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Opioid-like peptides and ghrelin mitigation of bariatric results depends on obesity level

Michał Dyaczyński[1], Marek Drożdż[1], Leontyna Wylężek[1], Colin G. Scanes[2], Anna Rzepa[3], Andrzej Cieśla[4], Krystyna Pierzchała-Koziec[5]

[1]*Municipal Hospital, Siemianowice Śląskie, Poland*
[2]*Center in Excellence in Poultry Science, University of Arkansas, Fayetteville, AR, United States*
[3]*2nd Department of General Surgery, Jagiellonian University Medical College, Krakow, Poland*
[4]*Institute of Experimental and Clinical Medicine, Faculty of Medicine, University of Rzeszow, Rzeszow, Poland*
[5]*Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Krakow, Poland*

Abstract

Introduction: Bariatric surgery, as the only effective treatment of obesity, has strong effects on the metabolism, and nervous and endocrine systems. Thus, based on the different opinions about the efficaciousness of morbid obesity treatments, the aim of the present study was to estimate the association of serum ghrelin and Met-enkephalin (native, five amino acids and cryptic, precursor of enkephalin) concentrations with body mass index (BMI) value in bariatric patients within 30 postoperative days.

Material and methods: The study was performed on 38 female patients divided into two groups: I — BMI lower than 40 kg/m^2 (n = 18) and II — BMI higher than 40 kg/m^2 (n = 20). Blood was taken before (–24 h), and 72 h and 30 days after the sleeve gastrectomy. Routine haematological, anthropometric, and metabolic parameters as well as thyroid-stimulating hormone (TSH), ghrelin, and Met-enkephalin values were measured in all patients.

Results: There were statistically significant differences between the two groups before the surgery in terms of TSH, both forms of Met-enkephalin, triglycerides concentrations, and activity of alanine transaminase (ALT), gamma-glutamyltransferase (GGTP), and C-reactive protein (CRP). After 72 h, the serum levels of cryptic Met-enkephalin and CRP, and activity of enzymes varied between the two groups of patients. Thirty days after the surgery, some metabolic and immune parameters were still different in both female groups in favour of patients with lover BMI. However, significant differences were noticed in the levels of ghrelin (increase), and native (decrease) and cryptic Met-enkephalins (increase).

Conclusions: The activity of endogenous peptides in bariatric patients is connected with the degree of obesity. Ghrelin level increases are negatively correlated with native Met-enkephalin changes shortly after bariatric surgery. The interplay of ghrelin and opioids might be considered as a predictor of postoperative weight loss success. **(Endokrynol Pol 2020; 71 (1): 27–33)**

Key words: *opioids; ghrelin; obesity; bariatric surgery*

Introduction

Obesity is a highly prevalent disease in the world, and it is caused by many different factors: an excess of nutrients, genetic background, lack of physical exercise, or disorder of the hypothalamo-gastrointestinal axis activity [1, 2]. Obesity is frequently subdivided into categories depending on a body mass index (BMI) value: class 1: BMI of 30 to < 35 kg/m^2; class 2: BMI of 35 to < 40 kg/m^2; and class 3: BMI of 40 kg/m^2 or higher. Class 3 obesity is sometimes categorised as "extreme" or "severe/morbid" obesity [2, 3]. In spite of many scientific studies the borders between the three classes of obesity are inconsistent, and widely used parameters are not able to indicate the reason/reasons for progressive obesity.

Currently, it may be concluded that the only effective treatment of obesity remains surgery. However, it must be pointed out that bariatric surgery has strong effects on the metabolism, and on the nervous and hormonal systems [3], because hunger and satiety are mediated by an interplay of nervous and endocrine signals at the central and peripheral levels [4]. Also, it is not clear why many patients are losing weight very slowly or even regaining the weight in a short time.

Since 2002 ghrelin has been considered as an important hormone stimulating the appetite in healthy mammals [5]; its effects have been correlated with the changes in synthesis, secretion, and concentration of opioid peptides in the blood, brain structures, and peripheral tissues in animals [6–8], which indicates a close relationship between these hormones.

Krystyna Pierzchała-Koziec, Katedra Fizjologii i Endokrynologii Zwierząt, Uniwersytet Rolniczy w Krakowie, Al. Mickiewicza 24/28, 30–059 Kraków, tel: (+48) 698 630 42; e-mail: rzkoziec@cyf-kr.edu.pl

Endogenous opioid peptides are expressed in the central nervous system, and in peripheral tissues, mainly the gastrointestinal tract, adrenals, heart, and pancreas [9]. Met-enkephalin, the most widely occurring opioid, exists in the blood and in the tissues as a native, five-amino-acid peptide with short half-life (less than 1 min) and in a larger form called cryptic Met-enkephalin (PENK). The ratio of cryptic/native Met-enkephalin significantly varies in animal models depending on tissue, physiological status, stress, and gender [10–12]. The synthesis of enkephalins, in all structures of the digestive tract and in the areas of the hypothalamus, responsible for regulation of feeding behaviour, provides evidence of the involvement of these peptides in the regulation of nutrition [13]. The orexigenic effect of enkephalins has been proposed by some investigators, which suggest that endogenous opioid peptides and their specific receptors could be considered as effective targets for counteracting obesity [14, 15].

Thus, based on the different opinions on the efficacy of morbid obesity treatments, the aim of the present study was to estimate the impact of the degree of obesity on the roles of ghrelin and Met-enkephalin in the short-term mitigation of bariatric surgery effects.

Material and methods

Bariatric surgeries — sleeve gastrectomy — were performed in the Municipal Hospital in Siemianowice Śląskie. Each patient signed an informed consent form. The reported investigations were carried out in accordance with the principles of the Declaration of Helsinki. Anthropometric parameters (Tab. 1) were measured in 38 qualified patients (age x = 30.4 years). Patients were divided into two groups: with BMI lower than 40 kg/m^2 (x = 35.8 ± 3.4, n = 18) and with BMI higher than 40 kg/m^2 (x = 46.1 ± 5.2, n = 20).

Blood samples were collected after overnight fasting: 24 h before and 72 h (3 days) and 30 days after the surgery.

The serum level of thyroid-stimulating hormone (TSH) was measured by the ELISA method. Ghrelin and Met-enkephalin levels in the blood were estimated by radioimmunoassay method (ghrelin-Phoenix Pharmaceuticals, Belmont, California, USA) and the method developed by Pierzchala and VanLoon for enkephalins [9]. Measurements of peptides were performed in the Department of Animal Physiology and Endocrinology at the University of Agriculture in Krakow.

The remaining parameters were estimated by the routine methods used in the hospital. For the normal range for all measurements the cut-offs for adult patients were used according to Synevo Laboratories.

Statistical analysis

Statistical analyses were performed by one-way ANOVA for multiple comparisons, or unpaired t-test to compare two mean values (Statistica 10 software StatSoft, USA). All data are expressed as means ± SEM. A value of $p < 0.05$ was considered statistically significant.

Results

Characteristics of parameters measured before the bariatric surgery are presented in Tables 1–3. There were statistically significant differences ($p < 0.05$) between two groups in term of TSH concentration, triglycerides, activity of alanine transaminase (ALT) and gamma-glutamyltransferase (GGTP), and C-reactive protein (CRP).

Statistical differences were observed after 72 h (Tab. 3) in the activity of ALT, GGTP, and white blood counts cells (WBC) counts. The serum level of CRP (Tab. 3) was dramatically elevated in the group with BMI < 40 kg/m^2 (18 times), and it was a much higher increase than in the patients from the group with BMI > 40 kg/m^2 (9 times, $p < 0.05$).

Thirty days after the bariatric surgery (Tab. 2) changes of total cholesterol, LDL, and triglycerides were observed in both groups. Significant differences (Tab. 3) were also observed in the activity of ALT, prolonged higher WBC, and high serum level of CRP.

The native Met-enkephalin level in the group with BMI < 40 kg/m^2 was significantly lower ($p < 0.05$) than in the blood of patients with BMI > 40 kg/m^2 at each measured point (Fig. 1) Blood levels of opioids measured three days after bariatric surgery were decreased in both groups by ~50% ($p < 0.05$). Thirty days after bariatric surgery Met-enkephalin levels increased but still were lower than before the operation in patients of both groups ($p < 0.05$).

The changes of cryptic Met-enkephalin (Fig. 2) in patients with BMI < 40 kg/m^2 had biphasic profile: decrease after three days by 21% ($p < 0.05$) and then

Table 1. ***Characteristics of parameters measured before bariatric surgery***

Parameter	BMI < 40 kg/m^2	BMI > 40 kg/m^2
Number of patients (female)	18	20
BMI [kg/m^2]	35.9 ± 3.4	46.1 ± 5.2*
Waist circumferences [cm] (min–max)	115 (83–144)	126 (104–146)
Hip circumferences [cm] (min–max)	129 (110–145)	134 (110–160)
Blood pressure [mm Hg] (systolic/diastolic)	144/98	142/91
HbA_{1c} (%)	5.4 ± 0.3	5.8 ± 0.4
Fe [mmol/L]	16.14 ± 1.8	13.27 ± 1.4
Ca [mmol/L]	2.09 ± 0.2	2.43 ± 0.2
B12 [pmol/L]	318.5 ± 21	377.3 ± 33
TSH [μIU/mL]	1.74 ± 0.1	2.43 ± 0.3*

–24 h, X ± SEM, *$p < 0.05$ between BMI. BMI — body mass index; HbA_{1c} — glycated haemoglobin; Fe — iron; Ca — calcium; TSH — thyroid-stimulating hormone; SEM — standard error

Table 2. *Comparison of parameters measured before and 30 days after bariatric surgery*

Parameter	BMI [kg/m^2]	Time	
		Before (–24 h)	After (30 days)
Total cholesterol [mmol/L]	< 40	5.36 ± 0.1	4.07 ± 0.1[a]
	> 40	5.61 ± 0.2	4.81 ± 0.2*
HDL [mmol/L]	< 40	1.58 ± 0.05	1.34 ± 0.03
	> 40	1.43 ± 0.07	1.42 ± 0.02
LDL [mmol/L]	< 40	3.3 ± 0.1	2.0 ± 0.1[a]
	> 40	3.5 ± 0.2	2.8 ± 0.2*
Triglycerides [mmol/L]	< 40	1.30 ± 0.1	1.22 ± 0.08
	> 40	1.60 ± 0.1*	1.68 ± 0.1*
Creatinine [μmol/L]	< 40	60.0 ± 3.1	67.0 ± 3.3
	> 40	68.1 ± 2.0	68.1 ± 4.2

–24 h *vs.* 30 days, X ± SEM, *p < 0.05 between BMI groups, [a]p < 0.05 between –24 h and 30 days); BMI — body mass index; HDL — high density lipoprotein; LDL — low density lipoprotein; SEM — standard error

Table 3. *Characteristic of parameters measured before (–24 h), 72 hours (+72 h) and 30 days (+30 days) after the bariatric surgery*

Parameter	BMI [kg/m^2]	Time		
		–24h	+72 h	+30 days
ALT [U/L]	< 40	21.1 ± 1.1	26.1 ± 1.3	33.3 ± 1.9[a]
	> 40	31.0 ± 2.3*	38.3 ± 2.1*[a]	41.6 ± 2.3*[a]
GGTP [U/L]	< 40	28.7 ± 1.2	35.5 ± 3.1[a]	26.8 ± 1.9
	> 40	36.1 ± 1.8*	23.1 ± 2.4*[a]	32.2 ± 2.1
Glucose [mmol/L]	< 40	5.01 ± 0.30	4.92 ± 0.20	5.49 ± 0.60
	> 40	5.95 ± 0.50	5.66 ± 0.40	5.76 ± 0.70
Haemoglobin [mmol/L]	< 40	8.25 ± 0.9	7.38 ± 0.8	7.94 ± 1.4
	> 40	8.62 ± 1.1	7.38 ± 0.9	8.49 ± 1.5
WBC × 10^9/L	< 40	7.01 ± 0.8	9.55 ± 1.2[a]	8.82 ± 1.1[a]
	> 40	8.70 ± 1.2	11.20 ± 1.3[a]	7.36 ± 0.9
CRP [mg/L]	< 40	7.01 ± 0.8	129.7 ± 10.3[a]	5.3 ± 0.4
	> 40	8.70 ± 1.2*	78.5 ± 8.7*[a]	6.2 ± 0.4[b]

X ± SEM, *p < 0.05 between BMI groups, [a, b]p < 0.05 between –24 h, +72 h (3 days), and 30 days; BMI — body mass index; ALT — alanine transaminase; GGTP — gamma-glutamyltransferase; CRP — C-reactive protein; WBC — white blood counts cells; SEM — standard error

increase after 30 days (p < 0.05). In contrast, cryptic Met-enkephalin level in the blood of females with elevated BMI, was much higher at –24 h (by 68%) and at +72 h (by 118%) than that seen in the second group (p < 0.05). After 30 days, the cryptic form of opioid was increased in this group to 17.1 ± 1.9 pmol/mL.

The differences in the changes of both enkephalin forms are clearly seen in Figure 3. The ratio of cryptic/native Met-enkephalin changed from 11.9 (–24 h) to 17.8 (+73 h) and to 43.7 (30 days) in the blood of females with BMI < 40 kg/m^2. In the plasma of patients with BMI > 40 kg/m^2 the ratio of cryptic/native enkephalins changed from 12.5 to 31.7 and to 25.9, respectively, at first, second, and third blood collection.

Blood levels of ghrelin (Fig. 4) were very similar in both patient groups before surgery. Three days after bariatric procedure the ghrelin level was increased by a comparable degree in all patients (35–45%, p < 0.05). After 30 days, the blood level of ghrelin in the patients with lower BMI was elevated to 43.8 ± 4.6 fmol/mL (p < 0.05). Unexpectedly, patients with BMI > 40 kg/m^2 manifested significantly higher increase of plasma ghrelin level up to 52.8+5.7 fmol/mL (p < 0.05).

Discussion

Obesity is connected with many changes of metabolic parameters. Qualification of patients for bariatric sur-

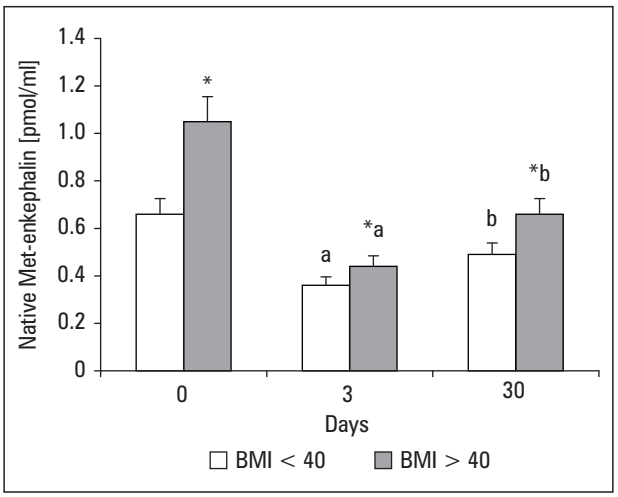

Figure 1. *Blood native Met-enkephalin levels [pmol/mL] in females before (0, –24 h) and after bariatric surgery (3 days, +72 h) and 30 days (x ± SEM, *p < 0.05 between BMI < 40 and BMI > 40 kg/m², different letter superscripts [a, b]p < 0.05 show the significant difference between results obtained at 0, 3 and 30 days in both BMI groups). BMI — body mass index; SEM — standard error of the mean*

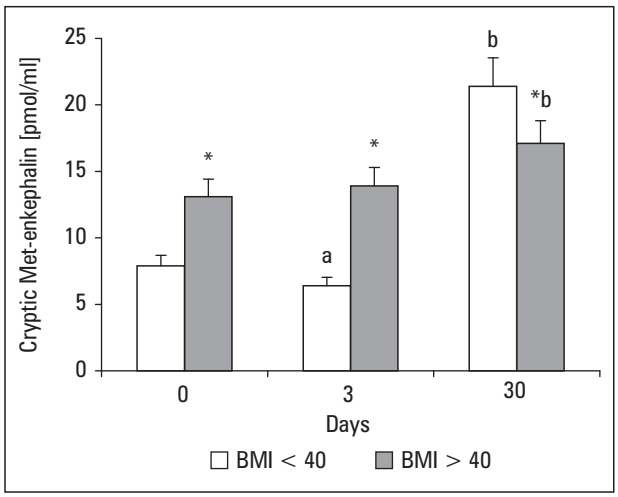

Figure 2. *Blood cryptic Met-enkephalin levels [pmol/mL] in females before (0, –24 h) and after bariatric surgery (3 days, +72 h) and 30 days (x ± SEM, *p < 0.05 between BMI < 40 and BMI > 40 kg/m², different letter superscripts [a, b]p < 0.05 show significant difference between results obtained at 0, 3 and 30 days in both BMI groups). BMI — body mass index; SEM — standard error of the mean*

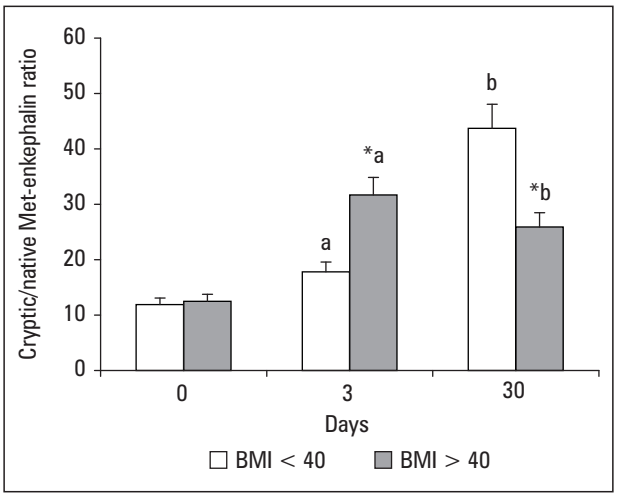

Figure 3. *Cryptic/native Met-enkephalin ratio values in females before (0, –24 h) and after bariatric surgery (3 days, +72 h) and 30 days (x ± SEM, *p < 0.05 between BMI < 40 and BMI > 40 kg/m², different letter superscripts [a, b]p < 0.05 show significant difference between results obtained at 0, 3 and 30 days in both BMI groups). BMI — body mass index; SEM — standard error of the mean*

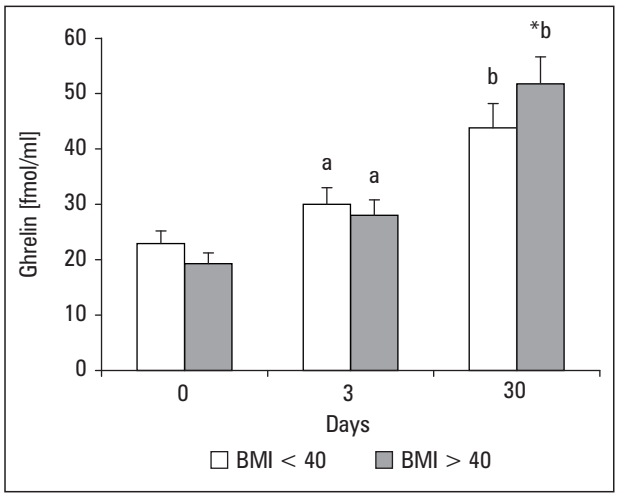

Figure 4. *Blood ghrelin levels [fmol/mL] in females before (0, –24 h) and after bariatric surgery (3 days, +72 h) and 30 days (x ± SEM, *p < 0.05 between BMI < 40 and BMI > 40 kg/m², different letter superscripts [a, b]p < 0.05 show significant differences between between results obtained at 0, 3 and 30 days in both BMI groups). BMI — body mass index; SEM — standard error of the mean*

gery must consider the lipid and carbohydrate panels and liver enzymes activity [16]. Analysis of triglycerides showed differences between the two groups of patients. Caution should be exercised in patients with BMI > 40 kg/m² and with significantly higher level of CRP even 30 days after bariatric surgery.

Accumulating evidences indicate that a state of chronic inflammation plays a crucial role in the pathogenesis of obesity-related metabolic dysfunction. Some data show that high BMI is connected with elevated CRP in obese patients. Bariatric surgery causes a significant decrease in the inflammation process, which is closely associated with adipose tissue loss. Santos et al. [17] found that three months after gastric banding surgery CRP was significantly decreased together with weight loss, increased iron absorption, and decreased triglyc-

eride activity. The present results indicate that significantly elevated CRP level in the blood of patients with BMI > 40 may be the effect of much slower body weight loss during the first 30 days after bariatric surgery.

In spite of the fact that the TSH level was within the reference range, the present study showed a positive correlation between the lower TSH level (1.74 ± 0.11 μIU/mL) and BMI below 40 as well as between the higher TSH level (2.43 ± 0.30 μIU/mL) and BMI above 40. Interestingly, higher TSH level was positively correlated with increased levels of ALT, GGTP, CRP, and triglycerides, clearly confirming that morbid obesity is connected with decreased energy expenditure by diminishing the degree/intensity of thyroid functions and metabolic processes [18].

It was noted that higher levels of TSH positively correlated with elevated concentrations of lipids [19]. In terms of metabolic syndrome prevention, some scientists suggest monitoring the TSH and thyroid hormones in overweight and obese children [19] and adolescents [20].

Bariatric surgeries have a strong impact on the metabolism, carbohydrates, and lipid turnover as well as on the hormonal system [3, 21, 22].

Endogenous opioid peptides are synthesized in all parts of the gastrointestinal tract, so each bariatric surgery has a strong impact on the enkephalin concentration and activity of the brain–gastrointestinal axis.

Some studies have suggested that endogenous opioids play a role in regulating consummatory behaviour and weight gain during access to palatable diets [15, 23]. Such results are interesting when viewed in the context of human genetic screens, strongly implicating opioid receptors in obesity phenotypes [24]. Additionally, antagonism of opioid receptors can in some circumstances be effective for reversing such phenotypes in preclinical and clinical studies. A previous study of our group showed that in obese human and animal experimental models (piglet, mouse) blood and tissue concentrations of Met-enkephalin were higher than in lean controls (unpublished data). In the present study, blood native Met-enkephalin levels were higher in females with BMI > 40 before and after bariatric surgery compared to values observed in patients with BMI < 40. Interestingly, native Met-enkephalin was decreased three days after the surgery in both groups of patients by 58% (BMI > 40) and 45% (BMI < 40) but at 30 days started to grow slowly; however, they were still lower than before the surgery. It may be suggested that the gastrointestinal tract is one of the main sources of enkephalin-releasing PENK and/or native forms to the peripheral blood [9]. On the other hand, during the 30 days, the native form of endogenous opioid peptides was probably released from other sources — adrenals, vagal nerve, pancreas or blood proteins — so its level increased. Bariatric surgery is also stressful for the patients and activates the hypothalamic-pituitary-adrenal axis. Increased release of corticoliberin from the hypothalamus stimulated synthesis and release of the adrenocorticotrophin from proopiomelanocortin. Paradoxically, proopiomelanocortin (POMC), the anorexigenic regulator of food uptake, is the precursor of enkephalins, which stimulate food intake [3].

Native Met-enkephalin is hydrolysed by specific aminopeptidases from the larger forms of peptide, called cryptic form or proenkephalin (PENK), which are present in tissues and blood proteins [9]. It seems probable that an alternative source of cryptic Met-enkephalin was more active in patients with lower BMI than in those with morbid obesity. Figure 2 clearly shows that the concentration of proenkephalin was increased 30 days after the bariatric surgery. The question arises why the cryptic form was not enzymatically processed to the native Met-enkephalin. It can be speculated that the aminopeptidase activity was diminished due to disturbed synthesis after bariatric surgery. Trying to prove this hypothesis we analysed the cryptic/native Met-enkephalin ratio, which was extremely increased at the end of the study in the group with lower BMI. Unexpectedly, the ratio was lower in the patients with higher BMI parallel with lower level of PENK and elevated concentration of native Met-enkephalin. These results clearly showed the differences in the metabolic processes, enzyme activity, and hormone concentrations dependent on the class of obesity.

Interestingly, the varied concentrations of opioid peptides were negatively correlated with ghrelin level 30 days after bariatric surgery.

Ghrelin levels in female patients from both studied groups were lower than those presented in previous research on non-obese humans [25]. After the bariatric surgery, blood ghrelin concentrations were increased dependently of the BMI levels: by 91% in the group with lower BMI and by 131% in the patients from group with higher BMI ($p < 0.05$). These changes were simultaneous with the decreased levels of native and elevated cryptic forms of enkephalin.

Ghrelin levels rise with prolonged fasting and fall after a meal; therefore, weight loss via calorie restriction increases ghrelin levels, which may contribute to the poor long-term efficacy of dietary manipulation to control obesity. The short- and long-term effects of bariatric surgery upon ghrelin levels are still unclear; different bariatric procedures appear to have variable effects upon ghrelin secretion [3]. Interestingly, sleeve gastrectomy may decrease circulating acylated ghrelin concentrations, possibly due to the removal of ghrelin-producing cells in the stomach [3]. Ghrelin

blood levels in obese individuals are much lower than in healthy humans, probably due to reducing the hormone transport into the brain [26] or ghrelin resistance [27]. Diet-induced weight loss increases ghrelin levels, which may be a barrier to sustaining long-term weight loss [28]. Ghrelin acts via the growth hormone secretagogue receptor 1A (GHSR-1A), a G-coupled receptor expressed in the brain and peripheral tissues [7]. Cabral et al. [28] showed that centrally administered ghrelin reaches and increases c-Fos levels in many brain areas with expressed GHSR-1A. They also found that peripheral ghrelin mainly reaches and activates brain areas that are close to circumventricular organs, allowing transportation of the circulating hormone to the brain. These results are in line with the findings of Banks [29], i.e. limited ghrelin brain accessibility is because its transport across the blood–brain barrier (BBB) in the brain-to-blood direction is by a saturable system, but blood-to-brain influx is very low. It has also been shown that the transportation of ghrelin to the brain was lower in obese individuals [29].

Holst et al. [30] found that GHSR-1A can act in a ghrelin-independent manner because this receptor is able to bind other ligands and heterodimerise with other G-protein-coupled receptors in order to modulate their activity.

GHSR-1A receptor, originally known as "orphan" receptor, stimulates growth hormone secretion and binds other growth hormone-releasing peptides (GHRP), e.g. Met-enkephalin and GHRP-6 [31]. Taken together, because the present study shows that in obese females the blood level of Met-enkephalin was elevated in parallel with decreased level of ghrelin, it can be postulated that these hormones interact in the regulation of appetite probably through the same receptor/receptors. However, question arises whether the enkephalin-ghrelin system, in spite of its orexigenic effect, will be a potential pharmacological target for new compounds regulating appetite in patients after bariatric surgery [32]. Future research must consider not only the role of ghrelin and opioid peptides, but also their impact on physiological, psychological, and genetic factors in weight loss failure or recidivism after bariatric surgery.

Conclusions

1. The activity of endogenous peptides in bariatric patients is connected with the degree of obesity.
2. Ghrelin level increases are negatively correlated with native Met-enkephalin changes shortly after bariatric surgery.
3. The interplay of ghrelin and opioids might be considered as a predictor of postoperative weight loss success.

Disclosure statement

The authors declare they have no conflict of interest.

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