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MASTERARBEIT / MASTER'S THESIS

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"A comparison of ghrelin values in healthy, obese and patients with eating disorders"

verfasst von / submitted by

Julia Bichl BSc

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Zusammenfassung

Hintergrund und Ziel:

Ghrelin ist ein Sättigungshormon welches im Magen freigesetzt wird und erstmals 1999 von Kojima et al. entdeckt wurde. Innerhalb der letzten Jahre wurde viel Forschung auf diesem Gebiet betrieben, bis jetzt konnten aber noch nicht alle Funktionen und die Wirkungsweise des Hormons geklärt werden. Dieser systematische Review untersucht Unterschiede in den Ghrelinwerten bei Gesunden, Übergewichtigen und Patienten mit Essstörungen. Zusätzlich wird auch versucht, die jeweiligen Durchschnittswerte in den Gruppen festzulegen.

Methode:

Die Literatursuche wurde im Internet in den folgenden Datenbanken durchgeführt: PubMed, Science Direct und Embase. Jede Studie musste definierte Einschluss- und Ausschlusskriterien erfüllen, um in der Arbeit verwendet zu werden.

Resultate:

Insgesamt konnten 24 Studien mit einer Gesamtpopulation von 1206 Personen die Kriterien erfüllen. Nach der Analyse aller Daten hat sich herausgestellt, dass Personen mit Essstörungen die Einzigen sind, bei denen Unterschiede in den Ghrelinwerten feststellbar waren. Die Unterteilung in Anorexia Nervosa (AN), Bulimia Nervosa (BN) und Binge Eating Disorder (BED) zeigten die folgenden Durchschnittswerte für Ghrelin: 1023 pg/ml, 701 pg/ml und 357 pg/ml. Für Gesunde und Übergewichtige konnte kein Durchschnittswert definiert werden, da die Werte jeweils eine enorme Streuung von 78 bis 819 pg/ml bei Gesunden und 67 bis 708 pg/ml bei Übergewichtigen aufwies.

Fazit:

Intensivere Forschung in Bezug auf standardisierte Verfahren zur Probenentnahme, Lagerung und der Messung von Ghrelin sind notwendig. Das stellt die Grundlage für die Vergleichbarkeit der Werte und die Definition eines Durchschnittswertes dar.

Keywords: Ghrelin, Durchschnittswerte, Gesunde, Übergewicht, Essstörungen.

Abstract

Background and objective:

Ghrelin is a novel gastric hormone that was first isolated by Kojima et al. in 1999. In recent years much research on this new hormone has been carried out, but still not all functions and modes of action have been resolved yet. This systematic review investigates the different ghrelin values of obesity patients, patients with eating disorders and healthy people. A further aim was also put onto defining mean ghrelin values for each of these groups.

Method:

Literature search was conducted using the following online in databases: PubMed, Science Direct, and Embase. Studies had to meet defined inclusion and exclusion criteria for being included in the systematic review.

Results:

A total of 24 studies including 1206 patients were identified to meet all inclusion criteria. After analysis only ghrelin values of patients with eating disorders showed statistical differences. Mean values for the subtypes of Anorexia Nervosa (AN), Bulimia Nervosa (BN) and Binge Eating Disorder (BED) were 1023 pg/ml, 701 pg/ml and 357 pg/ml, respectively. Mean values for healthy and obese patients were not able to be defined, as values covered a broad range from 78 to 819 pg/ml for healthy, and 67 to 708 pg/ml for obese patients.

Conclusion:

Further research is needed to create a standard performance regarding sample collection, preservation and sample preparation methods, and measurement techniques for ghrelin. This is the most important basis for comparable results and for the definition of mean ghrelin values.

Keywords: ghrelin, mean values, healthy, obese, eating disorders.

List of abbreviations

AC	Acylated Ghrelin
AGB	Adjustable Gastric Banding
AgRP	Agouti related Protein
AN	Anorexia Nervosa
AN-BP	Anorexia Nervosa - Binge Purging
ARC	Arcuate Nucleus
BED	Binge Eating Disorders
BES	Binge Eating Scale
BITE	Bulimic Investigation Test Edinburgh
BMI	Body Mass Index
BN	Bulimia Nervosa
BN-BP	Bulimia Nervosa - Binge Purging
BN-NP	Bulimia Nervosa - Non Purging
BPD	Billiopancreatic Diversion
CART	Cocaine and Amphetamine Regulated Transcript
CCK	Cholecystokinin
DSM-IV	Diagnostic and Statistical Manual of Psychiatric Disorders - Fourth Edition
ED	Eating Disorders
EDNOS	Eating Disorder Not Otherwise Specified
EDTA	Ethylene diamine tetra acetic
EIA	Enzyme Immunoassay
ELISA	Enzyme linked immunosorbent assay
FDA	Food and Drug Association

GHS-R1a	Growth Hormone Secretague Receptor 1a
GOAT	Ghrelin O-Acyltransferase
HCl	Hydrogen Chloride
HCN	Hydrogen Cyanide
LH	Lateral Hypothalamus
LSG	Laparoscopic Gastric Banding
mRNA	messenger Ribonucleotide Acid
NFI	No Further Information
NHLBI	National Heart Lung and Blood Institute
NPY	Neuropeptide Y
pH	potentia Hydrogenii
POMC	Propriomalonocortin
PRAN	Partially Recovered Anorexia Nervosa
PVN	Para Ventricular Nucleus
PWS	Prader Willi Syndrome
PYY	Peptide YY
QEWP	Questionnaire of Eating and Weight Patterns
RIA	Radioimmunosorbent Assay
RYBG	Roux-en Y Gastric Banding
T2DM	Type 2 Diabetes Mellitus
UAC	Unacylated Ghrelin
VMH	Ventromedial Hypothalamus
WHO	World Health Organization

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1. INTRODUCTION AND QUESTION

In the past few years increasing interest has been ascribed to the novel gastric hormone ghrelin. The term “ghrelin” consists of “ghre” which stands for “growth” in many Indo-European languages and “relin” which stands for “hormone-release”, one of the characteristic effects of ghrelin (Kojima et al., 1999). Through its multifunctional activities in pancreas, liver, hypothalamus, intestine, stomach, heart, and adipose tissue, intensive research has been done around this topic.

Ghrelin was discovered in 1999 as a hunger signal (Kojima et al., 1999) and until today still not all functions and modes of action, which are also attributed to ghrelin, have been described. Apart from these unsolved questions regarding the modes of action, there are still no average values defined. Indeed, many authors report negative correlations between ghrelin and Body Mass Index (BMI) as far as obese people are concerned. In contrast, anorectic patients have higher ghrelin values, but nevertheless, these findings do not contribute to define mean or standard values (Cummings et al., 2002). It was not possible to find any precise explanation regarding the normal range of ghrelin values, in case of obese patients for example, even conflicting results were highlighted.

After a general research in literature about ghrelin, it turned out that concerning different patient groups, there has not been done a systematic review before.

The topic about general standard values seemed to be the most interesting. This fact and, of course, the disagreement concerning ghrelin values represent the incentive to write a systematic review. Furthermore, this systematic review challenges the question whether ghrelin is different within three different patient groups. Given that ghrelin is a hunger signal, it suggests itself that patient groups with ED represent an interesting population. Treatment with ghrelin could possibly improve the symptoms of ED. Also the group of obese patients can provide new information about the reason for their non-satiation or maybe an excessively distribution of ghrelin, which could deliver the answer for being obese.

Should it be possible to discover these findings, ghrelin or anti-ghrelin hormones would play an important role in decreasing or even curing disorders, which are related to food intake or satiation. The third group will represent healthy patients, with whom the general range or value of ghrelin could be defined.

2. LITERATURE SURVEY

2.1 Ghrelin – the "hunger hormone"

Ghrelin, a gastric hormone, was first isolated in 1999 as the endogenous ligand for the growth hormone secretagogue receptor 1a – GHSR1a. This receptor consists of 336 amino acids and belongs to the group of G-protein coupled receptors (Kojima et al., 1999). The human ghrelin gene is located on chromosome 3 (Wajnrajch et al., 2000). There are two biological active peptides – ghrelin and des-Gln^{14}-ghrelin, whereas the latter is present just in small amounts in the stomach. The major active form is ghrelin (Kojima et al., 2001).

The human ghrelin gene generates a peptide of 117 amino acids, which is also called preproghrelin. There are several different products spawning from the precursor. The most metabolically relevant are acyl ghrelin (AC/AG) and des-acyl ghrelin (DAG or unacylated ghrelin – UAC/UAG) (Nishi et al., 2011). Acyl ghrelin consists of 28 amino acids with an n-octanoylation at Ser3. This modification is essential for ghrelin to bind to GHSR1a. Ghrelin O-acyl transferase (GOAT), identified by Gutierrez et al., is required for the metabolic activation of ghrelin. Octanoyl and decanoyl fatty acids are optimal for acyl modification and consequently the function of ghrelin (Gutierrez et al., 2008). Ghrelin mRNA is expressed in several tissues, but with the main distribution in the stomach, where ghrelin containing cells are found to a distinct endocrine cell type in the submucosal layer. These are called as X/A like cells, which represent a major endocrine population in the oxyntic mucosa. (Dornonville de la Cour et al., 2001)

In addition to the stomach, ghrelin is also expressed in other tissues like hypothalamus, pituitary, heart, the small and the large intestine, placenta, pancreas, kidney, testes, and lymphocytes. Besides the most important role of ghrelin as a growth hormone releasing factor, it also has functions including hypothalamic activities. It influences sleep and behavior, stimulates appetite, influences the pituitary gonadal axis, controls gastric motility, and acid

secretion. It also affects the cardiovascular system, the immune system and induces adiposity (Katergari et al., 2008). In the adipose tissue, ghrelin influences lipid oxidation and inflammation by a lowering effect and increases lipogenesis (Müller et al., 2015).

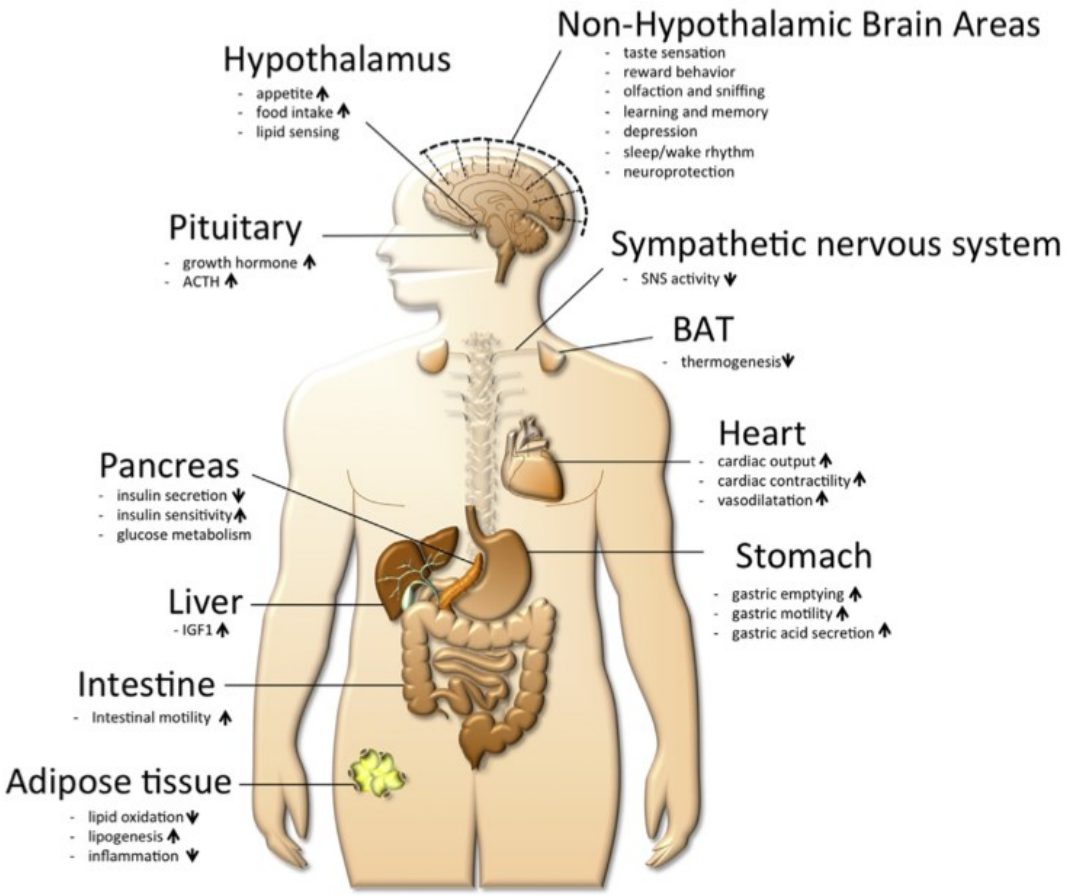

Figure 1: Schematic on ghrelin's physiological effects (Müller et al., 2015)

Ghrelin might also have an important role in taste sensation. Preproghrelin, PC1/3, ghrelin, GHSR and GOAT are expressed in type I - IV taste cells of mouse taste buds. The co-localization of ghrelin and its subtypes in taste cells suggest that ghrelin may work in an autocrine manner. Furthermore, GHSR null mice show significantly decreased taste responsivity to sour and salty tastants. These findings could bring greater understanding about the exact functioning of

taste sensitivity and how energy balance is linked to taste perception.(Shin et al., 2010)

Ghrelin levels change throughout the development of an individual. Highest levels are found during early postnatal life when growth and changes in food intake occur. These changes are regulated by somatotropin, the so-called “growth hormone” which, among other stimulators, can be influenced by ghrelin (Soriano-Guillen et al., 2004). Ghrelin levels also differ between men and women, whereas levels in women are higher in the late follicular stage compared to men´s levels (Barkan et al., 2003).

Ghrelin levels are negatively correlated with the BMI. There are decreased levels in obesity and increased levels in states of malnutrition, for example AN and BN. An exception is the Prader-Willi syndrome (PWS), the most common form of human syndromic obesity. It is characterized by severe hyperphagia, cognitive impairment, GH deficiency, hypogonadism, neonatal hypotonia, dysmorphic features (Burman et al., 2001) and high ghrelin levels. Cummings et al. reported ghrelin levels which were 4.5-fold higher in PWS subjects than in equally obese controls and 2.5-fold higher than in lean controls (Cummings et al., 2002).

Shiya et al. reported whether there are differences in ghrelin levels between patient groups of lean, obese and patients with type 2 diabetes mellitus (T2DM). They highlighted a negative correlation between ghrelin and BMI in all patient groups, therefore the negative correlation of ghrelin levels exists in non-diabetic and diabetic patients (Shiiya et al., 2002). In the following table basal ghrelin levels in pathological conditions are compared to healthy individuals.

Disease	Basal Ghrelin Levels Compared to Healthy Subjects
Acromegaly	↓ / ⟷
Prader-Willi Syndrome	↑
GH deficiency	↓ / ⟷
Obesity	↓
Anorexia Nervosa	↑
Bulimia Nervosa	↑
Diabetes	↑ in lean T2DM
	↓ in obese T2DM
	↓ in T1DM
Cardiovascular Disease	⟷ in CHF without cachexia
	↑ in CHF cachexia
Liver Disease	↑ in CLD
	↓ in HCC and NAFLD
Renal Failure	↑
Thyroid disorders	↓ in hyperthyroidism
	⟷ in hypothyroid
Surgical interventions to reduce weight	↓ shortly after BPD,
	↑ long-term after BPD
	↓ after RYGP
	↑ after ASGB
Cancer	↑ in cancer cachexia

↑ : Increased levels, ↓ : decreased levels, ⟷: no change

Table 1: Basal ghrelin levels in pathological conditions compared to healthy subjects (Katergari et al., 2008)

The table also shows that ghrelin levels change after surgical interventions for reducing weight. Shortly after a biliopancreatic diversion (BPD), a bariatric surgical treatment for obesity in which most of the stomach is removed (Medical Dictionary), ghrelin levels decreased. However, twelve month after the BPD, levels increased again, although BMI was reduced. (Garcia-Unzueta et al., 2005)

Ghrelin can be influenced by several factors, including meal caloric content and composition and the nutritional status of an individual. It increases during fasting as a sign of “hunger”, it peaks before meal intake and it is suppressed within an hour after meal ingestion. Direct nutritional sensing also influences levels. Carbohydrates, and to a lesser extent fat, have been shown to reduce ghrelin levels, whereas protein increases levels. A further important effect of ghrelin, compared to other gut hormones, is its orexigenic function. Ghrelin is the only gut hormone which stimulates feeding. (le Roux et al., 2005)

2.1.1 Ghrelin and food intake

The hypothalamic feeding regulation network consists of the ventromedial hypothalamus (VMH), the lateral hypothalamus (LH), the arcuate nucleus (ARC) and the paraventricular nucleus (PVN).

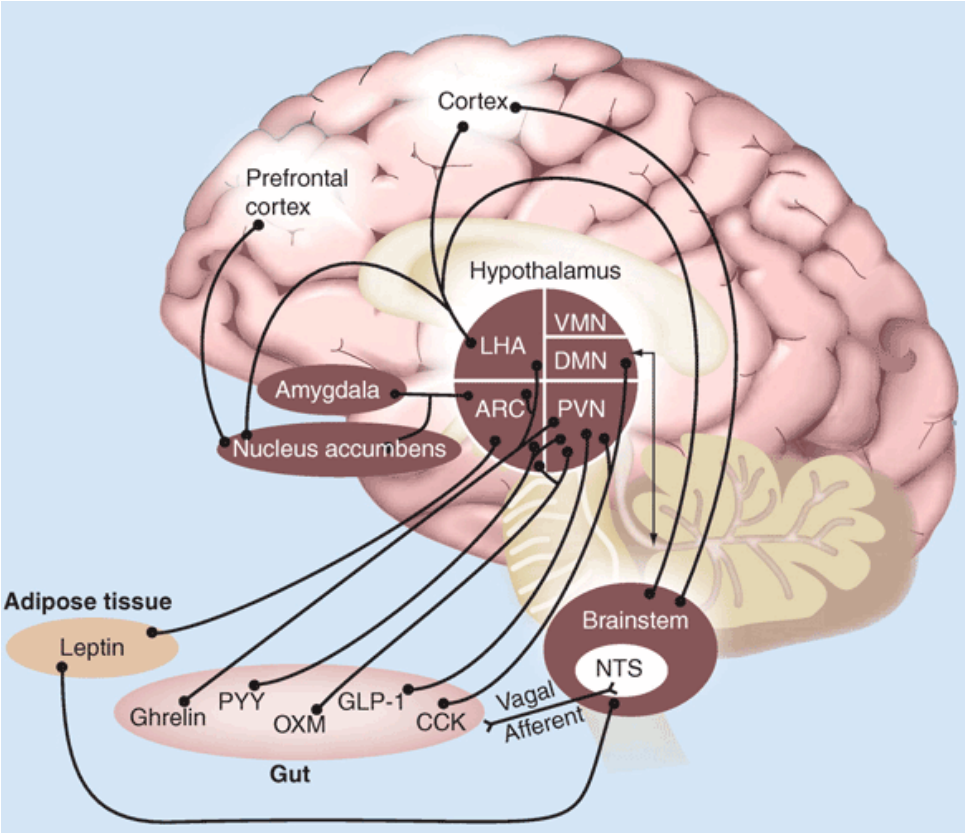

Figure 2:Hypothalamic Regulation of Appetite (Medscape)

There are two types of neurons, classified in increasing or inhibiting food intake. Agouti-related protein neuron (AgRP) and neuropeptide Y (NPY) increase food intake, and in contrast proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) inhibit food intake in the ARC. (Schwartz et al., 2000) Ghrelin influences food intake by activating NPY / AgRP neurons and inhibiting POMC / CART neurons. Other hormones like insulin, leptin, cholecystokinin (CCK) and peptide YY (PYY) lead to a suppression of food intake. (Chen et al., 2009)

In the following figure, a model of feeding regulation can be seen. The hormones leptin, insulin, CCK and PYY and their releasing location show that they stimulate an anorexigenic pathway and at the same time inhibit the orexigenic pathway. Ghrelin has the opposite function, whereas it stimulates the orexigenic pathway. This figure also shows that ghrelin, through its orexigenic function, could be a useful hormone for the treatment of food intake related diseases, for example EDs.

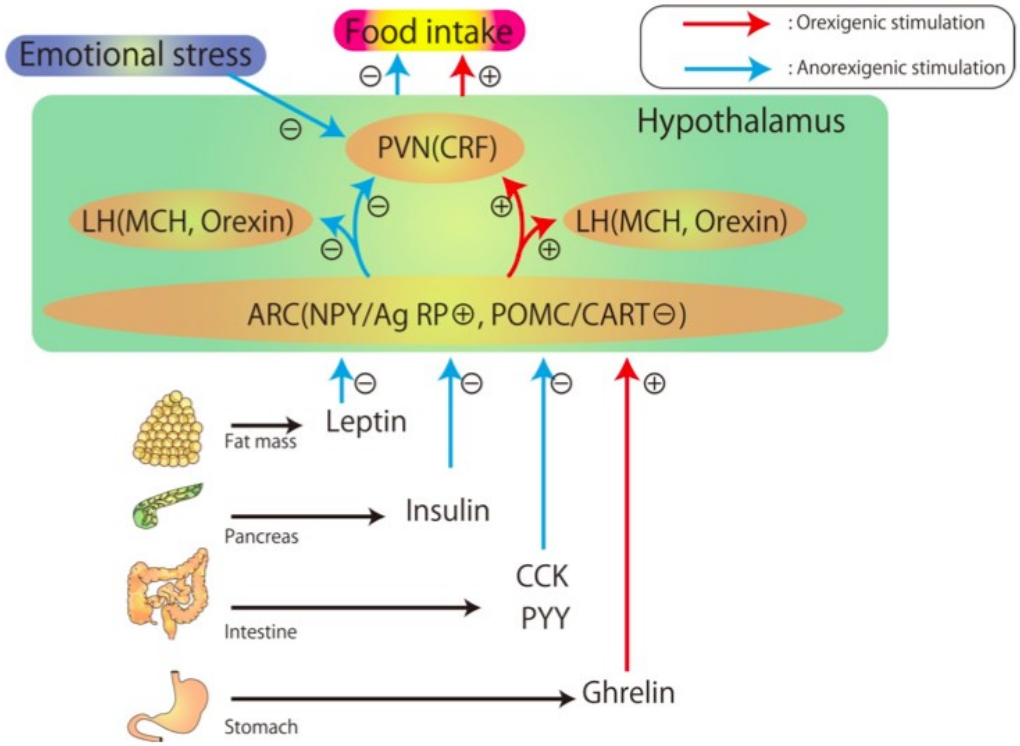

Figure 3: Feeding regulation (Yagi et al., 2012)

2.1.2 Measurement of Ghrelin

Within the last years, numerous ghrelin measurements have been done. Most studies use enzyme-linked immunoassays, also called ELISA, or radio immunoassays, RIA. Besides these common methods, a new approach for quantitative measurement of ghrelin is the multiplex analysis using Luminex technology (Kadas et al., 2014). Dependent on the method, ghrelin can be

analyzed in its total form, or separated as the active (AG) form and inactive (DAG) form. The following descriptions provide an overview of these methods.

Radio immunoassay (RIA)

S.A. Berson and R.S. Yalow developed the radioimmunoassay in the late 1950s for the determination of insulin in human serum. The principle is an isotope dilution technique and the method is based on the formation of an antigen-antibody complex. A mixture of radiolabeled antigen (tracer) and unlabeled antigen is added to a quantity of the antibody, which is insufficient to form the antigen-antibody complex with all available antigen (labeled and unlabeled). During the formation of this complex, both types of antigen will try to bind to the free binding sites of the antibody. Labeled and unlabeled complexes are formed in proportion to the amounts of respective antigen. The amount of bound antibody is inversely proportional to the quantity of unlabeled antigen. (Saha, 2010)

If the amount of unlabeled antigen is increased, the amount of bound antigen will decrease with an increase in the amount of free antigen left in the mixture. The ratio of bound to free antigen is a function of the concentration of unlabeled antigen in the RIA mixture, which is used in the determination of an unknown concentration of antigen in a sample by using a standard curve. (Saha, 2010)

There are two different ghrelin specific RIAs. While N-RIA recognizes the N-terminal, octanoyl-modified portion of ghrelin, also known as active ghrelin, C-RIA recognizes the C-terminal portion, which includes the active and des-acyl form, together called as total ghrelin. (Hosoda and Kangawa, 2004)

Enzyme-linked immunosorbent assay (ELISA)

The method of ELISA was developed in the 1960s. The assay is based on a principle of a conventional radioimmunoassay like RIA, but with the major difference that the antibodies are labeled with an enzyme and not with radioisotopes (Thompson, 2010). Enzyme-linked immunoassay, also known as

an enzyme immunoassay (EIA) is used to detect the presence of an antibody or antigen in a sample.

The principle of ELISA is that an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over this surface, to bind the antibody to the antigen. The antibody is then linked to an enzyme, and in the last step a substance is added, which the enzyme can convert to a detectable signal, in most assays a color change in a chemical substrate. This visible signal indicates the quantity of antigen in the sample, which is measurable with a spectrophotometric plate reader at specific wavelengths.

There are four different types of ELISA: direct, indirect, competitive and sandwich ELISA. The best results can be obtained with the sandwich ELISA, because highly purified, prematched capture and detector antibodies are used. The obtained signal provides very sensitive and highly specific data. (Sino Biological Inc)

Furthermore, depending on the company, which offers ghrelin ELISA/EIA kits, there are also different types for measuring ghrelin in its total or active form.

Luminex®

This technology uses antibodies which are captured and coupled to magnetic color-coded microspheres and tagged detection antibodies. The concentration or readout is the fluorescence from the bead, which is coding for one specific analyte in the multiplex approach, and the fluorescence from the detection antibody, which is the reflection concentration. This technique is relatively new and compared to the other two techniques RIA and ELISA not often used in clinical trails. In contrast to other techniques, this one offers the major advantage of multiplex analysis, which is able to analyze several substances at the same time. Furthermore, with Luminex® acylated values of ghrelin can be measured. Kadas et al. investigated the outcomes of ELISA and Luminex® technologies and their comparability. The absolute concentrations measured were in the same range, with a tendency to systematically higher (1.4-fold) concentrations through ELISA method. Kadas et al. showed that both

techniques indicate a good consistency and reliability for quantitative measurement of ghrelin. (Kadas et al., 2014)

2.2 Obesity

Nowadays obesity is one of the biggest health challenges of the 21st century. It is responsible for 10-13% of deaths in different parts of the world. The prevalence of obesity has nearly tripled in many countries since the 1980s, whereas particularly children are affected by a rising number of obesity. One in three 11-year-olds is overweight or obese. In 2013, 42 million children under the age of 5 were overweight or obese. In 2013, 39% of adults aged 18 years or older were overweight, and 13% were obese.

The World Health Organisation (WHO) definition of overweight and obesity is the following:

- Overweight: a BMI greater than or equal to 25
- Obesity: a BMI greater than or equal to 30.

The BMI is an index of weight for height that is used to classify underweight, overweight and obesity in adults. The following table shows the classification of BMI. (World Health Organisation)

BMI classification	
Underweight	< 18.5
Normal Range	18.5 - 24.9
Overweight	≥25.0
Preobese	25.0 - 29.9
Obese	≥30.0
Obese class I	30.0 - 34.9
Obese class II	35.0 - 39.9
Obese class III	≥40.0

Table 2: BMI classification (World Health Organisation)

Obesity is the result of an imbalance between calories consumed and calories burned. The main problem is the increased intake of energy-dense foods, which are high in fat, and at the same time an increasing level of physical inactivity.

A high BMI leads to many health consequences, which are:

- Diabetes
- Cardiovascular diseases, like heart disease and stroke
- Musculoskeletal disorders (osteoarthritis)
- Cancers (endometrial, breast and colon)

These diseases are also called non-communicable diseases. There is a positive correlation between them and the BMI.

2.2.1 Treatment possibilities

Lifestyle changes

Modification of diet, increasing physical activity and behavior are the first steps in weight-loss therapy. The National Heart Lung and Blood Institute (NHLBI) guidelines state that 10% of initial weight loss which is achieved over 6 months, is an appropriate target to decrease obesity related risk factors (National Heart Lung and Blood Institute, 1998). Weight loss through lifestyle changes is often very difficult to implement and maintain for any length of time. Therefore, medical interventions, like surgery or pharmacology may be necessary (Henry et al., 2013).

Bariatric surgery

Surgery can be a very effective option for weight reduction and a decrease in obesity correlated risk factors (National Heart Lung and Blood Institute, 1998). A bariatric surgery is only recommended in adults with a BMI > 35 kg/m^2 and T2DM, especially if the T2DM or other co-morbidities are difficult to control through lifestyle changes or pharmacology. A very important point is that patients who have undergone bariatric surgery need lifelong lifestyle support and also medical monitoring (American Diabetes Association, 2012). Bariatric surgery should always be the last instance for weight loss management.

There are different types of bariatric surgery, like adjustable gastric banding (AGB), Roux-en Y gastric bypass (RYGB) or laparoscopic sleeve gastrectomy

(LSG). Bariatric surgery alters the gut hormone profile and the neural activity. RYGB is the most practiced bariatric surgery, which leads to a significant and sustained weight loss in the long term. The mechanisms of action in RYGB are not well understood, but it is thought that it causes substantial and simultaneous changes in gut peptides, brain activation, the desire to eat and taste preferences. (Zhang et al., 2014)

RYGB has been shown to successfully impair the adaptive response of ghrelin to weight loss and it contributes to the capability of obese patients to maintain weight loss after this surgery (Morínigo et al., 2004).

Pharmacotherapy

The US Food and Drug Association (FDA) approved 4 weight loss drugs: Xenical, Contrave, Qsymia and Lorcaserin, which are divided into 2 types. The first type is a kind of fat absorption inhibitor, whereas only Xenical belongs to this type. It acts as a lipase inhibitor and decreases the absorption of fat by 30%. The other three drugs belong to the second type, which is an appetite suppressant. Lorcaserin, for example, is associated with significant weight loss and improved glycemic control in T2DM patients. Contrave is made up of two drugs – bupropion and naltrexone. Only a combination of these two drugs leads to a significant effect. Qsymia, also called Qnexa, consists also of two prescription drugs, phentermine and topiramate. The latter is an anti-convulsant in epilepsy patients, but has a side effect which leads to weight loss. Phentermine has been used for years to reduce obesity. The effect of Qsymia is the suppression of appetite. (Zhang et al., 2014)

Ghrelin and obesity

It is still not clear how the mechanism of ghrelin promotes hunger and leads to an increased energy intake. One theory is that gastric emptying is promoted by ghrelin and that this promotion leads to an increase of hunger. Furthermore,

gastric emptying is positively correlated with fasting plasma levels of ghrelin. (Tschop et al., 2001)

Ghrelin acts in the ARC of the hypothalamus by increasing hunger through the activation of the orexigenic NPY/AgRP neurons and by antagonizing the satiety effects of the hormone leptin through the inhibition of POMC neurons (Shintani et al., 2001). Ghrelin and leptin have a reciprocal relationship in the hypothalamus, which may play an important role in the regulation of appetite and feeding but the exact way of their interaction is not clearly understood yet (Saad et al., 2002).

In the treatment of obesity the use of GHS-R1a antagonists such as 2,4-diaminopyrimidine has been researched with promising results in decreasing appetite in animals (Xin et al., 2006). For the use of such antagonists as a treatment option in obesity for human, further research is needed, particularly for a better selectivity, potency and pharmacokinetic properties (Moulin et al., 2007).

2.3 Eating Disorders

EDs are associated with a wide range of adverse psychological, physical and social consequences. There are different types of EDs, like AN, BN, BED and their variants, whereas all feature serious disturbances in eating behavior and weight regulation. (National Institute of Mental Health)

According to the *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* (DSM-IV) AN and BN are the two specified EDs. The most common ED is the rest category “eating disorder not otherwise specified” (EDNOS), it is a heterogeneous, not well defined group of EDs, which includes syndromes of AN and BN, BED and purging disorder. (Smink et al., 2012)

Furthermore, AN is classified into two types, the restricting subtype (AN-R) and the binge/purge subtype (AN-BP). Also BN is classified into two subtypes, which is the binge/purge subtype (BN-BP) and the non-purging subtype (BN-NP) (Yagi et al., 2012). The restricting type of AN is the most commonly known type, whereby a person severely restricts their food intake. There can be many forms of restriction, for example with maintaining very low caloric count or eating only one meal per day. It may also follow obsessive and rigid rules like eating only the food with one special color. The binge eating or purging type is less recognized than the restricting type. In that case, a person restricts their intake as above but sometimes also with binge eating or purging behavior like self induced vomiting, misuse of laxatives, diuretics or enemas and over-exercising. (www.eatingdisorders.org)

EDs affect both genders, whereas rates among women and girls are two and a half times higher than among men and boys. EDs appear mostly during the teen years or young adulthood. They often coexist with other illnesses such as depression, substance abuse or anxiety disorders. AN is associated with the highest mortality rate of any psychiatric disorder and if a person does not receive treatment, symptoms can become life threatening. (National Institute of Mental Health)

Anorexia Nervosa

Symptoms:

- Extremely low body weight
- Severe food restriction
- Fear of gaining weight
- Unwillingness to maintain a normal body weight
- Lack of menstruation among girls and women
- Distorted body image

Bulimia Nervosa

Symptoms:

- Recurrent and frequent episodes of eating unusually large amounts
- Lack of control over these periods
- Forced vomiting
- Excessive use of laxatives or diuretics
- Excessive fasting, exercise or a combination of these behaviors
- Usually maintain a healthy or normal weight (unlike AN)
- Severe dehydration from purging of fluids
- Electrolyte imbalance

Binge Eating Disorder

Symptoms:

- People loose control over their eating
- No purging, excessive exercise or fasting
- Overweight, obese people
- High risk for developing cardiovascular disease and high blood pressure (National Institute of Mental Health)

2.3.1 Treatment of EDs

In the treatment of EDs it is helpful to consider the following treatment objectives: Firstly, to eliminate the pattern of binge eating and compensatory behaviors. Secondly, to have a normal eating pattern with regular, balanced meals. Thirdly, to identify any physical complications of the illness, like dental enamel erosion. Fourthly, to treat the psychological issues, which accompany the EDs, for example low self-esteem or body image dissatisfaction. Fifthly, to treat comorbid conditions like mood disorders and sixthly, one of the most important, to prevent relapse. Disease management is composed of psychological, nutritional and medical services, to have the best option for reaching the above mentioned objectives. (Yagi et al., 2012)

Ghrelin and Eating Disorders

It still remains unclear whether ghrelin plays an important role in the etiology of EDs or not but accumulating evidence suggests that it is possible that ghrelin has a therapeutic potential for patients with AN or cachexia. As Hotta et al. showed, intravenous ghrelin infusion of 3 µg/kg ghrelin, twice a day for 14 days, improved epigastric discomfort or constipation in 4 of 6 patients with AN. Furthermore, hunger scores evaluated by visual analogue scale questionnaires of these patients also increased significantly after ghrelin infusion. The most important result of the study was the increase of daily energy intake by 12-36% during ghrelin infusion, compared with the pretreatment period. (Hotta et al., 2009)

Analogously, research done by Broglio et al. demonstrated as well that intravenous ghrelin injection of 1 µg/kg was able to cause sensations of hunger in patients with AN (Broglio et al., 2004). Despite the positive correlation of ghrelin administration and the improvement of symptoms in AN patients, further studies are needed to use ghrelin as a possible therapeutic treatment option in eating disorders.

3. MATERIALS AND METHODS

3.1 Materials

The following databases were used for literature search: PubMed, Science Direct, and Embase. The literature search started at the beginning of November 2015 and lasted for four weeks, until December 2015. At the beginning, inclusion and exclusion criteria were defined and depending on them, search terms were fixed. For each database the following terms were used: “ghrelin and obesity”, “ghrelin and obese”, “ghrelin and anorexia nervosa”, “ghrelin and Bulimia Nervosa”, “ghrelin and eating disorders”, “ghrelin and healthy patients”.

Inclusion criteria:

- Human intervention
- Healthy, obese and patients with EDs (Anorexia Nervosa, Bulimia Nervosa and Binge Eating Disorder)
- Patients had to be fasting before measurement of ghrelin values
- Ghrelin values were measured in blood or plasma

Exclusion criteria:

- Children
- Adiposity induced surgeries
- Application of ghrelin or ghrelin mimetic before measurement
- Application of obesity induced medication
- Patients with Prader - Willi syndrome
- Type 1 diabetes and Type 2 diabetes
- Ghrelin values measured in saliva

3.2 Methods

The following flow diagram summarizes search criteria and outcome:

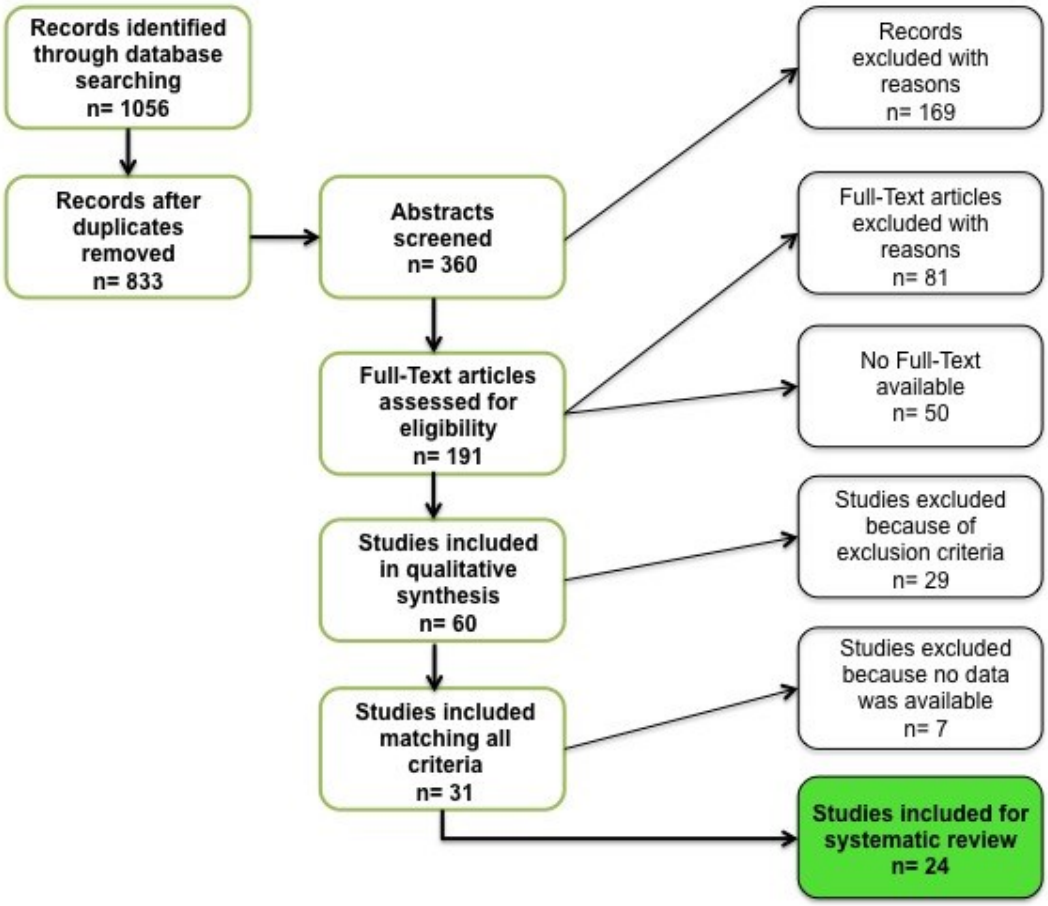

Figure 4: Flow Diagram of selected publications

After completing the search process, a total of 1056 hits were found. In the first exclusion step duplicates were removed and a total of 833 hits remained. These 833 studies were first filtered on the basis of the title and afterwards on basis of the abstract. Reasons for exclusion were for example the language, application of medications or ghrelin mimetic, surgeries or Type 1 diabetes. Another reason for exclusion was the non-availability of a full text and altogether, 50 studies were excluded for this reason. After this elimination, 60 studies met the inclusion criteria. These 60 studies were included in the qualitative synthesis of the literature search. After reading the studies, some of them were excluded

because of the wrong study population or non-fitting study protocol. One important inclusion criteria was that patients had to be fasted when ghrelin values were measured. In many studies, breakfast or other dishes were applied before measurement and in that case, values would not be comparable with other studies. Therefore only fasted values before application of meals were included. 7 studies had to be excluded because no exact data of ghrelin values were available. The measured data were only shown in graphs and it was not possible to get more detailed information.

In the end 24 studies were included in the quantitative synthesis of the literature search. These 24 studies were separated in three groups: healthy patients, obese patients and patients with EDs. For a better overview, a table with study characteristics was created for each group. These tables include the most important characteristics of each study. As far as these are concerned, the author and year, place of origin, number and gender of participants, and the classification into the three mentioned groups are considered as relevant. Moreover, subdivisions, which will be necessary for patients with eating disorders, are demonstrated. Further characteristics are mean BMI, age and, of course, mean ghrelin values.

During the selection of the studies, a framework for guidance – called PICO – was also used. In the following table the abbreviation for PICO is explained and the matched PICO framework is shown (Huang et al., 2006).

	Abbreviation	Matched for exclusion and inclusion criteria
P	Patient or Population	Healthy and obese people, people with EDs like Anorexia Nervosa, Bulimia Nervosa and Binge Eating Disorder
I	Intervention / Exposure	Measurement of ghrelin in sober patients
C	Comparator	Control group – healthy patients
O	Outcomes	Comparable ghrelin data

Table 3: Matched PICO framework

Translation into standard units

In most studies, the unit pg/mL was used to specify ghrelin values. In some cases other units like pmol/L, ng/mL or fmol/mL were used. To translate these units in the standard unit of pg/mL the following calculations were done:

$$pmol/\mathrm{L} = pg/\mathrm{mL} \times 0.296$$

$$pg\ /\ \mathrm{ml}\ = pmol/\ \mathrm{L}\ \times\ 3{,}371$$

$$fmol/\mathrm{mL}\ = pmol/\mathrm{L}$$

$$ng/\mathrm{mL}\ \times\ 1000\ = pg/\mathrm{mL}$$

The molecular weight of ghrelin is MW = 3370.9 Da (Ulasoglu et al., 2013).

The conversion factor between SI and conventional units, is 0.296 (Chanoine, 2005), and 3,371, respectively (Ulasoglu et al., 2013).

Figures and Tables

All data and consequently all figures and tables were created using SPSS or Excel. If not stated otherwise, all figures and tables were specifically created for the matter of this thesis.

4. RESULTS

4.1 General overview of analyzed data

24 studies with a number of 1206 participants were analyzed for this review. Separated into three groups, 354 people met the inclusion criteria for patients with EDs, 302 people belong to the group of healthy patients and the biggest group is represented by obese patients with a total of 550. Distribution between sexes was 73% for women and 27% for men. The participants were from the following regions: America (9 publications), Asia (10), Europe (3) and Australia (1), whereas one study had participants to one half each from USA and Asia.

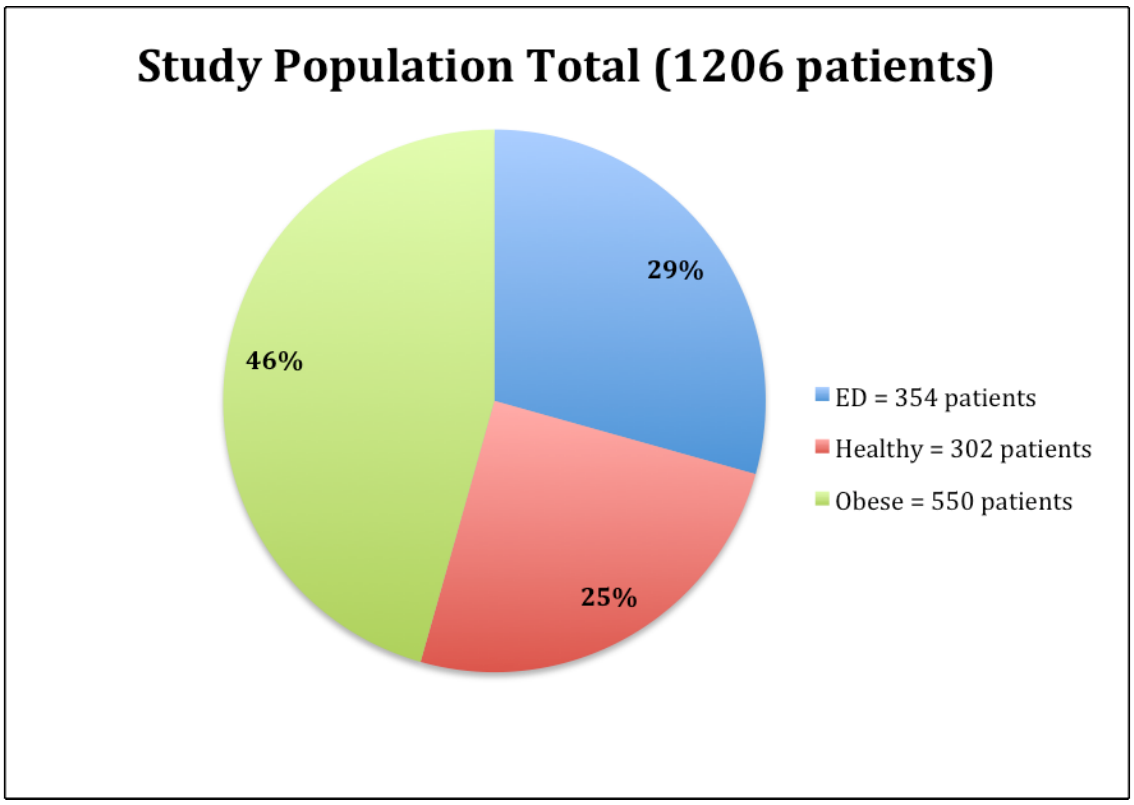

Figure 5: Study population in patient groups

All included studies covered research done between 2002 and 2013. Taking a closer look onto who investigates the effects of ghrelin, research led by Tanaka M., Monteleone P. and Nakazato M. was frequently found to occur among the selected studies.

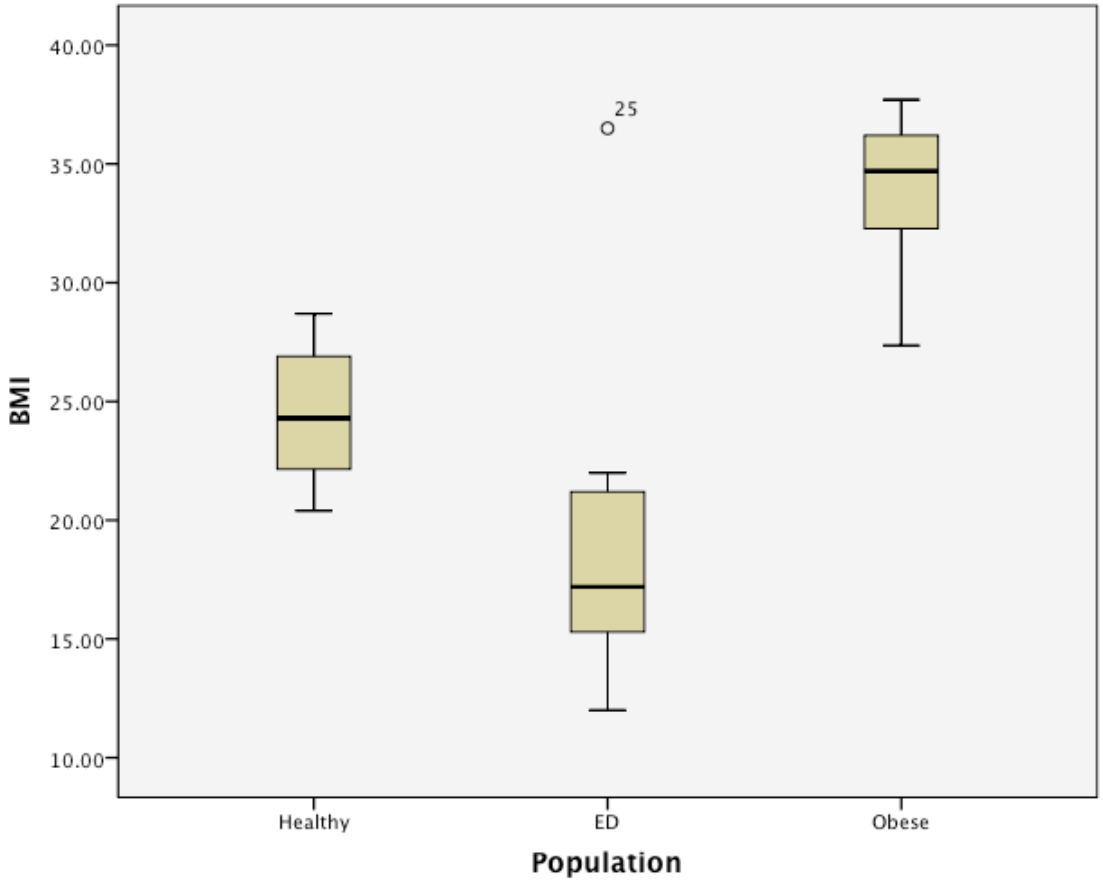

Figure 6: Boxplot of BMI in patient groups

To characterize the general study population more precisely, BMI was measured within the 3 groups of EDs, obese and healthy people. The average BMI of the healthy population was 24.5 kg/m^2. The obese population has an average BMI of 33.9 kg/m^2 and the population with EDs has an average BMI of 20.3 kg/m^2 (see Figure 6). The outlier (BMI of 36.5 kg/m^2) within Figure 6 represents participants with BED. More precise information about the study participants will be discussed in each of the groups below.

4.2 Healthy study participants

Study population

6 studies with a total of 302 patients (64 women and 238 men) met the search criteria. The population can be separated into their nationalities, depending on this classification 120 participants are Asian and 182 are American. The study by Matsunaga et al. includes a mixture of Caucasian and Japanese participants. Due to the same methods and sample preparation used in this research, a comparison of ghrelin data could be interesting. The average data is a BMI with 24.3 kg/m^2 and an age of 29.5 years. Further information can be seen in Table 4.

			N Gender			Ghrelin pg/ml	BMI	Age
Author & Year	**Country**	**Method**	F	M	Total	Healthy	Healthy	Healthy
Matsunaga et al.; 2007	Asia/US	RIA	-	189	189	706.3	25.1	44.7
Tong et al.; 2013	US	RIA	13	9	22	78.0	24.3	26.0
Akamizu et al.; 2004	Asia	RIA	-	18	18	627.9	21.0	22.7
Awar et al.; 2005	Asia	RIA	11	-	11	138.8	20.4	22.6
Brownley et al.; 2012	US	RIA	40	-	40	682.6	28.7	31.4
Crispim et al.; 2011	Brasil	ELISA		22	22	450.2	26.9	29.5
		Total	**64**	**238**	**302**	**Average in groups**	**24.3**	**29.5**

Table 4: Study characteristics of healthy patients

To have a broader comparing possibility, the control groups of the population with obese patients and patients with EDs are summarized. Through this factum, a much broader spectrum of data is available, whereas it should be possible to get a more precise mean value for healthy people.

Out of 24 studies there were 6 studies without a control group, whereas one was from the ED group and 5 from the obese group. With a total of 18 studies including 517 participants, ghrelin data are compared above. Out of this 18 studies, 14 were conducted with 267 women and 4 with 250 men, whereas in the group of EDs are only women.

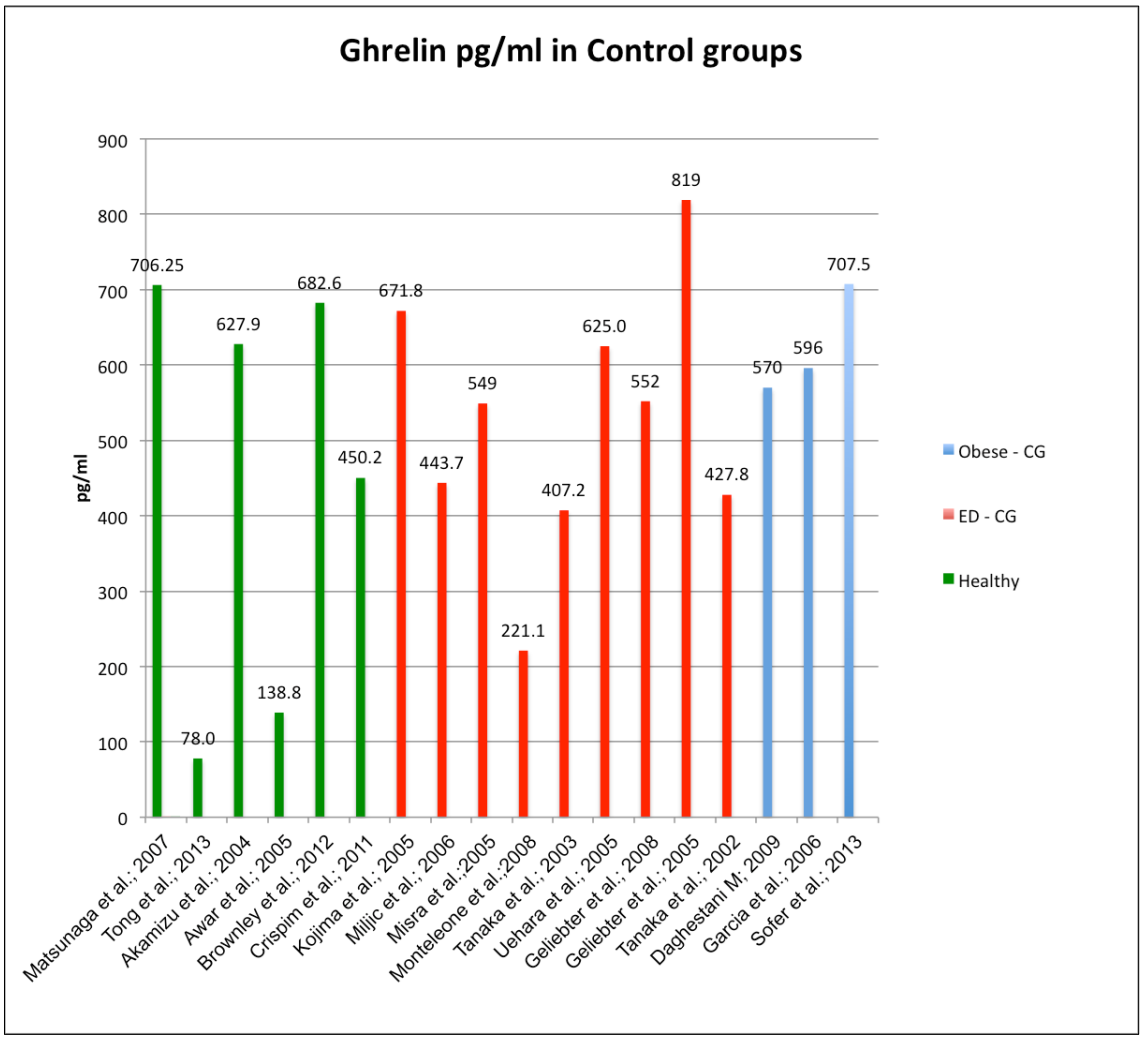

Figure 7: Ghrelin data of control groups

As it can be seen in Figure 7, ghrelin data range from a minimum of 78 pg/ml to a maximum of 819 pg/ml. Furthermore, on the basis of the scatterplot below, no trend is apparent between BMI and ghrelin data. For example with a BMI of about 20 kg/m^2 the ghrelin data is in the area from 200 pg/ml up to 700 pg/ml. Moreover, many studies declare that ghrelin data should be low in overweight patients and high in patients with AN (Cummings et al., 2002). After a detailed comparison of the three mentioned groups, it can be seen whether there is an agreement with the studies or not. However, a closer consideration of the following table shows that ghrelin data are greatly varying among control groups.

Furthermore, when considering the BMI as a single characteristic for ghrelin values, the scatterplot shows that there is no significant correlation between ghrelin and BMI. The Pearson correlation coefficient is 0.421, which means that there is a slight positive relationship, which would not be in accordance with other publications.

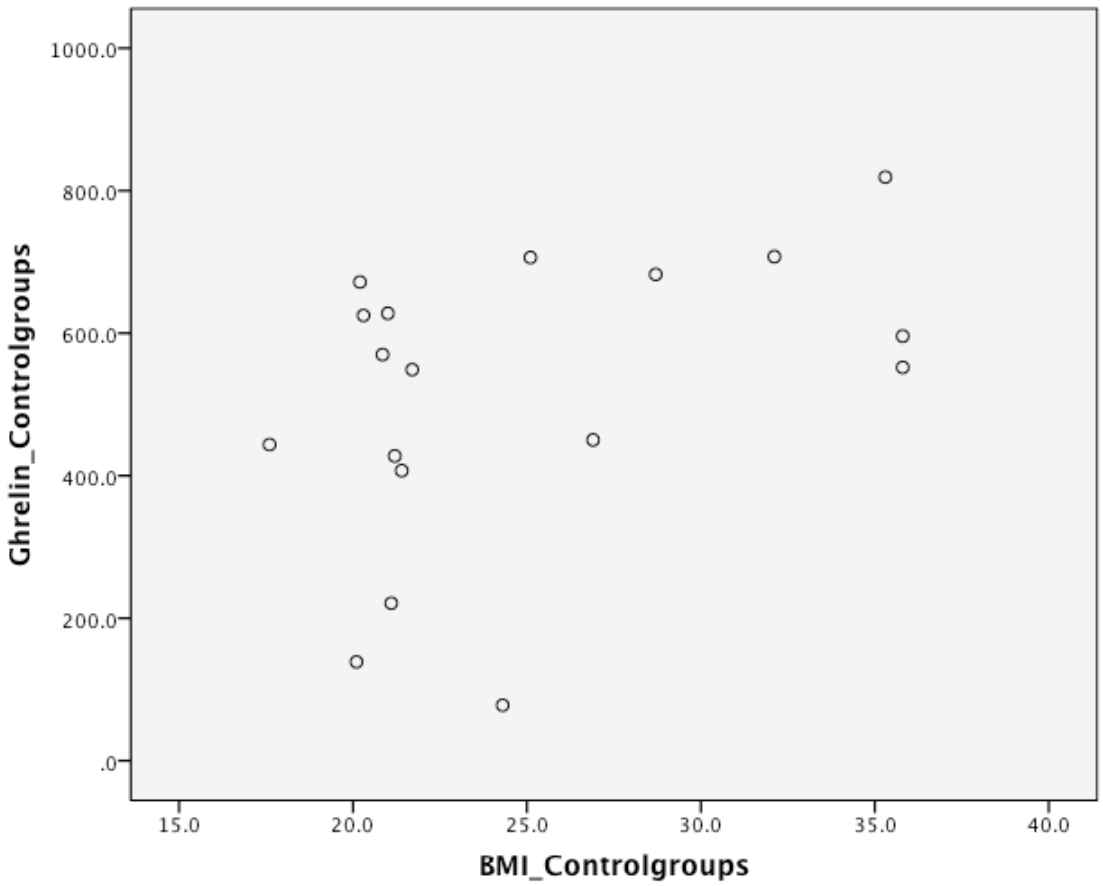

Figure 8: Scatterplot BMI - Ghrelin of Control groups

Inclusion and exclusion criteria for control participants

To have a better compromise between the ghrelin data of control groups, a short illustration of the participants has been done, showing the main inclusion and exclusion criteria, which had to be fulfilled by them.

Regarding the three patient groups, healthy control groups had to fulfill the following criteria: no medical diseases or psychiatric illnesses, no recent history of weight fluctuation or current use of medications, which influence appetite or weight, no history or clinical evidence of impaired fasting glucose or diabetes mellitus, no pregnancy or lactating and no use of oral contraceptives. A further exclusion criteria was the use of illegal drugs, alcohol or tobacco abuse.

The control patients in the group of EDs had in the main the same criteria, but with some further points, which are especially: no present or past history of EDs, a regular menstrual cycle, normal eating habits and their body weight had to be within 10% of ideal body weight. Geliebter et al. presented two studies, in which the control groups had a BMI larger than 30 kg/m^2, but the participants were declared to be healthy.

Control groups of the obese group had the same criteria as mentioned currently, to be fulfilled, with an exception that 2 studies from Garcia et al. and Sofer et al. had a control group with a BMI larger than 30 kg/m^2. Again these participants were declared to be healthy.

Comparison of ghrelin values

On the basis of the criteria for the control groups, ghrelin data of the participants should be comparable, nevertheless the values are varying strongly. When comparing the values of men and women, the mean ghrelin value of women with 431 pg/ml tended to be lower than those of men with 623 pg/ml. The mean age of all control patients is 29.7 years, at which women have a mean age of 28.2 years and men 34.9 years. With the lower mean ghrelin data and age of women it could be considered that there might be a correlation, but the Pearson correlation coefficient of age and ghrelin data is $r = 0.009$, that shows that there is no correlation between those two factors.

Awar et al. compared mean total fasting plasma ghrelin concentration across several publications and found levels that range from 286.5 to 837.7 pg/ml (Awar R., 2005). Considering Table 5 below, ghrelin levels are in a range of 78 to 819 pg/ml, which is a much broader range compared to Awar et al. Three publications are outside the range of Awar et al., when they are considered as spikes, levels are in the area of 407.2 to 819 pg/ml. The upper limit would be in agreement with Awar et al. Nonetheless, here rises the question why these values are up to 10 times higher regarding the lowest and highest one, because the healthy participants should be comparable on the basis of exclusion and inclusion criteria. Possible reasons could be the method of measurement, handling with the samples or individual differences in participants. All this and further reasons will be analyzed in detail in the discussion chapter.

Author & Year	Ghrelin pg/ml	Method
Tong et al.; 2013	78.0	RIA
Awar et al.; 2005	138.8	RIA
Monteleone et al.; 2008	221.1	ELISA
Tanaka et al.; 2003	407.2	RIA
Tanaka et al.; 2002	427.8	RIA
Miljic et al.; 2006	443.7	RIA
Crispim et al.; 2011	450.2	ELISA
Misra et al.;2005	549	RIA
Geliebter et al.; 2008	552	RIA
Daghestani M; 2009	570	ELISA
Garcia et al.; 2006	596	RIA
Uehara et al.; 2005	625.0	RIA
Akamizu et al.; 2004	627.9	RIA
Kojima et al.; 2005	671.8	RIA
Brownley et al.; 2012	682.6	RIA
Matsunaga et al.; 2007	706.25	RIA
Sofer et al.; 2013	707.5	ELISA
Geliebter et al.; 2005	819	RIA

Table 5: Ghrelin levels and methods

As it can be seen in Table 5, in most cases the method of RIA is used for measuring ghrelin values. When values of RIA and ELISA are compared, the mean values are 523 pg/ml for RIA and 487 pg/ml for ELISA. According to these average values, no big difference in the measurement method can be seen in this group of healthy and control participants.

As mentioned above, Matsunaga et al. measured ghrelin levels in Caucasian and Japanese patients. They investigated whether there are differences between the two ethnic populations. They examined 98 Caucasian residents and 91 Japanese residents and measured ghrelin through RIA. Ghrelin levels were significantly higher in the Caucasians, with values of 904.5 pg/ml and 508 pg/ml for Japanese. They did not expect this result, because BMI and waist circumference were higher in the Caucasian group, and therefore ghrelin should have been lower. Matsunaga et al. explained the higher levels through environmental and genetic factors. (Matsunaga-Irie et al., 2007)

This study shows that there could also be differences in the genetic background. Through this circumstance, also possible ethnic differences in ghrelin levels will be analyzed below.

After this general consideration of ghrelin data of healthy participants and control groups, it seems difficult to generate a mean value for ghrelin. On the following pages, the other two groups of eating disorders and obese patients are illustrated in detail. Hopefully, a mean ghrelin value or rather an area can be pointed out for each group.

4.3 Obese study participants

Study population

Altogether 7 publications met the inclusion and exclusion criteria for the systematic review. There is a total of 550 participants, of which 466 are female and 84 are male. Participants can be separated into an intervention group of 446 patients and a control group of 104. As it can be seen in Table 6, only three publications have an intervention and also a control group for comparison. The other four have only an intervention group. Furthermore, only the range of ghrelin in obese patients is reviewed in this chapter and the values of control groups are not discussed in detail.

			N Gender			N Groups		Ghrelin pg/ml		BMI		Age	
Author & Year	**Country**	**Method**	F	M	Total	Obese	CG	Obese	CG	Obese	CG	Obese	CG
Daghestani M; 2009	Asia	ELISA	122	-	122	62	60	360	570	31.6	20.9	24.0	24.0
Ellis et al.; 2012	US	ELISA	32	29	61	61	-	684.3	-	32.3	-	35.1	-
Garcia et al.; 2006	US	RIA	48	-	48	25	23	589	596	37.7	35.8	43.6	44
Jakubowicz et al.; 2012	Asia	ELISA	193	-	193	193	-	325.7	-	32.3	-	47.1	-
Katarzyna et al.; 2008	Poland	ELISA	37	-	37	37	-	66.9	-	36.5	-	40.7	-
Sumithran et al.; 2011	Australia	RIA	34	16	50	50	-	127	-	34.7	-	54.4	-
Sofer et al.; 2013	Asia	ELISA	-	39	39	18	21	707.5	707.5	34.2	32.1	43	42.5
		Total	**466**	**84**	**550**	**446**	**104**		**Average**	**34.2**	**29.6**	**41.1**	**36.8**

Table 6: Obese Study Characteristics

Regarding the nationality, 354 people are Asian, 109 are American, 50 are Australian and 37 are European. The average BMI of obese patients is 34.2 kg/m^2 and the average age is 41.1 years.

Inclusion and exclusion criteria

The following criteria had to be fulfilled by obese patients: no primary diseases, no pregnancy or the intake of oral contraceptives, no diet regimen, no smoking, no disorders of lipid or glucose metabolism, no medications that affect body weight (for example cholesterol medications), no weight change within the last months, no tobacco use and an irregular menstrual cycle. All patients were in

good health, with the single exception of a higher bodyweight than 30 kg/m^2, which is classified already as obesity (as described in Table 2).

Comparison of ghrelin values

Figure 9 shows the different ghrelin values, which range from the minimum of 66.9 pg/ml to a maximum of 707.54 pg/ml. The values are in a similar range like they have been in healthy patients. This is the first indication that ghrelin values do not differentiate between the first two groups described so far.

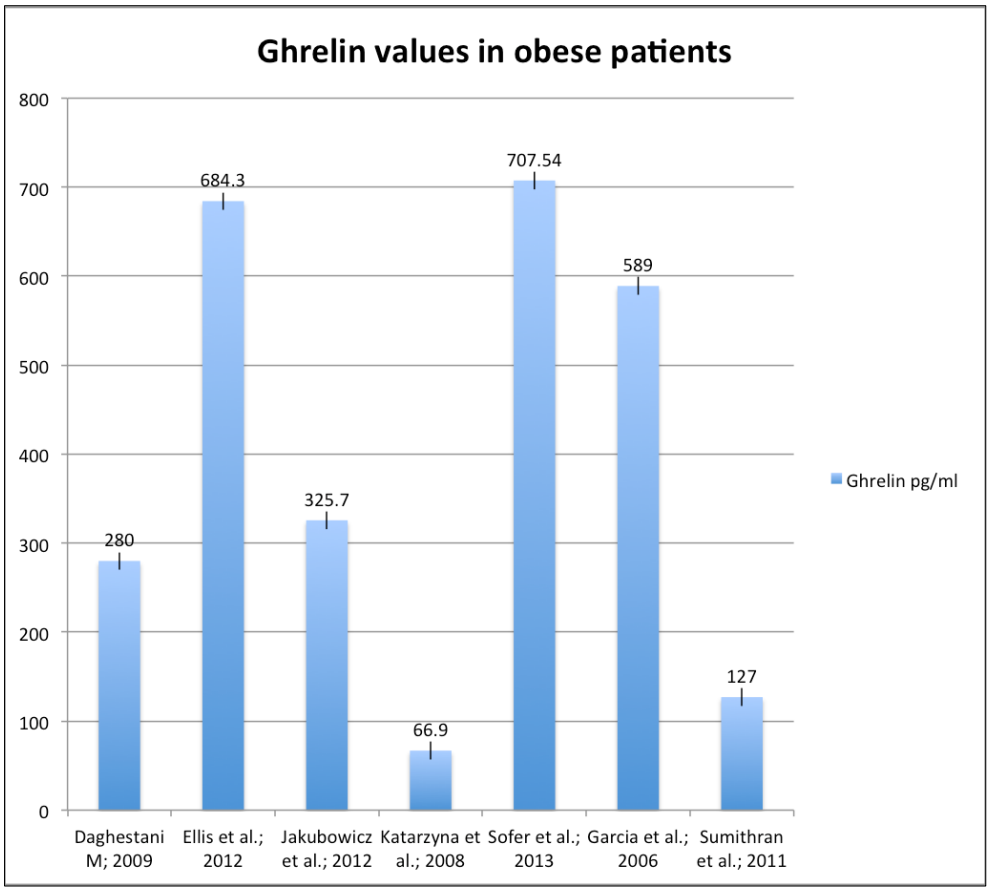

Figure 9: Ghrelin values of obese patients

When analyzing the different methods for measurement, it can be seen that five publications used ELISA and two publications used RIA for measuring ghrelin. The mean value for each method is 412.9 pg/ml for ELISA and 358 pg/ml for RIA, which shows again that there is no substantial difference between the two methods.

Another possibility of comparison is between men and women but unfortunately, in the publications of Ellis et al. and Sumithran et al., values are not specified separately for men and women, thereby there is only one publication from Sofer et al., where the values of men are stated with 707.5 pg/ml. The average value for women is 335.4 pg/ml. Women´s values tend to be lower, but this comparison has no significance, because there is only one value, which is representing the values of men. One plausible explanation for the gender combined data in the two publications, is that there was no significant difference between genders. On the other side, the lower values of obese women are confirmed with the lower values of healthy women in the chapter before. Regarding the ghrelin values, the age and BMI of the obese population, there is again no correlation determined, neither between ghrelin and BMI nor between ghrelin and age.

Sumithran et al. used a multiplex assay for the ghrelin measurement, which results in a simultaneously quantification of several peptide hormones at the same time. This fact can lead to a less accurate and precise measurement of the single hormones. Ghrelin is with a value of 127 pg/ml in the lower range compared to the other publications, this could be explained through the multiplex assay (Sumithran et al., 2011). However, the low value of ghrelin with 66.9 pg/ml in the study of Katarzyna et al. cannot be declared with the method, because they used ELISA with a sensitivity of less than 6 pg/ml (Katarzyna Mizia-Stec et al., 2008). In this case, the low value has to be explained in another way.

The highest value of ghrelin with 707.54 pg/ml was measured by Sofer et al., which also used the ELISA. Dependent on the method, it is not possible to explain high or low levels of the hormone. This point will be discussed more precisely in the discussion below. Interestingly, the values of control and intervention groups in the publication of Sofer et al. are nearly the same with 707.54 pg/ml for the intervention group and 707.53 for the control group. This could be explained through the similar BMI and age of the two groups or

through inaccurate handling with the samples or the implementation of the method. The similar values could also be a coincidence.

Summing up it can be said that the range of values in obese patients with 66.9 pg/ml to 707.54 pg/ml is in the same range of healthy people, which ranges from 78 pg/ml to 819 pg/ml. Neither a mean value for obese patients nor a different area for ghrelin can be determined compared to a healthy population. Until now, only half of the studies confirm the statement of Cummings et al. that ghrelin values should be low in obesity. The hypothesis of this systematic review that every patient group is defined through its own ghrelin area, cannot be approved with this data yet.

4.4 Participants with Eating disorders

11 studies met the criteria for the group of EDs. Altogether 354 participants were investigated, of whom nearly all were female. Only 8 participants were male, but this is not considered in Table 7 below, because all data regarding gender are stated together with the reason that there was no significant difference in the values. Furthermore, the data of 8 male participants would not have a strong force of expression adverse to the data of 346 female participants. Out of these 354 participants there is an intervention group with 243 and a control group of 111 patients.

When looking closely to the intervention group, it can be seen that there are several subcategories, which are AN, BN and BED. In these subcategories are further subdivisions, for example AN-R or AN-BP for the restricting type or binge-purging type of AN. BN-P and BN-NP stand for the purging and non-purging type. BED has no further subdivisions. More precise information for these subdivisions and their data are given below.

Going on with the general study characteristics, concerning the nationality, 155 patients are Asian, 93 are American, 61 are European, 25 are Serbs and 20 are Brazilian. The RIA method was used in 9 publications and ELISA in 2. When looking closer at the author information, it can be seen that two author groups, Geliebter et al. and Tanaka et al, are represented each with 2 publications. The comparison of ghrelin values could be interesting in this case, because disturbance factors like diverse handling with samples do not exist. Furthermore, each of them is investigating the same patient group, this means values should normally be in the same range.

Going on with general information it can be seen that the BMI is different in each of the 4 patient groups. The group of AN has its BMI with 15 kg/m^2, BN with 20.5 kg/m^2, BED with 36.6 kg/m^2 and the control group with 23.8 kg/m^2. All of them are in the expected range.

			Gender	N Groups				Ghrelin pg/ml				BMI				Age			
Author & Year	**Country**	**Method**	F	AN	BN	BED	CG	AN	BN	BED	CG	AN	BN	BED	CG	AN	BN	BED	CG
Kojima et al.; 2005	Asia	RIA	22	-	10	-	12	-	893.3	-	671.8	-	20	-	20.2	-	24.7	-	24.8
Miljic et al.; 2006	Serbia	RIA	25	15	-	-	10	821.9	-	-	443.7	14.6	-	-	17.6	25.0	-	-	22.5
Misra et al.;2005	US	RIA	40	22	-	-	18	784.0	-	-	549.0	16.6	-	-	21.7	16.2	-	-	15.1
Monteleone et al.;2008	Italy	ELISA	61	20	21	-	20	370.6	217.4	-	221.1	16.6	21.4	-	21.1	45	57.5	-	55.3
Tanaka et al.; 2003	Asia	RIA	86	40	31	-	15	1014.8	687.9	-	407.2	14.2	20.6	-	21.4	23.2	22.7	-	22.1
Uehara et al.; 2005	Asia	RIA	16	9	2	-	5	2062	-	-	625.0	15.3	-	-	20.3	20.2	-	-	19.4
Hotta et al.; 2009	Asia	ELISA	5	5	-	-	-	1085.5	-	-	-	13	-	-	-	26	-	-	-
Geliebter et al.; 2008	US	RIA	16	-	-	8	8	-	-	364	552.0	-	-	36.5	35.8	-	-	29.9	30.3
Tanaka et al.; 2002	Asia	RIA	26	-	15	-	11	-	1005.9	-	427.8	-	20	-	21.2	-	23.3	-	24
Carnier et al.; 2008	Brazil	RIA	20	-	-	20	-	-	-	6910	-	-	-	36.9	-	-	-	16.9	-
Geliebter et al.; 2005	US	RIA	37	-	-	25	12	-	-	350	819	-	-	36.3	35.3	-	-	28.8	33.1
		Total	**354**	**111**	**79**	**53**	**111**				**Average in Groups**	**15.0**	**20.5**	**36.6**	**23.8**	**25.9**	**32.1**	**25.2**	**27.4**

Table 7: ED Study Characteristics

Inclusion and exclusion criteria

All patients, except the two publications from Geliebter et al., were diagnosed on the basis of the "*Diagnostic and Statistical Manual of Psychiatric Disorders, Fourth Edition (DSM-IV)".* The "American Psychiatric Association" published this manual, and it covers all mental health disorders for children and adults. Furthermore, it lists known causes for these disorders, statistics in terms of gender, age of onset, prognosis and the optimal treatment approaches. The DSM uses a multiaxial approach for diagnose, consisting of five dimensions, which are related to different aspects of the disorders. (American Psychiatric Association)

The patients of Geliebter et al. were diagnosed with the "*Questionnaire of Eating and Weight Patterns (QEWP)*" which is a screening instrument for BEDs. After this questionnaire they followed a clinical interview with a clinical psychologist (Geliebter et al., 2005). Aside from having an ED, all patients were mentally and physically healthy. Patients were excluded, if they had any history of alcohol or drug abuse or gastrointestinal disease.

Comparison of ghrelin values

Figure 9 shows the different ghrelin values of each publication. On the basis of the color, the three subtypes of EDs can be recognized. Blue represents the group of AN, red stands for BN and green for BED. As it can be seen, Carnier et al. provides one spike with a value of 6910 pg/ml, which is the highest value compared to all other publications of this review. Aside from this enormous high value, ghrelin ranges from a minimum of 217.4 pg/ml to a maximum of 2062 pg/ml.

Compared to the groups of healthy and obese patients, this group represents a tendency for higher ghrelin values, especially in the group of AN, which is not in the range of the others. This is the first indication that after all, there are differences between the three patients groups. When the patients with AN are compared with their own control groups, there is yet another sign, which

approves the hypothesis, because values of AN are always higher than the controls. The same case can be seen in patients with BN, but in contrast, patients with BED do not support the hypothesis.

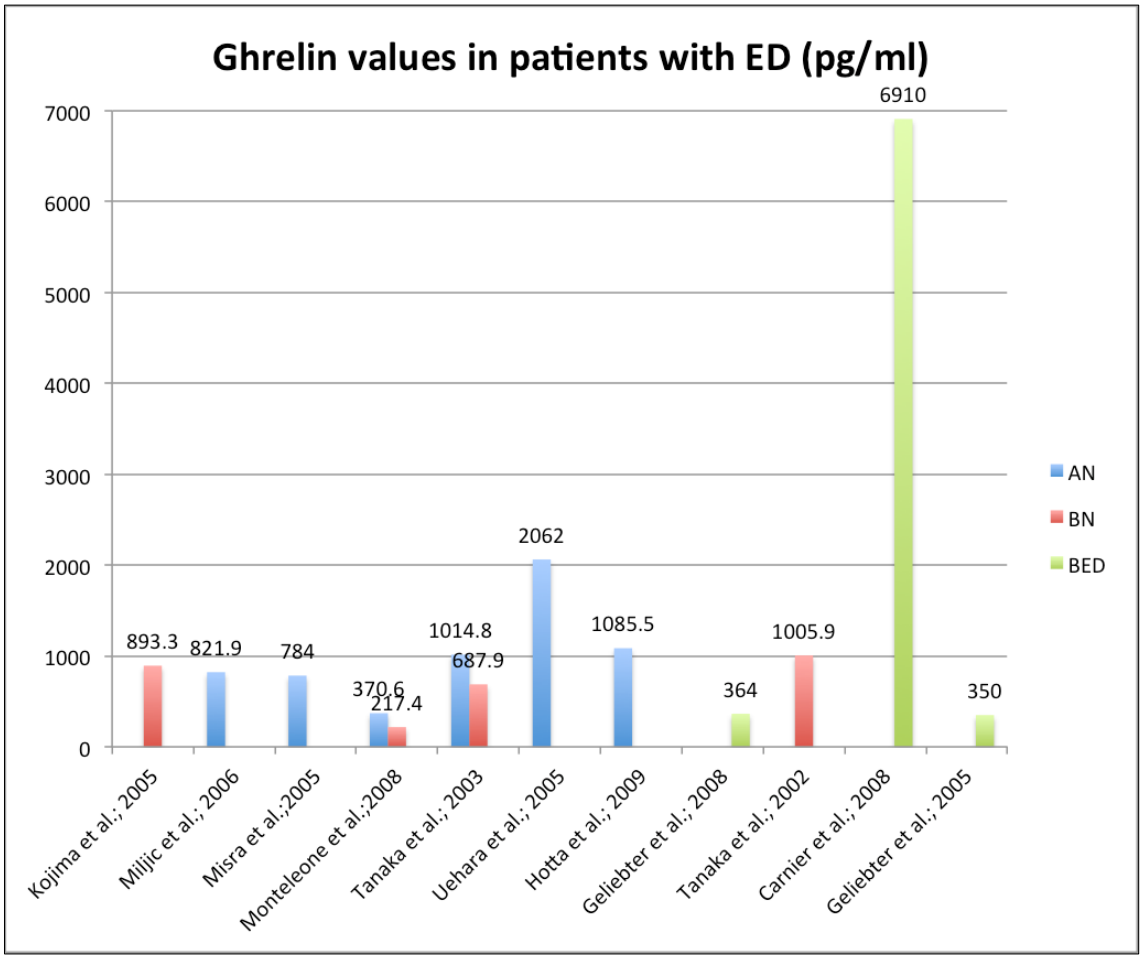

Figure 10: Ghrelin values of patients with ED

In the following calculations, the publication of Carnier et al. will be excluded, because the spike would falsify the results. The excluded publication will be examined separately, to retrace the high values of ghrelin.

The mean values for each subtype of ED were calculated as the following: AN with 1023 pg/ml, BN with 701 pg/ml and BED with 357 pg/ml. For the first time there is a trend for a negative correlation between ghrelin values and BMI. That means the higher the BMI, the lower the ghrelin values. The Pearson correlation coefficient is -0.54.

Overview and comparison of values within the subdivisions

As mentioned before, AN was categorized in the restricting and the binge-purging type. Furthermore, in the publication of Miljic et al. there was a category called *Partially Recovered Anorexia Nervosa* (PRAN). The patients in this group were characterized by a partially recovered body weight but with still existing amenorrhea *(Miljic et al., 2006).* When comparing the values in the publication of Miljic et al., AN-R patients have a ghrelin value of 958 pg/ml and the PRAN patients have a value of 686 pg/ml. With the lower value in the latter group, it could be said that these values are normalizing respectively, converging to the control group with a value of 444 pg/ml, because PRAN represents a group where patients are on the mend to cure AN. Unfortunately, this publication is the single one with a group of PRAN, so this value is not comparable with the other ones.

Going on with the comparison of ghrelin values within the subdivisions, Table 8 represents the exact data in each of them. This table is just for an overview, because there are partially to less data to compare each of the groups, and in this case it would not make sense. This is also the reason why the ghrelin data in the groups of AN and BN are not compared within their subdivisions. These are summarized as either AN or BN.

Author	Ghrelin pg/ml				
	AN-R	*AN-BP*	*PRAN*	*BN-P*	*BN-NP*
Kojima et al.; 2005				893	
Miljic et al.; 2006	958		686		
Misra et al.;2005	784				
Monteleone et al.;2008	371			217	
Tanaka et al.; 2003	865	1165		967	409
Uehara et al.; 2005		2062			
Hotta et al.; 2009	1085				
Tanaka et al.; 2002				1006	

Table 8: Ghrelin values in subdivisions

Nearly all publications, except for Monteleone et al., measured ghrelin values for patients with AN in the upper area. The publication of Monteleone et al.

measured generally lower values of ghrelin, but the values are significantly higher for patients with AN, compared to the control group (Monteleone et al., 2008). Within the categories of BN and their control group (CG) was no significant difference, nevertheless the significance between AN and CG approves the hypotheses for different values in the mentioned groups.

Tanaka et al. investigated in the publication from the year 2003 whether habitual binge/purge behavior influences circulating ghrelin levels. They measured ghrelin levels of women in 21 AN-R patients, 19 AN-BP patients, 18 BN-P patients and 15 sex matched healthy controls. AN-BP and BN-P patients had habitual vomiting at least twice a week and they were excluded if they had only purging behavior without vomiting. The frequencies of binge/purge cycles was about 6 times a week in the AN-BP and BN-P group. The frequency of binge eating episodes was about 5 times a week in the BN-NP group. Fasting plasma ghrelin in all subjects were negatively correlated with their BMI. Furthermore, mean plasma levels of ghrelin were significantly higher in the groups of AN-R, AN-BP and BN-P compared to BN-NP and control patients. Levels of AN-BP were also higher than in AN-R. In the groups of AN-BP and BN-P there was a correlation between ghrelin values and the frequency of binge/purge cycles, whereas there was no correlation between ghrelin levels and the frequency of binge-eating episodes in the group of BN-NP. Mean plasma ghrelin levels were higher in BN-P compared to BN-NP, though there were no significant differences in their nutritional parameters. (Tanaka et al., 2003)

On the basis of these results, Tanaka et al. showed that habitual binge/purge behavior may have an influence on ghrelin levels, whereas cycles with vomiting, as opposed to binge eating episodes without vomiting, may have greater influence (Tanaka et al., 2003).

A further publication of Tanaka et al., investigated in the year 2002, measured ghrelin levels in 15 patients with BN with binge eating and purging episodes at least 3 times a week. Ghrelin levels were significantly higher than in the control group and with a value of 1006 pg/ml also in the same range compared to the levels in the publication of 2003, with a value of 967 pg/ml. (Tanaka et al., 2002)

Indeed, the results are from the same working group and they are only comparable with each other, but the biggest advantage is that there cannot be a falsification through different methods of measurement or handling with the samples. Furthermore, there are the same inclusion and exclusion criteria for patients with BN. They are at the same age and have a similar BMI. Results show that levels of each control group with 407.2 pg/ml and 427.8 pg/ml are in the same range. Also the levels of BN-BP patients are similar, with 967 pg/ml and 1006 pg/ml.

The two publications from Geliebter et al. in the year 2005 and 2008 show that ghrelin values of patients with BED are also in the same range, with 364 pg/ml and 350 pg/ml. The criteria for BED patients were the same, because they were graded as BED patients through the Questionnaire on Eating and Weight Patterns (QEWP) in both publications. Regarding BMI and age, there were no differences. They all met the full criteria for BED, with reported binge eating episodes (characterized with more than twice the size of a normal meal) on two or more days a week for the past 6 months. Control patients also met the same criteria regarding BMI and age, but with values of 552 pg/ml and 819 pg/ml they differentiate more than in the intervention groups. The values of BED are in both publications lower, compared to the control groups, and Geliebter et al. suggest that BED may down regulate ghrelin levels. (Geliebter et al., 2005;Geliebter et al., 2008)

Summing up, it can be said that only patients with EDs represent different ghrelin values, which are dependent on their disease. After exact examination through this systematic review, the following values for the three groups of eating disorders were disposed. AN has its values around 1023.13 pg/ml, BN at 701.13 pg/ml and BED at 357 pg/ml. In the case of BED, the high value of Carnier et al. was excluded. Aside from this single publication, the other ones

were able to show that there are differences in ghrelin levels. A negative correlation was found regarding BMI and ghrelin. Unfortunately, in the other groups of healthy and obese patients it was not possible to determine a value or range for ghrelin.

4.5 Possible influences on ghrelin values

As it can be seen above, there are still some factors that represent possible influences on ghrelin values. In this review, there are different producers of ghrelin kits. Their sensitivity and comparability will be compared with each other. Handling with the samples is also an important point, for example the elapsed time from blood sampling to measurement. Another point could be the kind of tubes in which samples are stored. Furthermore, Matsunaga et al. have shown that there might be ethnic differences in ghrelin values, thus this will be another point that is analyzed with the data in this review.

Methods and Producers

All in all, there are six different producers used among all publications. The most common producers are Linco Research with 10 publications and Phoenix Pharmaceuticals with 7 publications. Four publications, one of Kojima et al., one of Akamizu et al. and two of Tanaka et al. used a ghrelin RIA kit established from the working group of Hosoda (Hosoda et al., 2000;Akamizu et al., 2004). Among these 21 publications, a comparison of values is possible, because there are several data available. Unfortunately, it is not possible to compare the data of the other three publications, because each of them used a different producer. Miljic et al. used a RIA ghrelin kit produced from Hammersmith Hospital, UK (Miljic et al., 2006). Hotta et al. used an ELISA kit produced from Mitsubishi Kagaku Iatron (Hotta et al., 2009). Exact information relating to producer, method and ghrelin value can be seen in Table 9 below.

In the publication from Uehara et al. it was not possible to find any further information regarding the producer of the used ghrelin kit. Unfortunately, it was not possible to get further information from these four mentioned ghrelin kits, nor the sensitivity or the range of measurement. The producers Linco Research and Phoenix Pharmaceuticals both offer ELISA and RIA for measuring ghrelin.

Each kit represents individual ghrelin ranges, which are the following:

Linco Research:

- ELISA: 50 – 5000 pg/ml
- RIA: 93 – 6000 pg/ml (www.merckmilipore.com)

Phoenix Pharmaceuticals:

- ELISA: 0 – 10000 pg/ml
- RIA: 10 – 1280 pg/ml (www.phoenixpeptide.com)

Author & Year	Producer	Method	Ghrelin pg/ml
Akamizu et al.; 2004	Developed by Hosoda et al.	RIA	627.9
Kojima et al.; 2005	Developed by Hosoda et al.	RIA	671.8
Tanaka et al.; 2003	Developed by Hosoda et al.	RIA	407.2
Tanaka et al.; 2002	Developed by Hosoda et al.	RIA	427.8
Miljic et al.; 2006	Hammersmith Hospital, London UK	RIA	443.7
Carnier et al.; 2008	Linco Research, USA	RIA	6910
Ellis et al.; 2012	Linco Research, USA	ELISA	684.3
Garcia et al.; 2006	Linco Research, USA	RIA	596
Sumithran et al.; 2011	Linco Research, USA	RIA	127
Sofer et al.; 2013	Linco Research, USA	ELISA	707.5
Matsunaga et al.; 2007	Linco Research, USA	RIA	706.25
Tong et al.; 2013	Linco Research, USA	RIA	78.0
Awar et al.; 2005	Linco Research, USA	RIA	138.8
Brownley et al.; 2012	Linco Research, USA	RIA	682.6
Crispim et al.; 2011	Linco Research, USA	ELISA	450.2
Hotta et al.; 2009	Mitsubishi Kagaku Iatron, Japan	ELISA	1085.5
Uehara et al.; 2005	n.f	RIA	625.0
Misra et al.;2005	Phoenix Pharmaceuticals, USA	RIA	549
Monteleone et al.;2008	Phoenix Pharmaceuticals, USA	ELISA	221.1
Geliebter et al.; 2008	Phoenix Pharmaceuticals, USA	RIA	552
Geliebter et al.; 2005	Phoenix Pharmaceuticals, USA	RIA	819
Daghestani M; 2009	Phoenix Pharmaceuticals, USA	RIA	570
Jakubowicz et al.; 2012	Phoenix Pharmaceuticals, USA	RIA	325.7
Katarzyna et al.; 2008	Phoenix Pharmaceuticals, USA	ELISA	66.9

Table 9: Kit and Producer Information

With table 10 on the next page it can be seen that the ranges are conform to the producers' kit manuals. NFI represents the groups where no further information was available. Apart from that issue, the groups of NFI and Hosoda represent

data that are in the same range compared to the other producers, so it can be supposed that they used similar kits.

Ghrelin_CoM

Producer	Mittelwert	Minimum	Maximum
Hosoda	502.2667	407.20	671.80
Linco Research	485.4333	78.00	904.50
Phoenix Pharmaceuticals	443.3857	66.90	819.00
nfl	695.5250	443.70	1085.50
Insgesamt	511.3696	66.90	1085.50

Table 10: Minimum – Maximum among producers

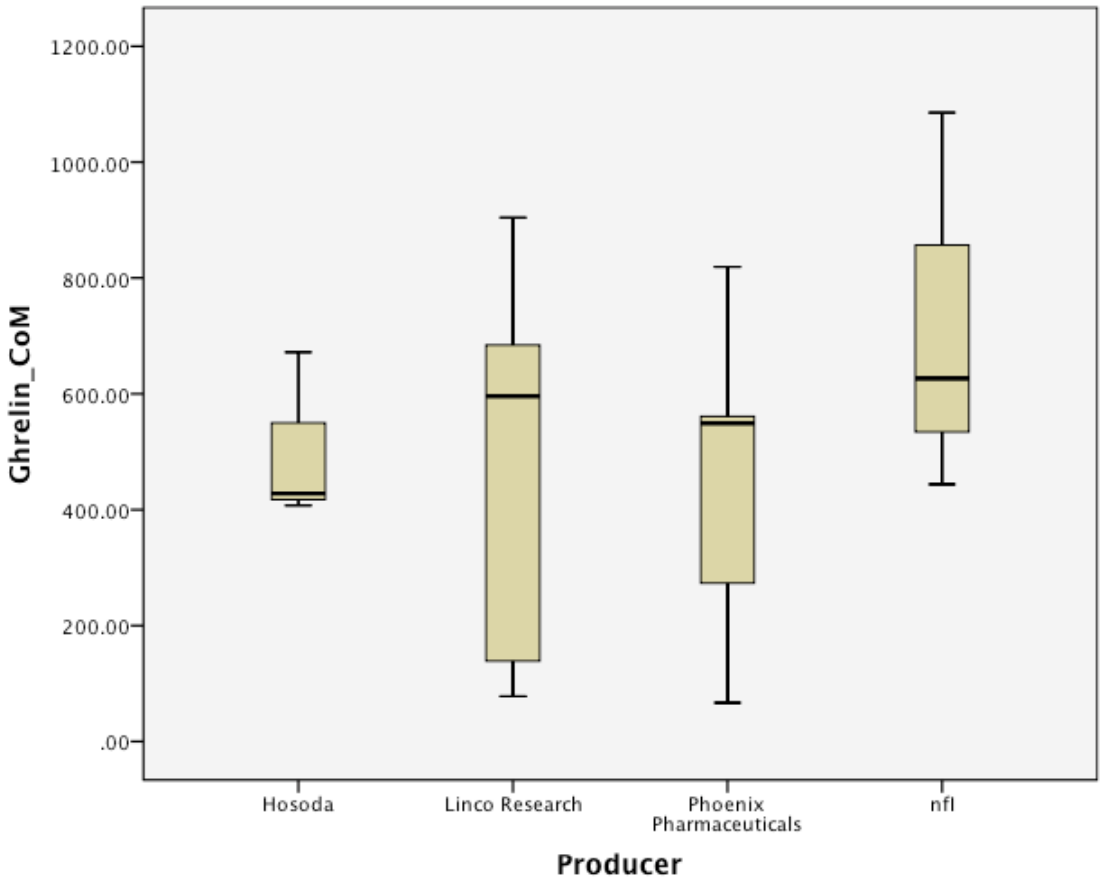

Figure 11:Ghrelin values among different producers

Sample preparation

More precise information regarding blood sampling and preparation was available in 17 publications. Ten publications collected their blood samples in tubes with EDTA and aprotinin. EDTA is the abbreviation for Ethylenediaminetetraacetic acid, that is an anticoagulant substance, commonly used for plasma preparation (Awar R., 2005). Aprotinin, also known as Trasylol, is a protease inhibitor and reduces bleeding (www.applichem.com). In three publications, EDTA and another protease inhibitor was added to the sample. Crispim et al., Hotta et al., Awar et al. and Tong et al used in addition to EDTA also HCN to acidify plasma. This step was done to stabilize the n-octanoyl modification of the serine residue and to prevent a rapid deacylation of ghrelin (Crispim et al., 2011). Jakubowicz et al. only used EDTA (Jakubowicz et al., 2012) and three publications from Sofer et al., Matsunaga et al. and Katarzyna et al. added no additional substances to the sample.

After the addition or non-addition of substances, samples were centrifuged and stored at -70 to -80 °C until measurement. Depending on the used substances, there are no differences between ghrelin values. Unfortunately, it was not possible to determine the time between blood collection and measurement. Matsunaga et al. is the single publication, which gave the information that they measured ghrelin within 60 minutes after blood collection (Matsunaga-Irie et al., 2007). This point could be interesting to discuss, because ghrelin is a hormone whose stability has not been investigated sufficiently yet.

Country of Origin

In the following analyzes, ghrelin data of healthy and obese patients and control groups of ED are represented, because previous comparisons showed no differences. In Table 11, ghrelin data among countries and total number of patients are shown. Asia and the US are representing the biggest groups with 436 and 282 participants, while the other ones are illustrated with a single publication each.

Country	Ghrelin pg/ml	N
Asia	554.1	436
US	608.2	282
Australia	127.0	50
Poland	66.9	37
Brazil	450.2	22
Italy	221.1	20
Serbia	443.7	10

Table 11: Ghrelin values and number of patients in different countries

In Figure 12 below mean values of ghrelin in each country are shown more clearly. The US represents the highest ghrelin mean value with 608.2 pg/ml, followed by Asia with 554.1 pg/ml, Brazil with 450.2 pg/ml and Serbia with 443.7 pg/ml. After a bigger interval, Italy follows with 221.1 pg/ml and Australia with 127 pg/ml. The lowest ghrelin value was achieved with only 66.9 pg/ml by Poland.

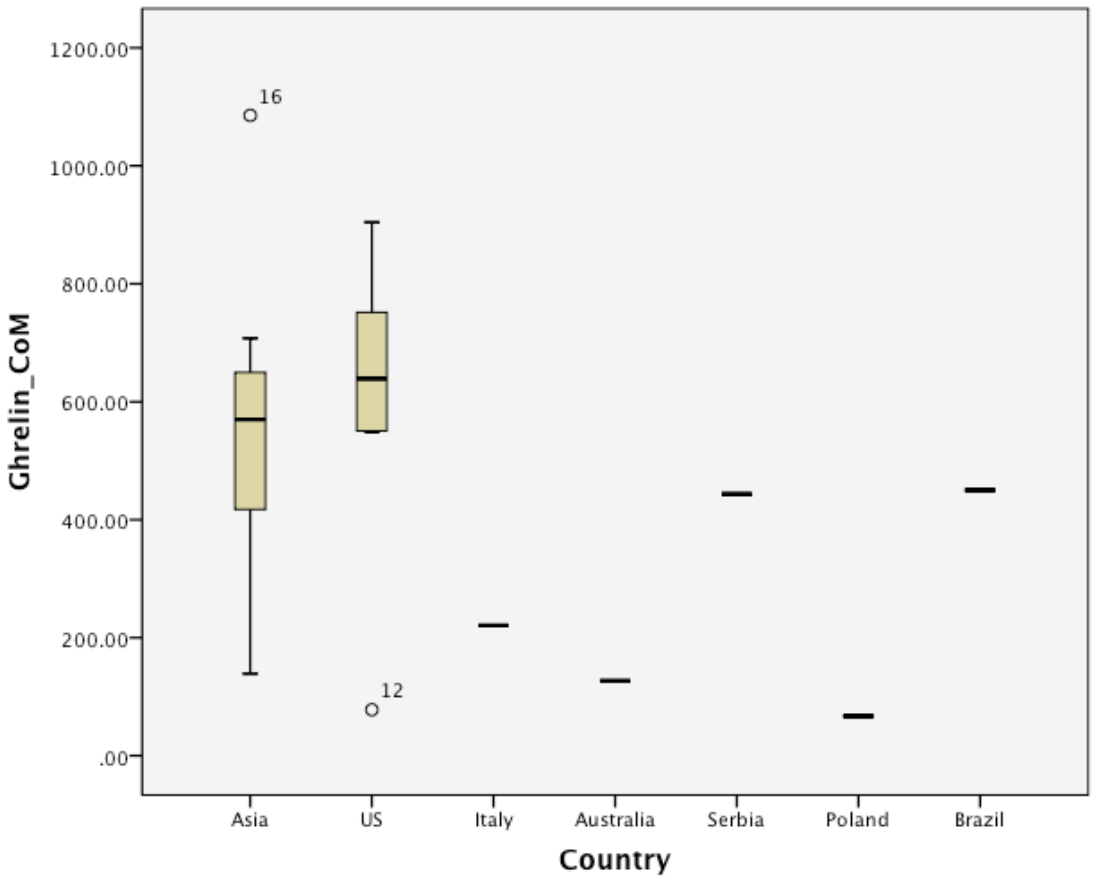

Figure 12: Mean ghrelin values among different countries

Body Mass Index and Age

In the following chart, all data of the 24 publications are used. Figure 13 shows the correlation between ghrelin and BMI among all participants.

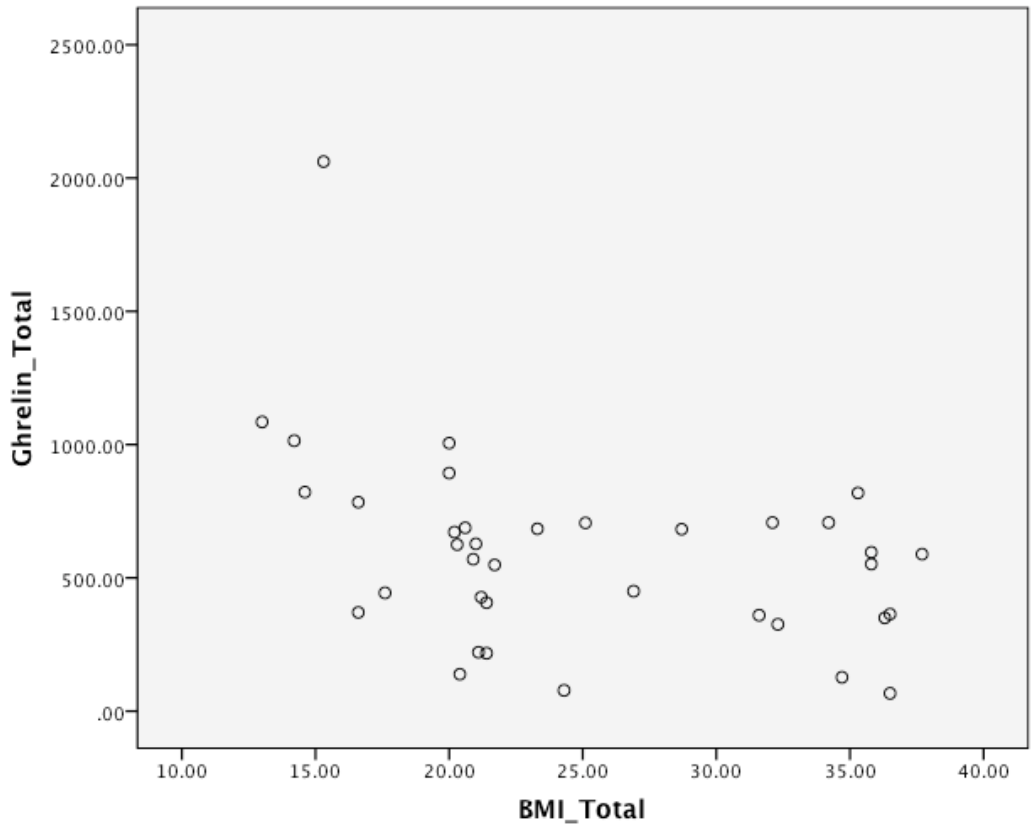

Figure 13: Correlation between Ghrelin and BMI in total population

Korrelationen

		BMI_Total	Ghrelin_Total
BMI_Total	Korrelation nach Pearson	1	-.394*
	Signifikanz (2-seitig)		.016
	N	37	37
Ghrelin_Total	Korrelation nach Pearson	-.394*	1
	Signifikanz (2-seitig)	.016	
	N	37	37

*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

Table 12: Correlation coefficient Ghrelin and BMI

It can be seen in Table 12 that there is a weak negative correlation. The Pearson correlation coefficient, which was calculated with SPSS, shows that even a significant correlation with -0.394 exists. The level of significance was assumed with 0.05.

Figure 14 below shows the correlation between ghrelin and age. There is an unexpected negative correlation, again with a level of significance of 0.05. The Pearson correlation coefficient is -0.377, as it can be seen on Table 13 on the next page.

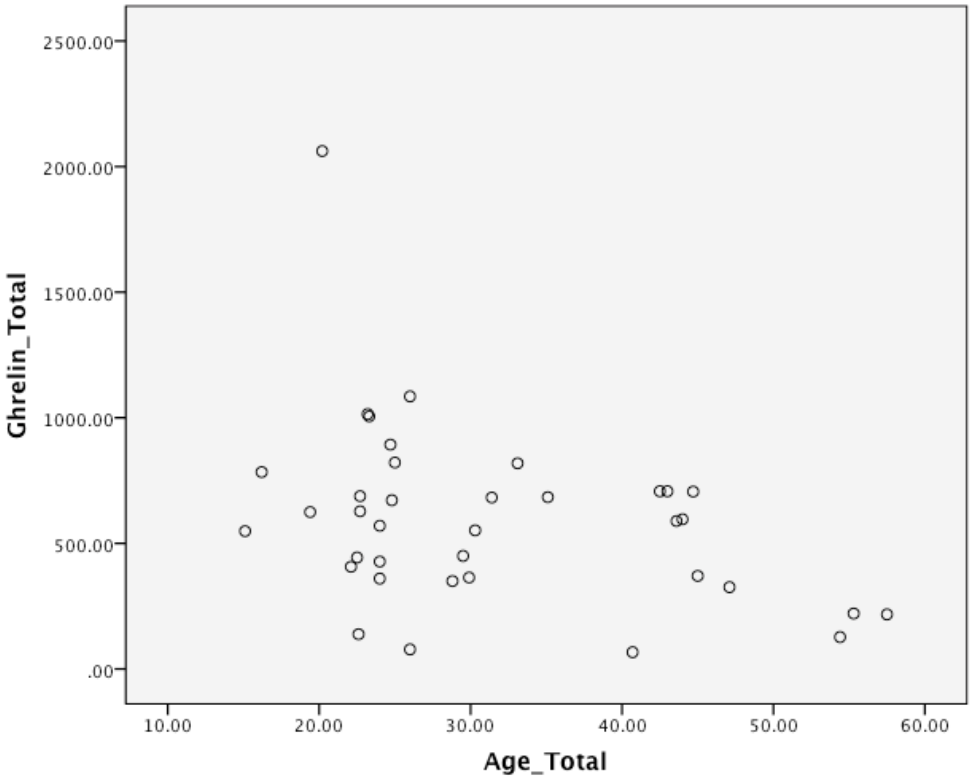

Figure 14: Correlation between Ghrelin and Age in total population

Korrelationen

		Ghrelin_Total	Age_Total
Ghrelin_Total	Korrelation nach Pearson	1	-.377*
	Signifikanz (2-seitig)		.021
	N	37	37
Age_Total	Korrelation nach Pearson	-.377*	1
	Signifikanz (2-seitig)	.021	
	N	37	37

*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

Table 13: Correlation coefficient Ghrelin and Age

Taking these facts into consideration, it could be concluded that ghrelin values are higher with an approximate age of 20 years, and lower with a higher age. Through the circumstance that there are no known authors who declare similar results regarding the connection of age and ghrelin, the question remains if this finding can be considered as meaningful.

Furthermore, the younger age group in this review is mostly represented by patients with EDs, and the older ones by obese patients, so this result could be expected, through the fact that ED patients have a lower BMI and obese patients a higher one.

After the comparison of possible influence factors on ghrelin values in those 24 publications, the following statements can be made. The used methods and also the different producers of the kit cannot be differentiated regarding ghrelin values. The values are altogether in the same range, also these where no further information was available. More attention should be given to the topic of handling with samples and sample preparation. The different substances that are added to plasma sample could have a great influence on the stability of ghrelin. Furthermore, the elapsed time from sampling to measurement, which was mostly not available here, raises a few questions concerning the storage life of the hormone.

5. DISCUSSION

As mentioned previously, there are still some unanswered questions, regarding the results that were found and the stability of the hormone. Before these questions will be answered, a short illustration of an individual measurement of ghrelin is done.

Individual measurement of ghrelin

The aim of the attempt was to investigate the behavior of the hormone after food intake. Therefore, a comparison between fasted ghrelin values and values shortly after breakfasting was conducted. In order to measure the ghrelin values, ELISA was used due to its common usage in this area. However, after two test series it turned out that values were absolutely not comparable with each other. After the first measurement, we obtained values in the area of about 100 pg/ml, but at the second attempt, values were outside the detectable range of the kit. Furthermore, problems occurred regarding the standard curve. Several standard values were deviating from the set point, which leads to the problem that the standard curve was not usable any more. It was unclear if mistakes occurred during the implementation of the method or whether blood samples were not stable enough up to measurement.

Stability and Methods

Gröschl et al. (2002) investigated preanalytical influences on the measurement and the stability of ghrelin. They tried to evaluate data on stability of ghrelin under different storage conditions and also the effect of repeated freezing and thawing cycles. Samples were stored at 25°C and 4°C. Ghrelin was measured with a commercial RIA from Phoenix. They investigated that ghrelin was stable up to three days when it was stored at 4°C, whereas the storage at 25°C for more than one day produced significantly lower results (Gröschl et al., 2002).

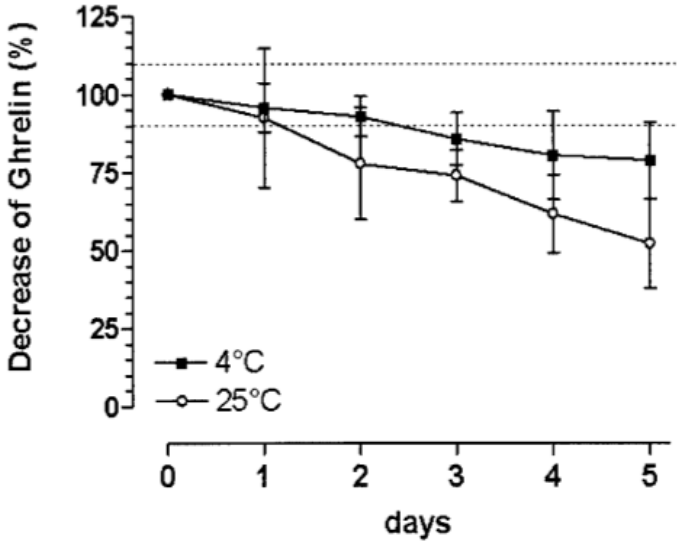

Figure 15: Stability of ghrelin after storage at 25 and 4°C for up to 5 days (Gröschl et al., 2002)

Repeated freezing and thawing should also not be a problem, for example if sample tubes are used several times for repeating an assay (Gröschl et al., 2002) .

These findings could also be an indication that samples should be cooled down immediately after the removal, if they are not used for measurement directly. Unfortunately, there were no exact specifications for the permitted elapsed time between measurement and withdrawal.

Hosoda et al. investigated the optimum collection and storage conditions for ghrelin measurements. They established two different forms of RIA, whereas N-RIA recognizes the octanoyl-modified portion, also known as active ghrelin and C-RIA recognizes the total form. They used different tubes, which contained disodium EDTA with aprotinin, disodium EDTA alone, heparin sodium or no anticoagulant (Hosoda et al., 2004).

Furthermore, samples were divided into two aliquots for incubation at either 4 or 37°C. There were comparable results for total ghrelin tested by C-RIA, but in contrast, the N-RIA gave significantly decreased ghrelin values at 37°C. Samples stored for 60 minutes at 37°C lost up to 35% of ghrelin. Ghrelin concentrations in tubes with heparin were significantly decreased. In contrast, when tubes with EDTA and aprotinin were used, the decreases in ghrelin

stability were smaller compared to the other three substances. Storage at 4°C also improved the stability. Hosoda et al. also investigates whether the pH has an influence on ghrelin stability. Ghrelin measured by C-RIA remained stable across different pH and storage temperature conditions. When measuring ghrelin by N-RIA, it was most stable at a pH of 3-4. Repeated freezing and thawing did not influence ghrelin values measured by C-RIA (Hosoda et al., 2004), which was also investigated by Gröschl et al.

On the contrary, when measuring ghrelin with N-RIA, values decreased significantly with each freeze-thaw cycle. After acidification, ghrelin remained stable. The acidification of ghrelin protects against degradation and improves the stability at pH 4. However, pH may not be under 2, because otherwise there would also be a decrease (Hosoda et al., 2004).

To sum up, it can be said that ghrelin measured by C-RIA is relatively stable at different storage conditions. When measuring ghrelin with N-RIA, stability can be received with acidification

Hosoda et al. also recommends the following standard procedure for the collection of blood samples:

- Collection of blood samples with EDTA and aprotinin is preferred
- Blood samples should be chilled and centrifuged as soon as possible, at least within 30 minutes after withdrawal
- Acidification is the best method for the preservation of ghrelin, thus 1 mol/L HCl or 10% of sample volume should be added to sample to adjust a pH around 4 (Hosoda et al., 2004)

In this systematic review, only four authors used acidification to stabilize ghrelin. Ten authors used EDTA and aprotinin, as it is recommended by Hosoda et al. Katarzyna et al. measured ghrelin without any additional substances. With a value of 66.9, ghrelin appears to be extremely low (Katarzyna Mizia-Stec et al., 2008). This low value could be explained by the non-acidification of the hormone.

In another publication of Gröschl et al. they investigated the comparability of two different ghrelin assays. They compared the two most commonly used commercial RIAs, purchased from Phoenix Pharmaceuticals and Linco Research. The samples were stored at -80°C and assayed as recommended by each producer. Both assays used 100 µL of the sample. There were differences in the concentration, whereas Linco Research gives the concentrations in ng/L and Phoenix Pharmaceuticals in pg/tube. There were also differences in the measured values. Phoenix ranges from 10 to 1240 ng/L and Linco measured values that were 10 times higher with 131 to 11666 ng/L. The linear regression, with a regression coefficient of 0.976 can be seen in Figure 15. The 10-fold difference was also observed when the calibrator of the Linco assay was replaced by the calibrator of the Phoenix assay or vice versa. The authors declare this discrepancy with a faulty determination by one (or both) of the producers. Aside from this finding, both assays appear analytically acceptable. Gröschl et al. also indicate that switching between the two producers is not recommended, especially not within one study. (Gröschl et al., 2004)

The findings in this review also show that the range of detectable ghrelin with 93-6000 pg/ml by Linco is higher than those of Phoenix with 10 to 1280 pg/ml. But in contrast to those findings, there were no significant differences between them, with 463.41 by Linco and 443.39 by Phoenix.

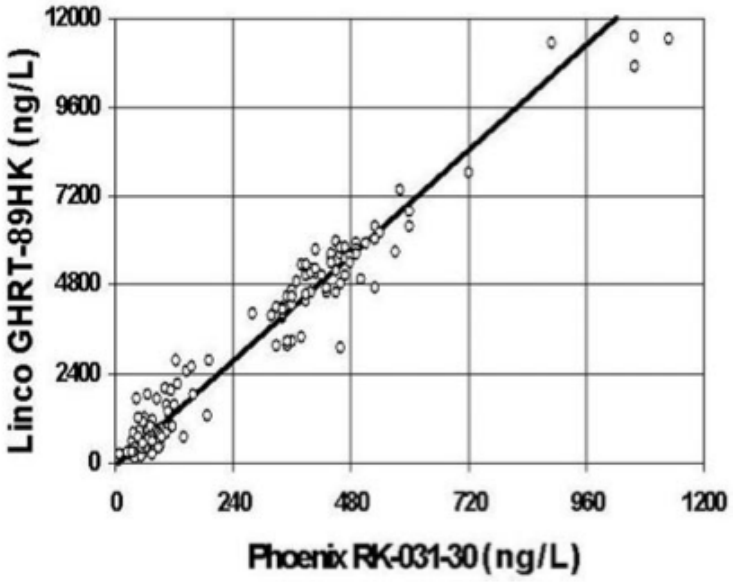

Figure 16: Linear regression between ghrelin concentrations from Linco and Phoenix (Gröschl et al., 2004)

Analysis of the publication from Carnier et al.

In this chapter the enormous high ghrelin value measured by Carnier et al. is analyzed. In contrast to the two publications of Geliebter et al., Carnier et al. provides contradictory respectively extremely high results for patients with BED. Carnier et al. investigated ghrelin values of 20 obese adolescents with symptoms of BN and BED, which were assessed using the Binge Eating Scale (BES) and Bulimic Investigation Test Edinburgh (BITE). They measured a ghrelin value of 6.91 ng/ml, which are 6910 pg/ml. Compared to the other results in this systematic review, it represents the highest value of ghrelin. The authors indicated in the publication that they have, like Geliebter et al. 2005, also a similar tendency with lower ghrelin levels and high levels of BMI. (Carnier et al., 2008)

Results in Table 7 show that they indeed have a high BMI, but with a value of 6910 pg/ml, ghrelin is anything but not low. In fact, this value can only be compared in this systematic review, and on the basis of the measurement

methods, it is by far the highest value. Carnier et al. measured ghrelin by RIA, which was provided by Linco Research, St. Charles, Miss., USA. Interestingly, when looking in the instruction manual of the ghrelin kit from Linco Research, they refer to a detection range with a minimum of 93 pg/ml to a maximum of 6000 pg/ml. Furthermore, they also suggest to repeat the measurement with a dilution of the sample, when values are outside the detectable range. (Linco Research)

After a short literature search for publications of Carnier et al., another study was found in which they investigated ghrelin levels in obese patients with EDs. Again ghrelin values in the same range of about 8 ng/ml were found. They reported their “low” values as appropriate for obese patients and also talked about an agreement with the publication of Reinehr et al., but with interesting results (Carnier et al., 2010).

This working group investigated ghrelin levels in obese children and used the same method and producer like Carnier et al. Reinehr et al. measured mean ghrelin levels of 1080 pg/ml, which would be in the detectable range of the kit and also in agreement with the values in obese patients in this review. (Reinehr et al., 2008) They might be a bit higher, but as mentioned in the chapter of healthy and obese patients, it was not possible to determine a range for them. Anyway, the important point in the comparison of these two publications is that Carnier et al. referred to the study of Reinehr et al. with the argument that their values would agree and be in the same range. After a comparison for my part, 8050 pg/ml and 1080 pg/ml are definitely not in the same range.

Comparison with other studies

Not only is this review problems in finding normal ghrelin values occurred, but also in other papers, for example published from Kosowicz et al. They investigated the technological difficulties in ghrelin assays, with the result that even when the used RIA kits from the same manufacturer, many laboratories got different results (Kosowicz et al., 2011). The following figure shows an overview of the range that was determined by them.

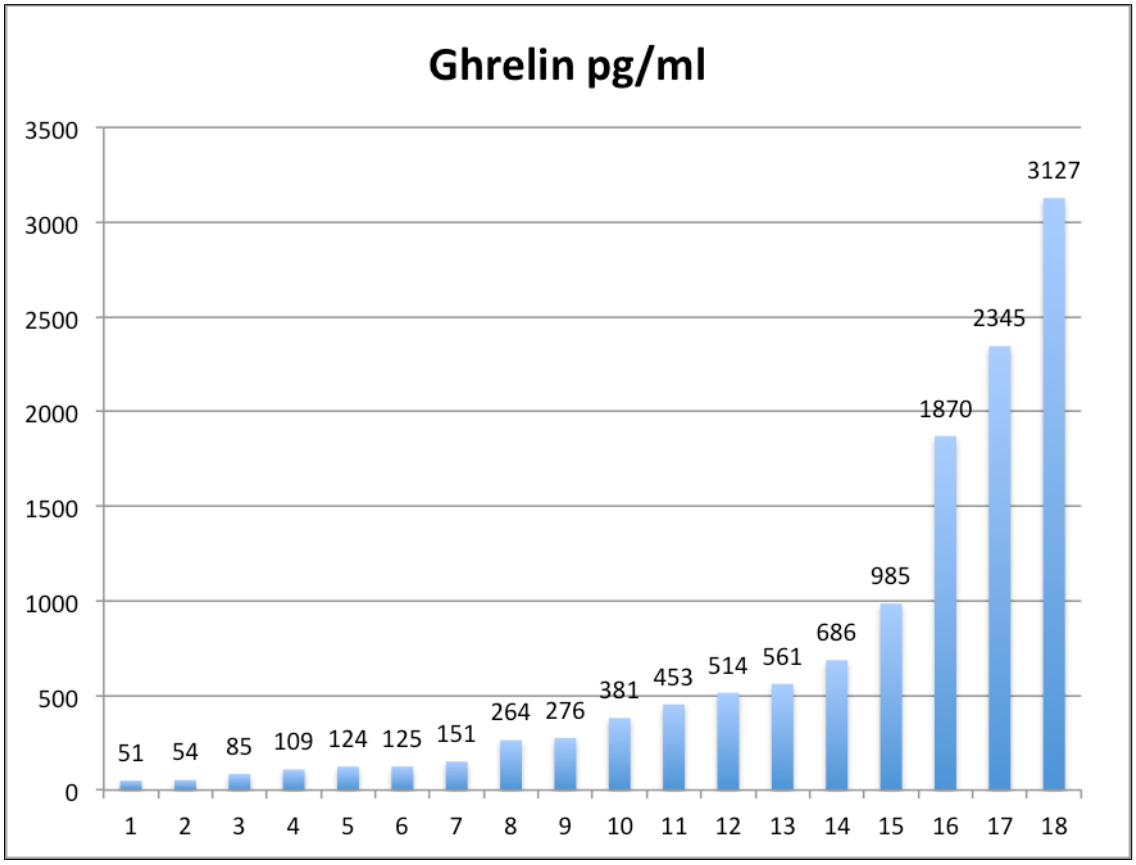

Figure 17: Ghrelin concentrations reported by various authors (Kosowicz et al., 2011)

It is obvious that ghrelin values are also scattering in such a wide range, like it was in this review. In most instances, values are in the range of 150 to 600 pg/ml, which is also close to the range described here. Kosowicz et al. declared that on the one hand, values below 100 pg/ml may result from improper handling of the reagents and also inappropriate conditions of transportation, storage and laboratory procedures, that require temperatures below 24°C.

On the other hand, values above 1000 pg/ml are not comprehensible for them, because the available ghrelin standard curve gives results in the range from 50 to 1000 pg/ml. (Kosowicz et al., 2011)

In the case of Carnier et al., the same unsolved question occurred, because they also measured values outside the reliable range. The authors never declare how they were able to measure such high values. Carnier et al. do not seem to be the only ones who measure values in an impossible or not reproducible range.

5.1 Limitations and Strengths

In this review there are some limitations that have to be considered. One important limitation is that, unfortunately, it was not possible to get access to all publications that met the inclusion criteria. As it can be seen in the Flow diagram (Fig. 4), 50 publications were not available for this review. These publications could have had further important findings. Furthermore, there were also 7 publications that had to be excluded because ghrelin values only were apparent through a diagram or figure in the study, and no exact data was stated. After contacting the authors to get further information regarding ghrelin data, no reply has been received so far.

Another limitation was the one time measurement of ghrelin. Through the fact that ghrelin is a hormone in which the stability is dependent on many factors, it would have been more reliable if samples were drawn and measured twice.

Furthermore, there could have been more male participants to succeed a better comparison between men and women, especially in the group of obese patients and those with eating disorders.

Disregarding these limitations, this systematic review can offer one important strength. It is the first systematic review about ghrelin values in the mentioned patients groups. A further strength is that inclusion and exclusion criteria were elected in a way, to make ghrelin values comparable within the three groups.

6. CONCLUSION

In this systematic review of 24 publications and altogether 1206 patients partially conflicting results were found. This was the first time that a systematic review about ghrelin values was undertaken. Due to this fact, there was no possibility to compare the found results. This master thesis should give an overview about ghrelin values in different patients groups.

The hypothesis that there are differences in ghrelin values among the mentioned three groups of patients cannot be approved completely. Within the group of patients with EDs that is approving the hypothesis, the subdivision in AN, BED and BN manifested differences of ghrelin values. Patients with AN have a mean ghrelin value of 1023 pg/ml, BN patients 701 pg/ml and BED patients 357 pg/ml. In addition to this classification of ghrelin values in ED patients, also a negative correlation between BMI and ghrelin was found. In the other two groups of healthy and obese patients no differences in ghrelin values were found. Moreover, it was not possible to create a mean value for each group because values were scattering in such a wide range. The area of ghrelin values for healthy and obese was the following: 78 - 819 pg/ml for healthy patients and 67 - 708 pg/ml for obese patients. Although there were exact inclusion and exclusion criteria, which should make the groups comparable with each other and on the other hand distinguishable from each other, ghrelin values are scattering in such a wide range that they are not assignable to any of the groups of healthy and obese patients.

Unfortunately, it was not possible to get new insights about the impact of ghrelin on obesity because we did not identify whether ghrelin values are high or low in these patients. In the case of EDs, there is an evidence that ghrelin is overexpressed through the permanent feeling of hunger. Hotta et al. and Broglio et al. also gave the evidence that ghrelin infusion might be a therapeutic option for patients with AN (Broglio et al., 2004;Hotta et al., 2009). Due to the reached increase in hunger sensations and energy intake, the administration of ghrelin could improve symptoms of EDs. It would be interesting if the opposite in obese patients was possible, for example through a suppression of ghrelin with anti-

ghrelin analogs. With a further decrease in ghrelin values the satiation period could be prolonged with the effect of less energy intake. To clarify this question definitely, more research has to be done in the future.

It was also not the first time that disagreements were found regarding ghrelin values. Kosowicz et al. also reported values with a high variation, although different authors used the same method and patient groups (Kosowicz et al., 2011). According to these findings, this systematic review illustrates the following problem: As long as there will be no standardized methods for blood sampling, preservation methods and storage conditions respectively duration, ghrelin values will not be comparable among different working groups.

Hosoda et al. was the first working group that recommended a standard procedure for the collection of blood samples (Hosoda et al., 2004). Furthermore, the various measurement types like RIA and ELISA should be standardized in a way, to make them comparable to each other.

Of what avail is it, when there are numerous publications with new findings about a hormone with interesting functions that are still not developed completely, and these findings cannot be compared with other ones? This review exposed that further research is needed. On the one hand, regarding ghrelin itself, and on the other hand, probably the more important one, regarding standardization procedures in measurement and preparation of samples.

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