979) "Endogenous opiates: through 1978." *Neuroscience and Biobehavioral Science* 3. Pg 285-299.
[15]Hosoda, H., Kojima, M., Mitzushima, T., Shimizu, S. and K. Kangawa. (2003) "Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing." *The Journal of Biological Chemistry* 278. Pg 67-70
[16]Brown, R. (2009) "Ghrelin." Endocrine Index. Colorado State University. <http://www.vivo.colostate.edu/> (Accessed 11/13/2009).
[17]Tomasetto, C., Karam, S.M., Ribieras, S., Masson, R., Lefebvre, O., Staub, A., Alexander, G., Chenard, M.P., Chenard, M.C. and Rio, M.C. (2000) "Identification and characterization of a novel gastric peptide hormone: the motilin-related peptide." *Gastroenterology* 119. Pg 395-405.
[18]Hosoda, H., Kojima, M., Matsuo, H. and Kangawa, K. (2000) "Purification and characterization of rat des-Gln14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor." *The Journal of Biological Chemistry* 275. Pg 21995-22000.
[19]Hosoda, H., Kojima, M., Matsuo, H., and Kangawa, K. (2000) "Ghrelin and des-acyl ghrelin: two major froms of rat ghrelin peptide in gastrointestinal tissue." *Biochemical and Biophysical Research Communications* 279. Pg 909-913.
[20]Broglio, Benso, A., Gottero, C., Prodam, F., Guana, C., Filtri, L., Arvat, E., Van der Lely, A.J., Deghenghi, R. and Ghigo, E. (2003) "Non-acylated ghrelin does not possess the pituitaric and pancreatic endocrine activity of acylated ghrelin in humans." *The Journal of Endocrinology Investment* 26. Pg 192-196.
[21]Samson, W.K., Lumpkin, M.D., Nilaver, G. and McCann, S.M. (1984) "Motilin: a novel growth hormone releasing agent." *Brain Research Bulletin* 12. Pg 57-62.
[22]Feighner, S.D., Tan, C.P., McKee, K.K., Palyha, O.C., Hreniuk, D.L., Pong, S.S., Austin, C.P., Figueroa, D., MacNeil, D., Cascieri, M.A., Nargund, R., Bakshi, R., Abramovitz, M., Stocco, R., Kargman, S., O'Neill, G., Van der Ploeg, L.H.T., Evans, J., Patchett, A.A., Smith, R.G. and Howard, A.D. (1999) "Receptor for motilin identified in the human gastrointestinal system." *Science* 284. Pg 2184-2188.
[23]Vergnano, A.M., Ferrini, F., Salio, C., Lossi, L., Baratta, M. and Merighi, A. (2008) "The gastrointestinal hormone ghrelin modulated inhibitory neurotransmission in deep laminae of mouse spinal cord dorsal horn." *Endocrinology* 149. Pg 2306-2312.
[24]Wajnrajch, M.P., Ten, I.S., Gertner, J.M. and Eibel, R.L. (2000) "Genomic organization of the human ghrelin gene." *The Journal of Endocrinology and Genetics* 1. Pg 231-233.
[25]Bednarek, M.A., Feighner, S.D., Pong, S.S., McKee, K.K., Silva, M., Warren, V.A., Howard, A.D., Van der Ploeg, L.H.T. and Heck, J.V. (2000) "Structure and function studies on the new growth hormone releasing peptide, ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretagogue receptor." *The Journal of Medical Chemistry* 43. Pg 4370-4376.
[26]Kishimoto, M., Okimura, Y., Nakata, H., Kudo, T., Iguchi, G., Takahashi, Y., Kaji, H. and Chihara, K. (2003) "Cloning and characterization of the 5`-flanking region of the human ghrelin gene." *Biochemical and Biophysical Research Communications* 305. Pg 186-192.
[27]Beaumont, N.J., Skinner, V.O., Tan, T.M., Ramesh, B.S., Byrne, D.J., MacColl, G.S., Keen, J.N., Bouloux, P.M., Mikhailidis, D.P., Bruckdorfer, K.R., Vanderpump, M.P. and Srai. K.S. (2003) "Ghrelin can bind to a species of high-density lipoprotein associated with paraoxonase." *The Journal of Biological Chemistry* 278. Pg 8877-8880.
[28]Dass, N.B., Munonyara, M., Bassil, A.K., Hervieu, G.J., Osbourne, S., Corcoran, S., Morgan, M. and Sanger. G.J. (2003) "Growth hormone secretagogue receptors in rat and human gastrointestinal tract and the effects of ghrelin." *Neuroscience* 120. Pg 443-453.
[29]Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S., Shirakami, G., Usui, T., Shimatsu, A., Doi, K., Hosoda, H., Kojima, M., Kangawa, K. and Nakao, K. (2001) "Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans." *The Journal of Clinical Endocrinology and Metabolism* 86. Pg 4753-4758.
[30]Sakata, I., Nakamura, K., Yamazaki, M., Matsubara, M., Hayashi, Y., Kangawa, K. and Sakai, T. (2002) "Ghrellin-producing cells exist as two types of cells, closed- and opened-type cells, in the rat gastrointestinal tract." *Peptides* 23. Pg 531-536.
[31]Leonetti, F., Silecchia, G., Iacobellis, G., Ribaudo, M.C., Zappaterreno, A., Tiberti, C., Iannucci, C.V., Perotta, N., Bacci, V., Basso, M.S., Basso, N. and Di Mario, U. (2003) "Different plasma ghrelin levels after laparoscopic gastric bypass and adjustable gastric banding in morbid obese subjects." *The Journal of Clinical Endocrinology and Metabolism* 88. Pg 4227-4231.
[32]Mozid, A.M., Tringali, G., Forsling, M.L., Hendricks, M.S., Ajodha, S., Edwards, R., Navarra, P., Grossman, A.B. and Korbonits, M. (2003) "Ghrelin is released from rat hypothalamic explants and stimulates corticotrophin-releasing hormone and arginine-vaso-pressin." *Hormone Metabolism Research* 35. Pg 455-459.
[33]Tschop M., Smiley, D.L. and Heiman, M.L. (2000) "Ghrelin induces adiposity in rodents." *Nature* 407. Pg 908–91334
[34]Cummings, D.E., et al. (2002) "Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery." *New England Journal of Medicine* 346. Pg 1623-1630.
[35]Rosicka, M., Krsek, M., Jarkovska, Z., Marek, J. and Schrieber, V. (2002) "Ghrelin - a new endogenous growth hormone secretagogue." *Physiological Research* 51. Pg 435-441.
[36]Van der Lely, A.J., Tschop, M., Heiman, M.L. and Ghigo, E. (2004) "Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin." *Endocrinology Review* 25. Pg 426-457.
[37]Hagemann, D., Meier, J.J., Gallwitz, B. and Schmidt, W.E. (2003) "Appetite regulation by ghrelin-a novel neuro-endocrine gastric peptide hormone in the gut-brain-axis." *Z Gastroenterol* 41. Pg 929-936.
[38]Wang, G., Lee, H.M., Englander, E. and Greeley, G.H., Jr. (2002) "Ghrelin-not just another stomach hormone." *Regulatory Peptides* 105. Pg 75-81
[39]Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Ueno, N., Makino, S., Fujimiya, M., Niijima, A., Fujino, M.A. and Kasuga, M (2001) "Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin." *Gastroenterology* 120(2). Pg 337-345.
[40]Date, Y., Kojima, M., Hosoda, H., Sawaguchi, A., Mondal, M.S., Suganuma, T., Matsukura, S., Kangawa, K. and Nakazato, M. (2000) "Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans." *Endocrinology* 141. Pg 4255-4261.
[41]Zigman, J.M. and Elmquist, J.K. (2003) "Minireview: from anorexia to obesity – the yin and yang of body weight control." *Endocrinology* 144. Pg 3749-3756.
[42]Holdstock, C., Engstom, B.E., Ohrvall, M., Lind, L., Sundbom, M. and Karsson, F.A. (2003) "Ghrelin and adipose tissue regulatory peptides: effect of gastric bypass surgery in obese humans." *The Journal of Clinical Endocrinology and Metabolism* 88. Pg 3177-3183.
[43]Groschl, M., Uhr, M. and Kraus, T. (2004) "Evaluation of the comparability of commercial ghrelin assays." *Clinical Chemistry* 50. Pg 457-458.
[44]Sugino, T., Hasegawa, Y., Jurose, Y., Kojima, M., Kangawa, K. and Terashima, Y. (2004) Effects of ghrelin on food intake and neuroendocrine function in sheep. *Animal Reproduction Science* 82-83. Pg 183-194.
[45]Friedman, L.S., Thistlewaite, F.C., Patel, K.Y., Yu, V.P.C.C., Lee, H., Venkitaraman, A.R., Abel, K.J., Calton, M.B.L., Hunter, S.M., Colledge, W.H., Evans, M.J. and Ponder, B.A.J. (1998) "Thymic lymphomas in mice with a truncating mutation in BRCA2." *Cancer Research* 58. Pg 1338–1343.
[46]Wu, J.T. and Kral, J.G. (2004) "Ghrelin: integrative neuroendocrine peptide in health and disease." *Annals of Surgery* 239. Pg 464-474.
[47]Hanada, T., Toshinai, K. and Date, Y. (2004) "Up-regulation of ghrelin expression in cachetic nude mice bearing human melanoma cells." *Metabolism* 53. Pg 84-88.
[48]Inui, A., Asakawa, A., Bowers, C.Y., Mantovani, G., Laviane, A., Meguid, M.M. and Fujimiya, M. (2004) "Ghrelin, appetite, and gastric motility: the emerging role of the stomach as an endocrine organ." T*he Journal of the Federation of American Societies for Experimental Biology* 18. Pg 439-456.
[49]Muccioli, G., Tschop, M., Papotti, M., Deghenghi, R., Heiman, M. and Ghigo, E. (2002) "Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity." *European Journal of Pharmacology* 440. Pg 235-254.
[50]Nagaya, N., Kojima, M. and Kangawa, K. (2006) "Ghrelin, a novel gowth hormone-releasing peptide, in the treatment of cardiopulmonary associated cachexia." *The Japanese Society of Internal Medicine* 45. Pg 127-134.
[51]Trudel, L., Tomasetto, C. and Rio, M.C. (2002) "Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat." *The American Journal of Physiological Gastrointestinal and Liver* 282. Pg G948-G952.
[52]Miller, D.W., Harrison, J.L., Brown, Y.A., Doyle, V., Lindsay, A., Adam, C.L. and Lea, R.G. (2005) Immunohistochemical evidence for an endocrine/paracrine role of ghrelin in the reproductive tissues of sheep. *Reproductive Biology and Endocrinology* 3. Pg 1477-1491.