Review article

Ghrelin and eating disorders

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Abstract

Background: Ghrelin is a potent hormone with central and peripheral action. This hormone plays an important role in the regulation of appetite, food intake, and energy balance. Studies have suggested that ghrelin is involved with eating disorders (ED), particularly bingeing and purging. Genetic variants have also been studied to explain changes in eating behavior. **Methods:** We conducted a literature review; we searched PubMed, Scientific Electronic Library Online (SciELO), and LILACS databases using the keywords "eating disorder", "ghrelin", "polymorphism", "anorexia nervosa", "bulimia nervosa", "binge eating disorder", and their combinations. We found 319 articles. Thirty-nine articles met the inclusion criteria. **Results**: High levels of ghrelin were found in patients with anorexia nervosa (AN), especially in the purging subtype (AN-P). There was also a positive correlation between fasting ghrelin level and frequency of episodes of bingeing/purging in bulimia nervosa (BN) and the frequency of bingeing in periodic binge eating disorder (BED). Some polymorphisms were associated with AN and BN. **Conclusion:** Changes in ghrelin levels and its polymorphism may be involved in the pathogenesis of EDs; however, further studies should be conducted to clarify the associations.

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Keywords: Eating disorders, ghrelin, ghrelin receptors, single nucleotide polymorphism, genetics.

Introduction

Eating disorders (ED) are characterized by severe changes in eating behavior[1-3]. Anorexia nervosa (AN), bulimia nervosa (BN) and binge eating disorder (BED) are EDs known for their high morbidity and mortality affecting mostly adolescents and young adult females and can lead to major biological, psychological and social complications[4-7]. AN is characterized by intense fear of weight gain, severe food restriction, low body weight and a distorted perception of the body image. BN is characterized by episodes of binge eating (uncontrolled consumption of a large amount of food in a short period of time) followed by inappropriate compensatory behaviors aimed at preventing weight gain (such as: self-induced vomiting, abuse of laxatives, diuretics, amphetamines and/or excessive physical activity), these episodes must occur at least once per week for three months. Finally, BED is characterized by episodes of binge eating as described previously but without the use of compensatory methods, as frequently quoted in BN[2,6].

Studies indicate a prevalence of ED ranging from 0.4% to 1.6%, with the highest frequency found in young women (between 18 and 32 years old)[2,4]. In Brazil, there is still a scarce number of epidemiological studies involving ED, even if the number of these studies has increased in recent years[8].

The etiology of an ED is complex and although widely studied, is still poorly understood. It is believed that the disease is multifactorial with a complex interaction of several factors: biological, psychological, sociocultural and family-related which are responsible for initiating and maintaining ED[9-11]. There is substantial evidence that genetic factors have up to an 80% stake in the etiology of AN[12], however, little is known about the molecular mechanism of these cases[13].

Most genetic studies on ED are focused on the investigation of candidate genes. Several genes that play an important role in appetite regulation and satiety are considered candidates and may be related to the development of ED[14-18], but the results of these studies are still inconsistent[19,20].

One of the major hormones involved in the regulation of food intake is ghrelin. Although there are many neuropeptides that stimulate food intake, ghrelin is the most established orexigenic peptide known until now[21].

Methods

We conducted a literature review to human studies in PubMed, Scientific Electronic Library Online (SciELO) and Lilacs databases, published between January 2000 and December 2014. The main keywords were used: "eating disorder" and "ghrelin", and filtered the results to the terms: "anorexia nervosa", "bulimia nervosa", "binge eating disorder", "polymorphism" and their combinations. The inclusion criteria were: 1) articles in English, Portuguese and Spanish; 2) articles that fully approached the topic ghrelin, eating disorders and their possible biological/genetic changes; 3) only studies in patients with diagnoses AN, BN and BED.

Three hundred and nineteen articles were found and only 39 contemplated these criteria (5 review articles, meta-analysis 1 and 33 experimental articles). Review articles and meta-analysis on the subject were consulted and cited in the discussion of this review, but for the presentation of data only original articles were used. We excluded studies in other languages and case reports as well, as articles that exclusively broached the topic obesity and ghrelin.

Results

The synthesis of these studies is presented in tables 1 and 2, sorted by month and year of publication. All data were taken from the original articles. To facilitate comparison we standardized the display of age and BMI and consider only one house after the comma without rounding.

Ghrelin and the regulation of appetite

The arcuate nucleus (ARC) of the hypothalmus and the brain stem are important regions involved in the regulation of appetite, body weight and energy balance[22]. The variety of hypothalamic appetite regulators are divided into two groups: The orexigenic types (appetite stimulators) which include the neuropeptide Y (NPY), the agouti-related peptide (AgRP), ghrelin, orexin and cannabinoids, while the anorectics (appetite suppressants) which include pro-opiomelanocortin (POMC), and cocaine and amphetamine regulated transcript (CART), thyrotropin releasing hormone (TRH), cortico-

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tropin releasing hormone (CRH), peptide YY (PYY), cholecystokinin (CCK) and glucagon-like-peptide (GLP 1), among other[23].

Ghrelin is a peptide of 28 amino acids, synthesized mainly by the oxyntic glands of the stomach[24]. It is acylated in the third residue which is a serine, the introduction of fatty acid (n-octanoyl) is essential for its activity[25].

It is one of the major signaling mechanisms for the start of the meal[26]. In humans, its concentration stays high during periods of fasting and periods that precede meals, falling soon after the start of food intake[27,28].

It is also involved in stimulating the secretion of growth hormone (GH) via the endogenous ligand of the GH secretagogue receptor (GHS-R)[29]. There are two subtypes of receptors, GHS-R1a, which is active, and GHS-R1b, a smaller isoform, which apparently has no biological activity[30]. This receptor (GHS-R) is present in various tissues including the anterior hypophysis and the hypothalamus, and in other areas of the brain, such as the hippocampus and gray matter. Because of its location, it has been suggested that GHS-R can modulate biological rhythms, mood, memory, learning and appetite[31].

Ghrelin is an orexigenic hormone that acts on the Central Nervous System (CNS) by activating the NPY/AgRP[32] neurons in the ARC via the GHS-R receptor. Thus, it promotes the production and secretion of other orexigenic neuropeptides that suppress neuronal activity of the POMC/CART, while stimulating food intake[33], this hormone undergoes a process of acetylation required to bypass the blood-brain barrier, making it suitable to connect to the GHS-R1a[34]. This acetylation converts the desacyl ghrelin (inactive form) into acyl ghrelin (active form)[35] and is catalyzed by the enzyme ghrelin O-acyltransferase (GOAT)[25] (Figure 1).

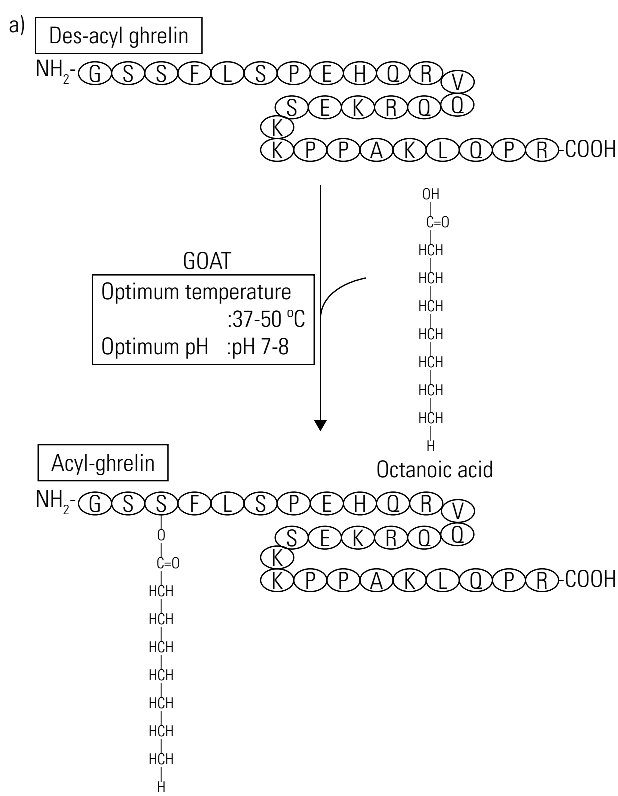

Figure 1. Ghrelin in its non-active form (desacyl ghrelin) being transformed into its active form (acyl ghrelin) by the enzyme ghrelin O-acyltransferase (GOAT). Adapted from Sato *et al.* 2012[80].

Ghrelin in EDs

The role of ghrelin has been extensively investigated in the etiology of obesity and contrary to what was expected, plasma levels seem to have an inverse correlation with the body mass index (BMI)[28,36]. Studies have shown that ghrelin levels are lower in obese subjects as compared to control subjects[36-39]. One study noted that the decrease in ghrelin after the meal was lower in obese individuals compared to normal weight individuals[40], and can thereby maintain the feeling of hunger. Studies with obese children also found low plasma ghrelin levels[41,42] and when these children have reduced 50% of their BMI, ghrelin levels remained lower in comparison to control subjects[41]. The same finding was observed in obese adults who normalized their BMI[43,44].

Studies conducted with AN patients found high levels of ghrelin in the plasma of these patients when compared with control of normal-weight individuals[39,45-49] which may suggest that this change may be an adaptive response to prolonged starvation[50]. Tolle *et al.* compared the levels of ghrelin plasma in 3 groups: healthy women considered thin (CT), who had a BMI similar to women with AN; patients with AN and women with normal weight (NW)[51]. It was demonstrated that ghrelin plasma concentrations in fasting patients with AN, was increased and remained high throughout the day (measured every 4 hours over a period of 24 hours) as compared to CT and NW. The study noted that these levels normalized after the patient gained the weight back, suggesting that in addition to body weight, levels of ghrelin may also be affected by the nutritional state[51]. Body fat instead of BMI has best explained the changes in the levels of ghrelin[47], some of the groups that had contradictory results between the correlation of BMI and ghrelin showed consistent results for body fat[37,45,52-54]. Studies have shown that ghrelin levels in patients with AN Restrictive (AN-R) have not been fully standardized, even after treatment[41,55-58].

Differences in ghrelin levels between subtypes of AN have also been reported. Tanaka *et al.* in 2003 found higher plasma levels of ghrelin in patients with AN Purging (AN-P) than in AN-R[59-61]. In 2004, the group of Tanaka replicated their findings in a later study which included a third subgroup of AN, a subgroup that required emergency hospitalization; in this group the patients were unable to eat and had an extreme loss of weight. It showed that the emergency group had higher plasma levels of ghrelin than AN-P, and that AN-P still had levels greater than the AN-R levels. The three groups experienced a decrease in their plasma levels of ghrelin after treatment, but patients with AN-P still kept the plasma levels of ghrelin higher than the control group at the end of rehabilitation[62]. In 2005 Troisi *et al.*, found higher levels of ghrelin during fasting in AN-R patients when compared to the AN-P patients[63]. However, the Troisi group compared data between patients with AN-P and BN, which probably had a higher BMI, which may explain the difference between the results of the two studies. There seems to be a relationship between ghrelin concentrations and patients with the compulsive/purging subtype for both AN (AN-P) and for BN[59-61]. However, this finding has still not reached a consensus, Monteleone *et al.* 2008[47] found no significant difference in the concentration of plasma ghrelin when fasting in groups with AN-R and AN-P. One explanation for these conflicting results is the method used to measure ghrelin and how it was performed, the preference for using plasma or serum can affect the levels obtained in different studies. The Monteleone study has confirmed this hypothesis; the study in 2008 obtained the result by screening for ghrelin plasma by way of the ELISA method (enzyme-linked immunosorbent assay). Whereas in 2010[64], in order to study patients with BN, they used the same test used by the group of Tanaka in 2003: the RIA (Radioimmunoassay) method and observed similar results, higher levels of ghrelin in these patients as compared to controls.

Tanaka *et al.* 2002[54] and Kojima *et al.* 2005[65], also observed elevated levels of fasting ghrelin in patients with BN. In addition, Tanaka in 2002 noted that ghrelin levels were negatively correlated with BMI and body fat percentage in both BN, as in the control group[54]. On the other hand, Nakazato *et al.* in 2004 found no significant difference between the levels of ghrelin plasma in patients with BN and the control group[66]. One possible explanation for this would be that Nakazato *et al.* 2004 measured ghrelin levels in the serum randomly between 11:00 am-12: 00 pm (postprandially), unlike Tanaka *et al.* 2002 who measured when fasting. When Kojima in 2005 measured the pre-and postprandial ghrelin, it was noted that the decrease in postprandial ghrelin was significantly attenuated in women with BN compared to the control group[66,67], generating a possible delay in the reduction of the hunger sensation in these patients.

Patients with BED tend to show a decrease in ghrelin when fasting[53,63,68] and a lower postprandial decline compared to the obese control group[68]. This decrease in ghrelin does not seem to reduce the propensity to gain weight in BED patients. Low ghrelin levels were also found in obese patients and seem to be more related to a sub-regulation of the release of ghrelin in response to excess weight and a lower postprandial decline, possibly acting to maintain the hunger[21].

A meta-analysis in 2009 found plasma concentrations in fasting and postprandial appetite hormones (gut hormones) in patients with AN, BN subtypes. It observed that in 8 studies analyzed, seven found elevated levels of plasma ghrelin in all diagnoses, with the exception of a single study[69].

In conclusion, the studies suggest that the changes found in ghrelin may be more related to the behavior of the binging and purging[60]. However, for the time being, it is still not clear as per whether ghrelin fundamentally participates as an important factor in the etiology of the EDs[70].

Ghrelin and the genes

The human ghrelin gene (GHLR, Gene ID: 51738)[71] which encodes ghrelin is located in the short arm of the chromosome 3 (3p25-26)[33]. Initially it was thought that it would have 4 exons (coding part of the gene), but subsequent studies have identified a number of additional exons in humans[72]. The precursor to ghrelin, the pre-proghrelin, is formed in the post-transcriptional process of GHLR, it consists of 518 pb encoded in a sequence of 117 amino acids, distributed over 23 amino acids of the signal peptide and 94 amino acids of pro-ghrelin, which include 28 amino acids of the mature ghrelin and over 66 additional amino acids[73], which include 23 of obestatin (a hormone with the antagonistic characteristics of ghrelin, which suppresses appetite and stomach activity)[74]. Therefore, ghrelin and obestatin are encoded by the same precursor gene (Figure 2).

The gene of the receptor (GHS-R, Gene ID: 2693)[71] was also located in the chromosome 3 (3q-26-31)[31]. The gene consists of two exons separated by one intron (non-coding part of the gene) (Figure 3). The exon 1 encodes the I-V transmembrane regions and exon 2 encodes the regions VI and VII[75]. The GHS-R gene encodes two types of mRNA: GHS-R1a and GHS-R1b[73]. The GHS-R 1a contains all 7 transmembrane regions and possess a high affinity with ghrelin, while the physiological role of GHS-R1b is not yet entirely clear[76].

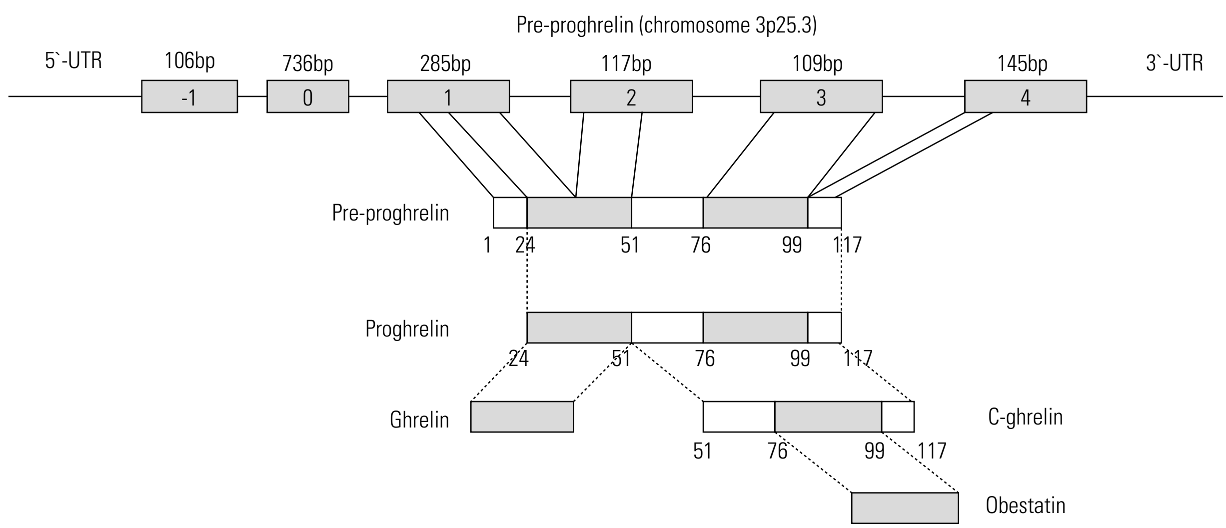

Figure 2. The human ghrelin gene (*GHLR*), also called the pre-proghrelin gene and its products. The upper boxes represent the exons, while the numbers at the bottom represent the amino acids. Adapted from Liu *et al.* 2011[70].

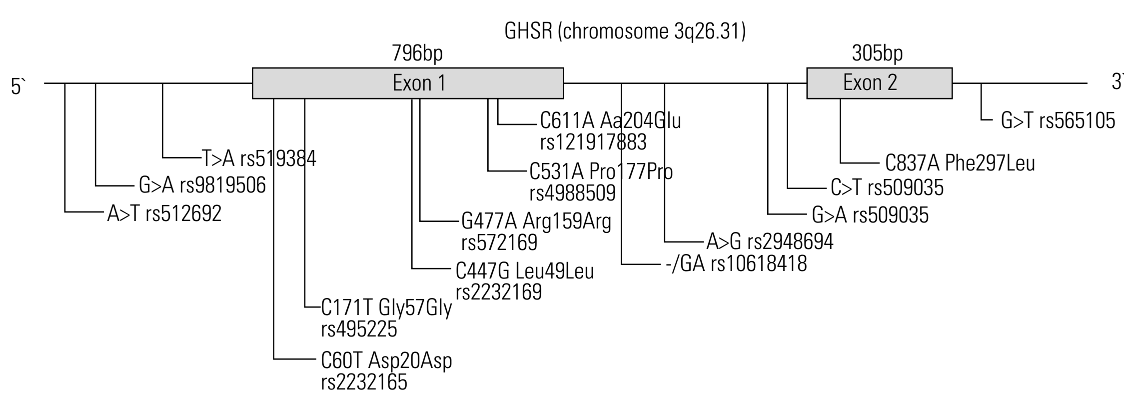

Figure 3. The growth hormone secretagogue receptor gene (*GHS-R*) and single nucleotide polymorphisms (SNPs) that are most researched in this gene, accompanied by their identification numbers. Adapted from Liu *et al.* 2011[70].

The gene of GOAT (MBOAT4; Gene ID: 619373)[71] is located in the chromosome 8 (8p12) and is expressed mainly in the stomach, in the pancreas and in lower concentrations in the bones[77,78]. This gene represents a new candidate gene in genetic research for investigating complex phenotypes[70] (Figure 4).

In table 2 below, you can see some studies that investigated single nucleotide polymorphisms (SNPs) in the ghrelin gene, in individuals with a diagnosis of an ED. It is noticeable that the studies were still inconclusive when the GHRL gene is investigated, these studies show different positive and negative associations with different EDs diagnoses[73,81,82,84,86]. However, when they analyzed the genes of the GHS-R and of the GOAT some studies have found a positive association between polymorphisms and the EDs[79]. In this sense, only two studies have found a positive association between polymorphisms in the GHS-R and in the GOAT with BN and AN respectively, which is that of Miyasaka *et al.* 2006[83] and Muller *et al.* 2010[85].

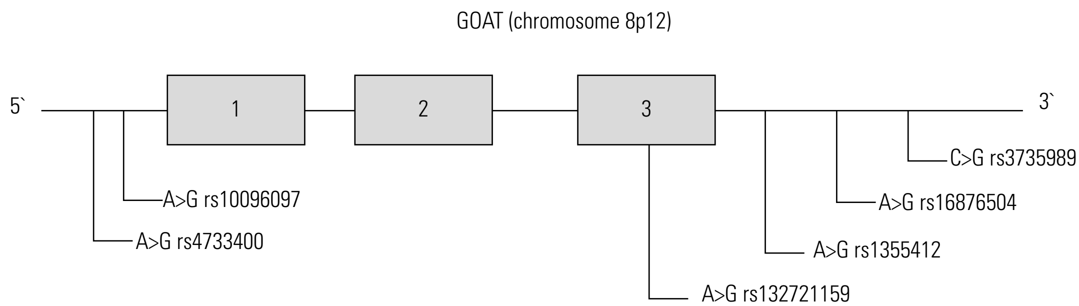

Figure 4. The enzyme ghrelin O-acyltransferase gene (*GOAT*) and single nucleotide polymorphisms (SNPs) that are most researched in this chromosome, accompanied by their identification numbers. Adapted from Liu *et al.* 2011[70].

Table 1. Studies of ghrelin plasma in differents EDs diagnoses, ordered by month and year of publication

Authors and year	Diagnoses studied	Hypotheses/ Objectives	Sample	Age years (mean ± SD)	BMI kg/ score (mean ± SD)	Measurement ghrelin	Collection of the blood sample	Main analyzes used statistics	Results
Ariyasu *et al.* 2001[48]	AN and GP	To estimate the plasma ghrelin in humans, ghrelin -LI fasting and after the meals	N			RIA	Overnight fasting plasma	Student's t-test, linear regression analysis	The concentration of ghrelin was higher in AN, found a negative correlation between ghrelin levels and BMI compared to female CO
			33 GP	68,0 ± 4,0	23,3 ± 2,8				
			31 AN	25,0 ± 1,0	DNS				
			Control Group						
			61 CO (35 female)	26,0 ± 1,0 (DNS female)	20,7 ± 0,3 (20,4 ± 0,4 female)				
Otto *et al.* 2001[46]	AN	Investigate the involvement of ghrelin in the pathogenesis of EDs, analyze circulating levels of ghrelin and its possible correlations with clinical parameters before and after weight gain	N			RIA	Overnight fasting plasma	DNS	The concentration of plasma ghrelin was higher in AN, after partial improvement, there was a decrease in circulating ghrelin (25%). Negative correlation with Delta BMI
			36 AN	25,0 ± 1,2	15,2 ± 0,2				
			Control Group						
			24 CO	31,0 ± 1,4	22,9 ± 0,4				
Shiiya *et al.* 2002[37]	OB, AN and DM2	Research on ghrelin in metabolic balance, measurement of plasma ghrelin responses in plasma ghrelin in CO and DM2 and investigation of 24 hours of circulating ghrelin profile	N			RIA	Fasting plasma and postprandial (0, 3, 5, 10, 15, 30, 60 and 120 min)	ANOVA, *post hoc* Fisher's test, linear regression analysis	The concentration of plasma ghrelin was high in the AN, and low in BN, it was negatively correlated with BMI
			17 AN	22,2 ± 2,3	14,2 ± 0,5				
			11 OB	35,1 ± 3,7	30,4 ±1,2				
			42 DM2	58,5 ±1,6	DNS				
			Control Group						
			28 CO	30,4 ± 4,1	22,7 ± 0,4				
Tanaka *et al.* 2002[54]	BN	Concentration of plasma ghrelin fasting will be increased in the BN or show some specificity regarding the pathology	N			RIA	Overnight fasting plasma	Student's t-test, linear regression analysis	The concentration of ghrelin was greater in BN. Ghrelin fasting was negatively correlated with BMI and% body fat
			15 BN	23,3 ± 5,3	20,0 ± 2,9				
			Control Group						
			11 CO	24,0 ± 1,9	21,1 ± 1,2				

continuation

Authors and year	Diagnoses studied	Hypotheses/ Objectives	Sample	Age years (mean ± SD)	BMI kg/ score (mean ± SD)	Measurement ghrelin	Collection of the blood sample	Main analyzes used statistics	Results
Tolle *et al.* 2003[51]	AN	Measuring plasma levels of ghrelin in the AN before and after renutrition in women CT and CO	N			RIA	Fasting plasma and postprandial (hours: 800, 1200, 1600, 2000, 2400 and 400 h)	ANOVA, t-test	High plasma levels of ghrelin in the AN, which remained high throughout the day and decreased after renutrition. The CT group had intermediate levels of ghrelin
			9 AN	17,2 ± 0,9	14,6 ± 0,4				
			7 CT	23,3 ± 3,1	15,7 ± 0,4				
			Control Group						
			10 CO	23,2 ± 1,1	21,5 ± 0,7				
Tanaka *et al.* 2003a[59]	AN BN	The presence and frequency of purging behaviors can influence the levels of ghrelin	N			RIA	Overnight fasting plasma	Linear regression analysis, ANOVA, *post-hoc* Fisher's test	Mean plasma ghrelin was higher in the AN-R, AN-P and BN-P, was significantly higher in AN-P than AN- R. Ghrelin fasting was negatively correlated with BMI and % body fat
			21 AN-R	21,8 ± 8,9	13,9 ± 1,9				
			19 AN-P	24,6 ± 5,5	14,4 ± 2,1				
			18 BN-P	22,7 ± 5,0	20,0 ± 2,1				
			13 BN-NP	22,7 ± 6,5	21,2 ± 3,9				
			Control Group						
			15 CO	22,1 ± 3,4	21,4 ± 11,0				
Nedvidkova *et al.* 2003[45]	AN	Study the response of plasma ghrelin, to food intake, meal volume and nutritional value	N			RIA	Fasting plasma and postprandial (30, 60, 90, 120 min)	Unpaired t-test, Mann-Whitney rank Test, correlations Spearman's, ANOVA, Dunnet´s test	The concentration of plasma ghrelin was two times higher in the AN, and was negatively correlated with % body fat. There was no change in the concentration of ghrelin in the AN after 2 h meal
			5 AN	24,3 ± 2,6	15,2 ± 1,5				
			Control Group						
			6 CO	22,9 ± 4,7	21,6 ± 1,2				
Tanaka *et al.* 2003b[60]	AN	Measure plasma concentrations of ghrelin between subtypes of AN	N			RIA	Overnight fasting plasma	Linear regression analysis, ANOVA, *post-hoc* Scheffe's test	The plasma levels of ghrelin were higher in AN-R and AN-P. Ghrelin was even higher in the AN-P as compared to the AN-R. Ghrelin fasting was negatively correlated with BMI in AN
			19 AN-R	20,1 ± 4,9	13,6 ± 1,5				
			20 AN-P	21,9 ± 4,7	13,7 ± 1,9				
			Control Group						
			11 CO	21,0 ± 1,9	21,4 ± 1,2				
Monteleone *et al.* 2003[52]	BN	Study of ghrelin and leptin responses meals in BN and CO	N			RIA	Fasting plasma and postprandial (0, 45, 60, 90, 120 and 180 min)	ANOVA, 2-way ANOVA with repeated measures e *post-hoc* Turkey´s, correlation Pearson´s	Found no difference between groups in fasting ghrelin. Noted that the postprandial ghrelin remained increased in the BN (90, 120 and 180 min). Ghrelin fasting was negatively correlated with body fat
			9 BN-P	24,2 ± 2,3	21,7 ± 3,4				
			Control Group						
			12 CO	24,5 ± 2,6	21,5 ± 1,8				
Tanaka *et al.* 2003c[61]	AN	The differences in eating behavior can influence the secretion of ghrelin and insulin in AN	N			RIA	Fasting plasma and postprandial (0, 30, 60, 120 and 180 min)	ANOVA, *post-hoc* Scheffe's test, Kruskal–Wallis, chi-square statistic	The baseline ghrelin in AN-R and AN-P was significantly higher compared with CO. In the AN-P was found delayed recovery levels of ghrelin postprandially (120 and 180 min)
			11 AN-R	18,5 ± 1,4	13,3 ± 0,4				
			9 AN-P	20,9 ± 1,4	13,8 ± 0,5				
			Control Group						
			10 CO	21,0 ± 0,6	21,4 ± 0,4				

continuation

Authors and year	Diagnoses studied	Hypotheses/ Objectives	Sample	Age years (mean ± SD)	BMI kg/ score (mean ± SD)	Measurement ghrelin	Collection of the blood sample	Main analyzes used statistics	Results
Soriano-Guillen *et al.* 2004[41]	OBCH, AD and AN	To investigate the role of ghrelin in the EDS analysis of baseline ghrelin level in OBCH and AN and the weight loss effect	N			RIA	Overnight fasting plasma	Student´s t test, ANOVA with repeated measures, *post-hoc* Scheffe's test, correlation analysis	Ghrelin levels were decreased in OBCH and not normalized after weight reduction. Also found increased levels in AN
			26 OBCH	8,0 ± 1,3	SD 4,4 ± 1,8				
			16 AN	17,0 ± 1,6	SD -2,2 ± 0,4				
			Control Group						
			21 CH	6,3 ± 3,0	SD 0,1 ± 1,0				
			20 AD	17,2 ± 0,4	SD 0,3 ± 0,8				
Misra *et al.* 2004[49]	AN	Ghrelin values may be higher in AN than in healthy adolescents	N			RIA	Overnight fasting plasma	Student´s t-test, Wilcoxon's test, chi-square statistic	Ghrelin levels were higher and decreased postprandially ghrelin was also high in AN-R
			19 AN-R	16,1 ± 1,1	16,9 ± 1,6				
			Control Group						
			20 CO	15,4 ± 1,8	21,8 ± 3,7				
Nakazato *et al.* 2004[66]	BN	Determination of serum ghrelin levels and compare with the BDNF reported in a previous article	N			EIA	Postprandial (11:00-12:00 am)	Student's t-test, Mann-Whitney, correlation Pearson's	There was no significant correlation between the levels of ghrelin and BDNF
			18 BN (BN-P e BN- NP)	21,6 ± 4,0	20,4 ± 2,1				
			Control Group						
			21 CO	21,4 ± 1,7	20,0 ± 1,5				
Tanaka *et al.* 2004[62]	AN	Measuring ghrelin and GH in AN during treatment to evaluate the effect of nutritional rehabilitation of these substances in	N			RIA	Fasting plasma	Linear regression analysis, ANOVA, *post-hoc* Sheffés, Kruskal-Wallis, chi-square statistic	The fasting ghrelin was found too high in AN -E group, and the high in AN-P and AN-R before treatment. It remained high in AN-R during the treatment and after the treatment it maintained high only in the AN-P group. The concentration of ghrelin was negatively correlated with BMI before and during treatment
			7 AN-E	18,1 ± 1,2	11,1 ± 0,3				
			14 AN-R	18,4 ± 1,3	13,1 ± 0,2				
			13 AN-P	25,0 ± 1,3	14,5 ± 0,3				
			Control Group						
			9 CO	21,5 ± 0,9	21,5 ± 0,4				
Kojima *et al.* 2005[65]	BN	To investigate the changes in plasma ghrelin and PYY postprandial after the meal in the BN and CO	N			RIA	Overnight fasting plasma and postprandial (0, 30, 60, 120 and 180 min)	Student's t-test, ANOVA with repeated measures, correlation Pearson's	Concentration of plasma ghrelin was high in the BN and remained high after the meal
			10 BN-P	24,7 ± 1,5	20,0 ± 0,6				
			Control Group						
			12 CO	24,8 ± 0,8	20,2 ± 0,5				
Monteleone *et al.* 2005[53]	BN, BED and OB	To investigate the changes of plasma ghrelin in EDs	N			RIA	Overnight fasting plasma	Kruskal-Wallis, Mann-Whitney, correlations Spearman's	Plasma ghrelin reduced in BED and OB, but found no changes in BN. Ghrelin was negatively correlated with body weight, BMI and body fat in all sample
			13 BED NO	26,9 ± 8,0	25,8 ± 2,5				
			34 BED OB	33,6 ± 9,1	39,8 ± 4,9				
			56 BN-P	23,4 ± 4,3	21,9 ± 3,8				
			Control Group						
			28 OB	38,4 ± 14,1	38,1 ± 6,3				
			51 CO	22,6 ± 3,1	21,7 ± 2,3				
Monteleone *et al.* 2005[67]	BN	Investigate the total PYY and ghrelin responses after a high fat meal in BN and CO	N			RIA	Fasting plasma and postprandial (0, 45, 60, 90, 120 and 180 min)	ANOVA, 2-way ANOVA with repeated measures, *post hoc* Turkey, multiple regression analysis	There was no difference in the concentration of ghrelin fasting. The postprandial ghrelin remained higher in BN
			9 BN-P	24,5 ± 2,6	21,5 ± 1,8				
			Control Group						
			10 CO	24,2 ± 3,9	21,7 ± 3,4				

continuation

Authors and year	Diagnoses studied	Hypotheses/ Objectives	Sample	Age years (mean ± SD)	BMI kg/ score (mean ± SD)	Measurement ghrelin	Collection of the blood sample	Main analyzes used statistics	Results
Stock *et al.* 2005[39]	AN and OB	PYY may be higher in AN and the response of PYY, ghrelin, GIP and satiety to mixed meals can be impaired in AN and obesity	N			RIA	Fasting plasma and postprandial (15, 60, 90, 120, 180 and 240 min)	ANOVA, *post-hoc* Bonferroni, correlation, Wald test, correlation Pearson's	Ghrelin was found lower in obesity, with respect to CO and AN. The response of ghrelin in each group showed a significant difference over time
			10 AN	16,5 ± 0,4	16,3 ± 0,4				
			10 OB	14,2 ± 0,3	34,4 ± 2,0				
			Control Group						
			10 CO	14,8 ± 0,3	20,2 ± 0,4				
Geliebter *et al.* 2005[68]	BED and SBED	BED patients have higher levels of postprandial ghrelin, as a gastric emptying rate slower and less that the postprandial CCK than CO	N			RIA	Overnight fasting plasma and postprandial (-15, 0, 5, 15, 30, 60, 120 min)	GLM, *post-hoc* Turkey´s	Plasma ghrelin was smaller and had a smaller decline after the meal in the BED compared to CO
			11 BED	29,0 ± 8,4	36,6 ± 6,2				
			14 SBED	28,6 ± 6,7	35,9 ± 5,3				
			Control Group						
			12 OB	33,1 ± 8,7	35,3 ± 5,5				
Otto *et al.* 2005[58]	AN	To investigate the suppression of postprandial ghrelin in AN during weight gain	N			RIA	Overnight fasting plasma and postprandial (20 and 60 min)	ANOVA of repeated measurement, Wilcoxon test	Increased levels of ghrelin in AN fasting and there was a significant decrease after the weight gain
			20 AN	25,6 ± 1,0	15,1 ± 0,3				
			Control Group						
			6 CO	28,8 ± 1,0	21,1 ± 0,7				
Troisi *et al.* 2005[63]	AN, BN and BED	To investigate the relationship between plasma ghrelin, cortisol, thyroid hormones and dietary patterns of AN, BN and BED. Analyzing the groups To by the criterion of bingeing and purging	N			RIA	Overnight fasting plasma	ANOVA, Student's t-test, *post-hoc* Sheffé, Stepwise's regression	High plasma concentrations of ghrelin in the AN, BN and BED were found. EDs concentrations of plasma ghrelin was negatively correlated with BMI. Positive correlation between the concentrations of ghrelin and disordered eating behavior.
			13 AN-R	26,6 ± 6,7	15,9 ± 2,3				
			16 BN (AN-P e BN-P)	29,2 ± 11,4	26,0 ± 7,5				
			21 BED (BN-NP e BED)	38,0 ± 11,9	33,0 ± 7,8				
			Control Group						
			23 CO	25,5 ± 3,2	21,24 ± 1,8				
Janas-Kozik *et al.* 2007[55]	AN	To investigate the involvement of the AN dysfunction during treatment of ghrelin	N			RIA	Fasting plasma	Student's t-test and Spearman's correlation	The concentration of ghrelin was high in the AN-R and not fully stabilized after treatment. Negative correlation with total plasma ghrelin and BMI in AN-R after treatment
			30 AN-R	18,0 ± 2,0	15,1 ± 1,4				
			Control Group						
			20 CO	18,5 ± 0,5	21,4 ± 2,1				
Nakahara *et al.* 2007[56]	AN	Measure ghrelin, PYY3-36, glucose and insulin after a meal to evaluate the effect of nutritional status in AN during hospitalization	N			RIA	Overnight fasting plasma and postprandial (0, 30, 60, 120 and 180 min)	ANOVA and *post-hoc* Sheffé, 2-way ANOVA with repeated measures	Plasma ghrelin fasting was higher in AN, after treatment decreased but remained higher compared to CO
			14 AN-R	24,6 ± 6,0	12,4 ± 1,7				
			Control Group						
			12 CO	25,7 ± 6,7	22,3 ± 2,2				
Monteleone *et al.* 2008[47]	AN and BN	Measure circulating levels of ghrelin/obestatin and evaluating its relationship with anthropometric and clinical measures in BN, AN and CO	N			ELISA	Overnight fasting plasma	Shapiro Wilk normality test, ANOVA, Pearson´s correlation	The concentration of ghrelin was higher in the AN, regardless of subtype. No difference to the BN was found. The concentration of plasma ghrelin in the AN had a positive correlation with body fat and BMI
			21 AN (AN-R e AN-P)	23,4 ± 7,5	16,6 ± 1,6				
			21 BN	26,2 ± 7,1	21,4 ± 3,3				
			Control Group						
			20 CO	23,6 ± 5,5	21,1 ± 2,2				

continuation

Authors and year	Diagnoses studied	Hypotheses/ Objectives	Sample	Age years (mean ± SD)	BMI kg/ score (mean ± SD)	Measurement ghrelin	Collection of the blood sample	Main analyzes used statistics	Results
Monteleone *et al.* 2010[64]	BN	To investigate the ghrelin response in "misleading" feedback on BN and CO	N			RIA	Fasting plasma and postprandially (0, 15, 30, 45, 90 and 120 min)	ANOVA, 2-way ANOVA with repeated measures and *post-hoc* Turkey's, Pearson's correlation	In BN ghrelin was high postprandially. The response of ghrelin was positively correlated with the frequency of bingeing and purging weekly and disease duration
			6 BN-P	DNS	DNS				
			Control Group						
			7 CO	DNS	DNS				
Terra *et al.* 2013[57]	AN	Studying levels of circulating adipocytokines in AN and CO	N			ELISA	Overnight fasting plasma	Student's t-test, Pearson's correlation, linear regression analysis	There was no difference in the concentration of ghrelin fasting. Negative correlation with BMI and the plasma ghrelin in AN-R after treatment.
			28 AN-R	27,4 ± 1,4	16,8 ± 0,2				
			Control Group						
			33 CO	32,6 ± 1,3	21,8 ± 0,3				

AD: adolescents; AN: anorexia nervosa; AN-E: anorexia nervosa with emergent hospitalization; AN-P: anorexia nervosa purging type; AN-R: anorexia nervosa restritive type, ANOVA: analysis of variance (one-way); BED: binge eating disorder; BN: bulimia nervosa; BN-P: bulimia nervosa purging type; BN-NP: bulimia nervosa nonpurging type; BNDF: brain-derived neurotrophic factor; BMI: body mass index; CCK: cholecystokinin; CH: Childs; CO: controls; CT: constitutionally thin subjects; DM2: *diabetes mellitus* type 2; DNS: data had not shown; ED: eating disorder; EIA: enzyme immunoassay; ELISA: enzyme-linked immunosorbent assay; GH: growth hormone; Ghrelin-LI: ghrelin-like immunoreactivity; GIP: gastric inhibitory polypeptide; GLM: generalized linear model; GP: gastrectomized patients; NO: non-obese patients; OB: obese patients without eating disorders; OBCH: obese childs without eating disorders; PYY: peptide YY; RIA: radioimmunoassay; SD: BMI curves above Spanish standards; SBED: subthreshold binge eating disorder.

Table 2. Studies of candidate genes for polymorphisms in the ghrelin gene (GHRL), the ghrelin O-acyltransferase (GOAT) and the GH secretagogue receptor (GHS-R) in EDs diagnoses

Authors and Year	Diagnoses studied	Hypotheses	Gene	Polymorphisms	N	Control Group	Associations	Conclusion	Country of Study
Cellini *et al.* 2006[73]	AN and BN	To analyze whether polymorphisms of the ghrelin gene which may be involved in the etiology of the EDs.	GHRL	-Gln90Leu, -Leu72Met, -Arg51Gln,	-366 AN -326 BN -529 AN and BN family trios	342 Control Subjects	No association was found	Unlikely that these polymorphisms are related to EDs in the European population	Europe
Ando *et al.* 2006[81]	AN and BN	Is Ghrelin involved in the etiology of the EDs?	GHRL	-Leu72Met, -3056 T>C (rs2075356)	-131 AN-R -97 AN-P -108 BN	300 Control Subjects	Found in the two polymorphisms for BN	These polymorphisms may be involved in the etiology of ED	Japan
Monteleone *et al.* 2006[82]	AN and BN	Functional variations in the ghrelin gene may contribute to genetic susceptibility to ED or modulate some aspect of the phenotype of the EDs	GHRL	-Arg51Gln, -Leu72Met	-114 BN -31 AN-R -29 AN-P	119 Control Subjects	No association was found	Suggest that these polymorphisms of ghrelin should not contribute to the genetic vulnerability for AN or BN	Italy
Miyasaka *et al.* 2006[83]	AN, BN and EDNOS	Investigate a new polymorphism in the GHS-R because the polymorphism in GHRL-Leu72Met was not previously detected in the Japanese population	GHS-R	-rs495225 (171C to T)	-96 AN -116 BN -16 EDNOS	284 Control Subjects	Found for BN	This polymorphism may be a risk factor for BN	Japan
Dardennes *et al.* 2007[84]	AN	To analyze whether polymorphisms of the ghrelin gene which may be involved in the etiology of the EDs.	GHRL	-Gln90Leu, -Leu72Met,	-114 AN-R and related -90 AN-P and related	Does not exist	Found in the AN-P in the Leu72Met	Genetic analyses with simultaneous genetic-biological determinants may help explain the high degree of heritability and the standard pathophysiological description of the EDs	France

continuation

Authors and Year	Diagnoses studied	Hypotheses	Gene	Polymorphisms	N	Control Group	Associations	Conclusion	Country of Study
Muller *et al.* 2010[85]	AN	Verify whether genetic variants of GOAT are involved in the etiology of AN	GOAT	-rs1355412, -rs10096097, -rs16876504, -rs3735989, -rs13272159, -rs4733400	-543 AN	612 Control Subjects	AN found in the G/G genotype for the SNP rs10096097	GOAT Genetic variation may be related to the etiology of AN	Germany
Kindler *et al.* 2011[86]	AN, BN and BED	Genetic factors are likely to contribute to the biological vulnerability to the EDs	GHRL	-Arg51Gln, -Leu72Met, -Gln90Leu	-46 AN -30 BN -38 BED	164 Control Subjects	No association was found	Previouse positive associations with polymorphisms of the ghrelin gene could not be replicated	Austria

Conclusion

In recent years, ghrelin has been an object of study in the EDs, but we haven't had any clear conclusions about its role in these pathologies. Genetic research could bring a different perspective and provide a new direction for research.

The studies suggest that some polymorphisms in the ghrelin gene, mainly in the genes of GHS-R and the GOAT, may be involved in the pathogenesis of the EDs and possibly related to the behavior of binge eating and purging. However, this is a case of only two studies, further work should be conducted with larger samples addressing the need to compare the polymorphisms found between the three main types of eating disorders (AN, BN and BED) in order for greater clarity in the associations.

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